A Listing of Important Endocrine Science Papers

This listing of key studies on endocrine science is not intended to be comprehensive or exhaustive. These studies are significant because of their high utility or their analysis which provides perspectives on the science and policy debate surrounding endocrine issues. They also represent a body of literature that was not included in the Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals (Gore et al., 2015). This selection of studies includes peer-reviewed articles that present concepts on the importance of dose and potency; address the theory that the endocrine system is too complex to allow estimations of safe levels of exposure to chemicals that may have potential endocrine activity; and discuss scientific approaches for integrating evidence for setting priorities and reaching decisions on potential hazards and risk.

1. Principles of Pharmacology and Toxicology Also Govern Effects of Chemicals on the Endocrine System. H. Autrup;, et al., Toxicol. Sci. (2015) 146(1): 11-15.

Authored by 15 distinguished academic scientists from the EU and the U.S., this article addresses a contention made by some that current paradigms used in toxicology and in hazard identification and risk characterization of chemicals are inadequate to protect people and wildlife from endocrine-disrupting chemicals (EDCs). The authors argue that chemicals with hormonal activity can be assessed by the well-evaluated health risk characterization approach used by governments and industry for many years.

Keywords: Endocrine Disruptors; Endocrine Toxicology; Risk Assessment; Regulatory; Policy; Endocrine-disrupting Chemicals (EDCs)

http://toxsci.oxfordjournals.org/content/146/1/11.full

2. Critical comments on the WHO-UNEP State of the Science of Endocrine Disrupting Chemicals – 2012. J.C. Lamb, et al., Regul. Toxicol. Pharmacol. (2014) 69(1): 22–40.

This article, written by U.S., Canadian and EU experts, provides a critique of a 2012 World Health Organization (WHO) report which purports to be an update to the highly regarded
2002 WHO State of the Science of Endocrine Disrupting Chemicals. First, the authors conclude the new WHO report is not a state-of-the-science review and does not follow the 2002 WHO recommended a Weight of Evidence (WoE) approach. Second, the authors note the report often presumes that endocrine disruption occurs based on exposure or a potential mechanism despite a lack of evidence to show that chemicals are causally established as endocrine disruptors. Additionally, causation is inferred by the presentation of a series of unrelated facts, which collectively do not demonstrate causation. Third, trends in disease incidence or prevalence are discussed without regard to known causes or risk factors; endocrine disruption is implicated as the reason for such trends in the absence of evidence. Fourth, dose and potency are ignored for most chemicals discussed. Finally, controversial topics (i.e., low-dose effects, non-monotonic dose response) are presented in a one-sided manner and these topics are important to understanding endocrine disruption. Overall, the authors conclude the 2012 report provides less

than a balanced perspective, and does not accurately reflect the state of the science on endocrine disruption.

Keywords: Endocrine-disrupting Chemicals (EDCs); Weight of Evidence (WoE); Causation; State of the Science; Disease Trends; Low-dose Effects; Non-monotonic Dose Response (NMDR)

http://www.sciencedirect.com/science/article/pii/S0273230014000269

3. A Critique of the European Commission Document, "State of the Art Assessment of Endocrine Disruptors." L.R. Rhomberg, et al., Crit. Rev. Toxicol. (2012) 42(6): 465–473.

The authors critique a document titled "State of the Art Assessment of Endocrine Disrupters" (SOA Assessment) that was commissioned by the EU Directorate-General for the Environment to provide a basis for developing scientific criteria for identifying endocrine disruptors and reviewing and possibly revising the European Community Strategy on Endocrine Disrupters. The authors note that the SOA Assessment takes an anecdotal approach rather than attempting a comprehensive assessment of the state of the art or synthesis of current knowledge. To have accomplished the latter, the document would have had to (i) distinguish between apparent associations of outcomes with exposure and the inference of an endocrine-disruption (ED) basis for those outcomes; (ii) constitute a complete and unbiased survey of new literature since 2002 (when the WHO/IPCS document, "Global Assessment of the State-of-the-Science of Endocrine Disruptors" was published); (iii) consider strengths and weaknesses and issues in interpretation of the cited literature; (iv) follow a WoE methodology to evaluate evidence of ED; (v) document the evidence for its conclusions or the reasoning behind them; and (vi) present the evidence for or reasoning behind why conclusions that differ from those drawn in the 2002 WHO/IPCS document need to be changed. The authors conclude that, in its present form, the SOA Assessment fails to provide a balanced and critical assessment or synthesis of literature relevant to ED.

Keywords: Endocrine-disrupting Chemicals (EDCs); Weight of Evidence (WoE); Causation; State of the Science

http://informahealthcare.com/doi/abs/10.3109/10408444.2012.690367

4. Potency Matters: Thresholds Govern Endocrine Activity. C.J. Borgert, et al., Regul. Toxicol. Pharmacol. (2013) 67(1): 83-88.

This article argues that thresholds exist for endocrine active substances and, therefore, safe levels of exposure to these substances can be established for protecting the health of humans and wildlife. Whether thresholds exist for endocrine active substances, and for endocrine disrupting effects of exogenous chemicals, has been posed as a question for regulatory policy by the EU. This question arises from a concern that the endocrine system is too complex to allow estimations of safe levels of exposure to any chemical with potential endocrine activity, and a belief that any such chemical can augment, retard, or disrupt the normal background activity of

endogenous hormones. However, the authors conclude that vital signaling functions of the endocrine system require it to continuously discriminate the biological information conveyed by potent endogenous (i.e., internal to the body) hormones from a more concentrated background of structurally similar, exogenous (i.e., external to the body) molecules with low hormonal potential. This obligatory ability to discriminate important hormonal signals from background noise can be used to define thresholds for induction of hormonal effects, without which normal physiological functions would be impossible. From such thresholds, safe levels of exposure can be estimated. This brief review highlights how the fundamental principles governing hormonal effects – affinity, efficacy, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.

Keywords: Endocrine Active Substances (EAS); Endocrine Pharmacology; Hormone Affinity; Hormone Efficacy; Hormone Potency; Thresholds; Endocrine Disruption

http://www.sciencedirect.com/science/article/pii/S0273230013001025

5. Hypothesis-driven Weight of Evidence Framework for Evaluating Data within the U.S. EPA's Endocrine Disruptor Screening Program. C.J. Borgert, et al., Regul. Toxicol. Pharmacol. (2011) 61(2): 185-191.

The authors discuss why WoE approaches are preferred and often used to critically examine, prioritize, and integrate results from different types of studies to reach general conclusions. The authors conclude that for assessing hormonally active agents, WoE evaluations are necessary to assess screening assays that identify potential interactions with components of the endocrine system, long-term reproductive and developmental toxicity tests that define adverse effects, mode of action studies aimed at identifying toxicological pathways underlying adverse effects, and toxicity, exposure and pharmacokinetic data to characterize potential risks. The authors propose a hypothesis-driven WoE approach for hormonally active agents and illustrate the approach by constructing hypotheses for testing the premise that a substance interacts with various components of the endocrine system and for evaluating data within the U.S. Environmental Protection Agency‟s (EPA) Endocrine Disruptor Screening Program (EDSP).

Keywords: Weight of Evidence (WoE); Endocrine Disruptor Screening Program (EDSP); Endocrine Disruption; Regulatory Framework

http://www.sciencedirect.com/science/article/pii/S0273230011001528

6. Information Quality in Regulatory Decision-making: Peer Review versus Good Laboratory Practice. L.S. McCarty, et al., Environ. Health Perspect. (2012) 120(7): 927-934.

This article presents a review of Good Laboratory Practice (GLP) and the peer review publication process, and their relative contributions to ensuring information quality for regulatory decision-making. Some scientists who are engaged in the endocrine issue debate have expressed extreme disappointment that their research, which has not been conducted according

to GLP standards, was unfairly given less weight by regulators. These scientists have been critical of GLP and have alleged that GLP is merely an expensive record keeping and study reporting requirement. The authors of this review conclude that the highly critical view of GLP is not supported by published analyses pointing to subjectivity and variability in peer-review processes. Although GLP is not designed to establish relative merit, it is an internationally accepted quality assurance, quality control method for documenting experimental conduct and data. The authors conclude that neither the peer review process nor GLP are completely sufficient for establishing relative scientific soundness. However, changes occurring both in peer-review processes and in regulatory guidance resulting in clearer, more transparent communication of scientific information point to an emerging convergence in ensuring information quality. The solution to determining relative merit lies in developing a well-documented, generally accepted WoE scheme to evaluate both peer-reviewed and GLP information used in regulatory decision making where both merit and specific relevance inform the process.

Keywords: Information Quality; Validity; Peer Review; Regulatory Decision Making; Good Laboratory Practices

http://ehp.niehs.nih.gov/1104277/

7. State of the Science Evaluation: Non-monotonic Dose Responses as They Apply to Estrogen, Androgen, and Thyroid Pathways and EPA Testing and Assessment Procedures. U.S. Environmental Protection Agency, June 2013

Given allegations that EDCs may not display traditional dose-response curves (i.e., they may behave differently at lower doses) and the consequent implication that current approaches for regulating chemicals may not be adequate for EDCs, EPA chartered a team of U.S. Food and Drug Administration (FDA) and EPA scientists to develop a state of the science evaluation of the degree to which NMDRs are evidenced in the scientific literature and the extent to which they may impact EPA‟s chemical testing and risk assessment policies and procedures. They found that NMDRs after exposure to synthetic chemicals do occur in biological systems, but are generally not common. Where NMDRs were observed, they were more likely to occur at the molecular level rather than at the level of the subsequent adverse outcomes. Current testing approaches successfully identify the potential for hazards due to exposure to the chemical of concern and, based on the current evaluation, are highly unlikely to mischaracterize a chemical that has the potential to adversely affect the endocrine system due to an NMDR. EPA sought and received comments on their technical report from an external advisory group and is in the process of addressing comments prior to issuing a final report.

Keywords: Endocrine Disruption; Non-monontonic Dose Response (NMDR); State of Science; Low Dose Effects; Regulatory Framework

http://epa.gov/ncct/download_files/edr/NMDR.pdf

8. Low-dose Effects and Non-monotonic Dose-responses of Endocrine Disrupting Chemicals: Has the Case Been Made? L.R. Rhomberg, J.E. Goodman, Regul. Toxicol. Pharmacol. (2012) 64(1): 130–133.

The authors critique a paper by Vandenberg et al. (2012) which claimed that "most if not all [endocrine-disrupting chemicals (EDCs)] are likely to have low-dose effects" and "non-monotonicity is a common occurrence after exposures to hormones and EDCs in cell culture and animals and across human populations." The authors note, for example, Vandenberg et al. present examples as anecdotes without attempting to review all available pertinent data, selectively citing studies without evaluating most of them or examining whether their putative examples are consistent and coherent with other relevant information. They assume that any statistically significant association indicates causation of an adverse effect, and their limited evaluation of specific studies is not done uniformly (i.e., studies with positive results are evaluated differently than those with null results). They also do not evaluate whether exposures in studies are truly "low-dose" and relevant to humans. They propose a number of different NMDR curves, but do not consider reasons for why they should be expected to apply generally across species. Overall, the authors conclude that Vandenberg et al. put forth a number of asserted illustrations of their two conclusions without providing sufficient evidence to make the case for either, and while overlooking evidence that suggests the contrary.

Keywords: Endocrine-disrupting Chemicals (EDCs); Low-dose Effects; Non-monotonic Dose–response (NMDR); Weight of Evidence (WoE)

http://www.sciencedirect.com/science/article/pii/S0273230012001262

9. In utero and Lactational Exposure to Bisphenol A, In Contrast to Ethinyl Estradiol, Does Not Alter Sexually Dimorphic Behavior, Puberty, Fertility, and Anatomy of Female LE Rats. B.C. Ryan, et al., Toxicol. Sci. (2010) 114(1): 133-48.

This study, conducted by scientists from the EPA, was designed to determine if maternal exposure to relatively low oral doses of oral contraceptive ethinyl estradiol (EE2) or bisphenol A (BPA) in utero and during lactation would alter sexual behaviors or alter the age of puberty or reproductive function in female rat offspring. It was intended to test whether BPA could cause such effects and particularly whether BPA and the oral contraceptive pill could cause more harm at low doses to test the low dose hypothesis. Pregnant rats were exposed to a wide range of doses of EE2 (0.05-50 microg/kg/day), or BPA (2, 20, and 200 microg/kg/day) from day 7 of gestation to postnatal day (PND) 18, and the female offspring were studied. EE2 (50 microg/kg/day) caused a number of adverse effects both in structure and function, but only at doses in the range of those that were pharmacology relevant. By contrast, BPA caused no adverse effects.

Keywords: Endocrine Disruption; Ethinyl Estradiol; Bisphenol A; BPA; Rat Reproductive Toxicology; Sexually Dimorphic Behavior; Low Dose Effects

http://toxsci.oxfordjournals.org/content/114/1/133.long

10. Risk Assessment of Endocrine Active Chemicals: Identifying Chemicals of Regulatory Concern. R. Bars, et al., Regul. Toxicol. Pharmacol. (2012) 64(1): 143–154.

A European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) task force was formed to propose scientific criteria on how to identify and evaluate endocrine activity and disruption within EU regulations on pesticides, biocides and industrial chemicals. The resulting ECETOC technical report and an associated workshop with participation by academic, regulatory and private sector scientists presented a science-based concept on how to identify endocrine activity and disrupting properties of chemicals for both human health and the environment. Specific scientific criteria for the determination of endocrine activity and disrupting properties that integrate information from both regulatory (eco) toxicity studies and mechanistic/screening studies were proposed. These criteria combined the nature of the adverse effects detected in studies which give concern for endocrine toxicity with an understanding of the mode of action of toxicity so that adverse effects can be explained scientifically. A key element in the data evaluation is the consideration of all available information in a WoE approach. However, to be able to discriminate chemicals with endocrine properties of low concern from those of higher concern (for regulatory purposes) the task force recognized that the concept needed further refinement. Following a discussion of the key factors at a second workshop, the task force developed further guidance, which is presented in this paper. For human health assessments these factors include the relevance to humans of the endocrine mechanism of toxicity, the specificity of the endocrine effects with respect to other potential toxic effects, the potency of the chemical to induce endocrine toxicity and consideration of exposure levels. For ecotoxicological assessments the key considerations include specificity and potency, but also extend to the consideration of population relevance and negligible exposure.

Keywords: EU Legislation; Endocrine Disrupting Properties; Weight of Evidence (WoE); Adversity; Human Relevance; Specificity; Potency; Population Relevance

http://www.sciencedirect.com/science/article/pii/S0273230012001237

http://toxsci.oxfordjournals.org/content/68/1/121.full

11. Trends in Global Semen Parameter Values. H. Fisch and S.R. Braun, Asian J. Androl. (2013) 15: 169–173;

The authors conclude that allegations of a worldwide decline in semen parameter values have not withstood scientific scrutiny. Reported declines in semen parameter values are likely to be either highly local phenomena with an unknown etiology or the result of methodological errors arising from attempts to observe highly variable physical attributes (semen characteristics) with relatively low-resolution tools (retrospective analysis of nonrandomized study populations). The authors summarize methodological flaws in an influential 1992 paper and review studies that have been published since 1992. Of the 35 major studies of time trends in semen quality reviewed, eight (a total of 18,109 men) suggest a decline in semen quality; 21 (112,386 men) show either no change or an increase in semen quality; and six (26,007 men) show ambiguous or

conflicting results. The cause (or causes) of the geographical and temporal variations in semen parameter values reported by these diverse studies deserve further investigation. The authors conclude that the data supporting a role for „endocrine disruptors‟ in the alleged „decline‟ in semen parameters is weak.

Keywords: Sperm Quantity; Sperm Quality; Global Trends; Methodological Errors; Endocrine-disrupting Chemicals; EDCs

http://www.asiaandro.com/news/upload/20130912-aja2012143a.pdf

12. The Screening of Everyday Life Chemicals in Validated Assays Targeting the Pituitary-gonadal Axis. H. Tinwell, et al., Regul. Toxicol. Pharmacol. (2013) 66(2): 184–196.

The authors tested ten structurally diverse chemicals (vitamins C, B9, B6, B3, sucrose, caffeine, gingerol, xanthan gum, paracetamol, and ibuprofen) deemed intrinsic to modern life but not considered as endocrine active, in several commonly used screening assays designed to identify industrial chemicals as endocrine active. They found that most of the chemicals tested as endocrine active in at least one of the screening tests. They concluded that to avoid regulation of an overwhelming number of chemicals, a WoE approach, combining hazard identification and characterization with exposure considerations, is needed to identify those chemicals of real regulatory concern.

Keywords: Endocrine Activity; Pubertal Development; Everyday Life Chemicals; Hazard Characterization; Exposure Considerations

http://www.sciencedirect.com/science/article/pii/S0273230013000548

13. Developing Scientific Confidence in HTS-derived Prediction Models: Lessons Learned from an Endocrine Case Study. Cox et al. Regul Toxicol Pharmacol. 2014 Aug; 69(3):443-50. doi: 0.1016/j.yrtph.2014.05.010.

Scientific confidence in high-throughput screening (HTS) methods needs to be established prior to regulatory use. This investigation focused on a case study of classification models originally developed by the EPA that used HTS results to predict in vivo endocrine endpoints. EPA‟s classification models were independently recapitulated, and then extended to more robust cross validation models. Cross validation models (based on a set of endocrine ToxCast™ assays and guideline in vivo endocrine screening studies) were shown to have balanced accuracies from 79% to 85% for androgen and estrogen, but only 23% to 50% for thyroid and steroidogenesis. These results indicate the estrogen and androgen models are quite promising for initial use in setting priorities for endocrine screening. Based on the lessons learned, the authors proposed a framework for organizing and documenting scientific confidence in HTS assays and the prediction models derived therefrom.

Keywords: High-throughput Screening (HTS); Predicting Endocrine Activity;

http://www.ncbi.nlm.nih.gov/pubmed/24845243

14. An Exposure:Activity Profiling Method for Interpreting High-throughput Screening Data for Estrogenic Activity - - Proof of Concept. Becker RA et al. Regul Toxicol Pharmacol. 2015 Apr; 71(3):398-408 doi: 10.1016/j.yrtph.2015.01.008.

This article illustrates how to integrate information on exposure with results of rapid high-throughput in vitro screening assays for estrogenic activity. This exposure:activity profiling is accomplished by calculating the exposure:activity ratios (EARs) using human exposure estimates and AC50 values for a range of chemicals tested in a suite of seven estrogenic assays in ToxCast™ and Tox21. Additional context is provided by comparing chemical-specific EARs to the EAR of the ubiquitous dietary phytoestrogen, genistein (GEN) to calculate relative estrogenic exposure:activity quotients (REEAQ) . For risk-based prioritization, substances with small EARs and REEAQs would indicate low priority for further endocrine screening or testing.

Keywords: Endocrine Screening; High-throughput Screening (HTS);Integrating Exposure With Bioactivity; Risk-Based Prioritization ;

http://www.ncbi.nlm.nih.gov/pubmed/25656492

15. Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. Browne P et al. Environ Sci Technol. 2015 Jul 21;49 (14):8804-14. doi: 10.1021/acs.est.5b02641.

The authors report on the performance of a model developed using HTS1 assays of estrogenic activity to predict the uterotrophic response in vivo in rodents. This computational model, the ToxCast ER AUC model, integrates results from 18 separate assays to derive bioactivity scores. The model was shown to be 86% to 93% accurate in predicting reference chemicals. In addition, the model predicted results of the EDSP Tier 1 guideline and other uterotrophic studies with 84% to 100% accuracy. This performance is sufficient for EPA to accept the ToxCast ER model data for chemicals as alternatives for EDSP Tier 1 ER binding, ER transactivation, and uterotrophic assays.

Keywords: High-throughput Screening (HTS); Predicting Estrogenic Activity; ToxCast; Uterotrophic Response; Endocrine Disruptor Screening Program (EDSP)

http://www.ncbi.nlm.nih.gov/pubmed/26066997

16. **Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor. Judson RS et al. 2015, Toxicological Sciences 148(1):137-154**

Further documentation of the ToxCast ER AUC model is detailed in this report. Details include documentation of how the model performs in predicting ER agonist or antagonist activity, as well as approaches to identify false positive responses caused by assay interference. In applying this method to 1,812 commercial and environmental chemicals, 111 (6.1%) were predicted to be ER active in agonist or antagonist mode. The authors discuss how this model can be used to

distinguish ER active chemicals with environmental chemicals with human exposure potential from non-ER active substances. Substances with negligible activity would be deprioritized, while prioritized substances would be candidates for additional in vivo endocrine screening.

Keywords:
High-throughput Screening (HTS); Predicting Estrogenic Activity; ToxCast; Endocrine Disruptor Screening Program (EDSP)

https://doi.org/10.1093/toxsci/kfv168

17. Does GLP Enhance the Quality of Toxicological Evidence for Regulatory Decisions? Borgert C, et al. Toxicological Sciences 151(2):206-213

The authors respond to a recent commentary on assessing dose-response relationships for endocrine disrupting chemicals. The authors of the commentary contend that study quality scoring systems giving primacy to good laboratory practices (GLP) are biased against non-GLP studies from the literature and are merely record-keeping exercises to prevent fraudulent reporting of data from non-published guideline toxicology studies. In their response, Borgert and colleagues argue that while both GLP and non-GLP studies should be utilized in the assessment of chemicals, the use of standardized test guidelines and GLP promotes consistency, reliability, comparability, and harmonization of various types of studies used by regulatory agencies worldwide. They point out that it is not an either/or choice to use GLP or non-GLP data in a regulatory context and that all literature should be evaluated for reliability and relevance in a systematic and transparent manner.

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