



Published in final edited form as:

Endocr Disruptors (Austin). 2015 ; 3(1): . doi:10.1080/23273747.2015.1069916.

Assessing Human Health Risk to Endocrine Disrupting Chemicals: a Focus on Prenatal Exposures and Oxidative Stress

Kari Neier¹, Elizabeth H. Marchlewicz¹, Dana C. Dolinoy^{1,2}, and Vasantha Padmanabhan^{1,3,*}

¹Department of Environmental Health Sciences, University of Michigan, Ann Arbor, Michigan 48109

²Department of Nutritional Sciences, University of Michigan, Ann Arbor, Michigan 48109

³Department of Pediatrics, University of Michigan, Ann Arbor 48109

Abstract

Understanding the health risk posed by endocrine disrupting chemicals (EDCs) is a challenge that is receiving intense attention. The following study criteria should be considered to facilitate risk assessment for exposure to EDCs: 1) characterization of target health outcomes and their mediators, 2) study of exposures in the context of critical periods of development, 3) accurate estimates of human exposures and use of human-relevant exposures in animal studies, and 4) cross-species comparisons. In this commentary, we discuss the importance and relevance of each of these criteria in studying the effects of prenatal exposure to EDCs. Our discussion focuses on oxidative stress as a mediator of EDC-related health effects due to its association with both EDC exposure and health outcomes. Our recent study (Veiga-Lopez et al. 2015)¹ addressed each of the four outlined criteria and demonstrated that prenatal bisphenol-A exposure is associated with oxidative stress, a risk factor for developing diabetes and cardiovascular diseases in adulthood.

Keywords

EDCs; oxidative stress; prenatal programming; organizational effects; risk assessment

INTRODUCTION

Endocrine disrupting chemicals (EDCs), such as bisphenol A (BPA) and phthalates (e.g. diethylhexyl phthalate, DEHP), are ubiquitously present in the environment and humans, and have been the subject of rigorous scientific investigation in recent years due to the potential for a variety of adverse health outcomes.² EDCs act as hormone agonists or antagonists, interfere with signaling mechanisms, and consequently disrupt hormonal homeostasis and developmental processes. Importantly, in utero exposure to EDCs have the potential to alter developmental trajectories of offspring, thus influencing health and disease

*Correspondence to: Vasantha Padmanabhan, PhD, 7641A, Med Sci II, University of Michigan, Ann Arbor, MI 48109, Phone: 734-647-0276, ; Email: vasantha@med.umich.edu.

Disclosures: The authors have no conflicts of interest associated with the present manuscript. No conflict of interest is declared.

status later in life, illustrating the concept of the developmental origins of health and disease (DOHaD).³

There is continuing debate regarding the health risks posed by exposures to EDCs.⁴ To better understand human risk from developmental exposure to EDCs, the following criteria should be considered: 1) characterization of target health outcomes and their mediators, 2) study of exposures within the context of critical periods of development, 3) accurate estimates of human exposure and use of human-relevant doses in animal studies, and 4) cross-species comparisons for establishing weight-of-evidence for adverse health outcomes (Figure 1). Our recent study¹ investigating perinatal BPA exposure in mouse, rat, and sheep models, as well as in human pregnancy and infant samples, applied these principles in evaluating oxidative stress and free fatty acid (FFA) outcomes, thus providing a framework for conducting future studies aimed at evaluating implications of developmental EDC exposures on life course health effects. Here we review the criteria outlined above to describe its importance in evaluating human health risk to EDCs, with a focus on oxidative stress as a mediator of adverse health outcomes.

OXIDATIVE STRESS AS A MEDIATOR OF EDC-RELATED HEALTH OUTCOMES

Identifying and assessing mediators of health outcomes, which are effectors in the pathway of exposure to disease, is essential for characterizing the impact of developmental insults on health outcomes. For example, inflammation has been identified and extensively studied as a potential mediator of obesity, diabetes, and premature birth.^{5,6} Recently, oxidative stress has emerged as an investigative mechanism for EDC toxicity.

Oxidative stress is classically defined as an imbalance in oxidant and antioxidant species within a system in which oxidant species are predominant.⁷ Interestingly, although oxidative stress and exposure to EDCs have been associated with many of the same health effects, they are rarely studied in parallel. For example, both oxidative stress and EDC exposures have been associated with metabolic syndrome, insulin resistance, diabetes, obesity, and cardiovascular complications.^{8,9} In one study, Houstis et al.¹⁰ provided evidence of a causal relationship between increased production of reactive oxygen species (ROS), molecules that cause oxidative damage and induce oxidative stress, and insulin resistance in mice. In another study, Alonso-Magdalena et al.¹¹ demonstrated that prenatal exposure to BPA induced insulin resistance in mice. Both oxidative stress and EDCs have also been implicated in intrauterine growth restriction and preterm birth, two risk factors associated with adult onset metabolic diseases.^{12,13} However, only a few studies have simultaneously examined oxidative stress pathways mediating the impact of prenatal EDC exposure on health outcomes using the sensitive measures for measuring markers of oxidative stress described here.

Despite the overlaps in health outcomes, oxidative stress has only recently been studied as a potential mediator of EDC-related outcomes. Recent studies have reported associations between a variety of different EDCs and oxidative stress.¹⁴⁻¹⁶ For example, phthalate-induced reproductive toxicity has been linked to oxidative stress in both rats and

humans,^{13,17} while perinatal exposure to thimerosal, tributyltin and benzene have been implicated in production of oxidative stress in rats and mice.^{14–16} Given its role in disease development together with increasing evidence supporting a link to EDC exposures, oxidative stress is worthy of further study, particularly in the context of prenatal exposures.

Measures of Oxidative Stress

A wide variety of measures are used to evaluate oxidative stress, each with distinct applications and interpretations. Many measures of oxidative stress can be obtained using minimally invasive methods (e.g., blood draw and urine collection), making them relatively easy to implement in both animal and human studies. Others involve direct analysis of a target organ or tissue. Careful consideration of the EDC and health outcome(s) of interest is essential in both choosing the measurement to implement and in interpreting results. While elevated ROS levels represent a hallmark of oxidative stress, ROS are short-lived and difficult to accurately measure and interpret.¹⁸ Therefore, more stable measures or markers of oxidative stress, such as covalently modified amino acids and proteins, are needed for accurate characterization of oxidative stress. The stability of these markers is dependent on a variety of factors, but under steady state conditions, they can generally be interpreted as representative of the oxidative stress environment of the cells or tissues at the time of collection.

Historically, immunoassay-based methods for assessing markers of oxidative stress such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) have been widely used to measure oxidative stress. However, state of the art analytical techniques have been developed in recent years for quantification of specific biomarkers of oxidative stress and the link between EDC exposures and chronic disease outcomes. Below, we discuss a wide range of highly sensitive analytical methods available for measuring stable markers of oxidative stress and their relevance in studying developmental exposures to EDCs. Many of these methods are yet to be applied for the study of developmental programming following prenatal EDC exposure (see Table 1).

Oxidation products of amino acids—Recent studies have utilized products of oxidized aromatic amino acids in proteins as a measure of oxidative stress. These products are commonly measured in plasma, serum, urine, and tissues, and can be representative of either systemic or target organ oxidative stress.¹⁸ However, to accurately analyze products of amino acid oxidation, the target tissue should be perfused with an antioxidant buffer prior to tissue harvest and sample collection, and samples stored in this buffer at -80°C until analysis.¹⁸ Oxidized amino acid products can then be measured using liquid chromatography (LC) or gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS), and with high performance liquid chromatography (HPLC). HPLC may result in coelutions of similar compounds, and is therefore not as specific as MS.^{1,18}

Modified tyrosine residues are one of the most commonly measured oxidation products due to their stability and usefulness in identifying specific oxidation pathways.¹⁸ These molecules represent both oxidized phenylalanine and tyrosine residues.¹⁸ Examples of oxidized tyrosine amino acid products include o,o-dityrosine (DiY), 3-chlorotyrosine (ClY),

and 3-nitrotyrosine (NY). DiY is formed upon oxidation and radical formation of a tyrosine residue and can be formed through both nitrosative and oxidative pathways.¹⁸ Recently, DiY has been found to be elevated in metabolic pathologies, including atherosclerosis and hyperlipidemia.¹⁹ Since EDCs, such as BPA, have also been linked to similar metabolic conditions⁸, measuring DiY following prenatal exposure to EDCs would be useful to identify oxidative stress pathways.

CIY, a tyrosine modification catalyzed by myeloperoxidase, is associated with macrophage activation and acute inflammation.^{18,20} Elevated levels of CIY are associated with similar health effects as those associated with elevated DiY, such as atherosclerosis and cardiovascular disease.^{20,21} Thus, CIY may also be of interest to investigators studying EDCs.

NY is formed by nitration of tyrosine and is mediated by reactive nitrogen species and is characteristic of nitrosative pathways.¹⁸ Increased levels of NY have been linked with a variety of negative health outcomes associated with preterm birth, such as perinatal asphyxia.²² Results from several studies have suggested that estrogenic compounds may impact NY formation, indicating a role for NY in response to EDCs. For example, a study performed in estrogen receptor- α (ER α) knock out mice demonstrated that impaired ER α signaling causes an increase in NY production and metabolic dysregulation.²³ This link between estrogen signaling and NY demonstrates the usefulness of measuring NY levels in exposure studies involving EDCs that bind estrogen receptors.

Oxidation products of lipids—Products of lipid peroxidation represent another set of common measures of oxidative stress. These compounds are typically less stable than amino acid products due to rapid reactions following initial oxidation; nonetheless, lipid peroxidation products may have specific biological functions and are detectable in a variety of biological matrices,^{18,24} but these sensitive analytical techniques have not yet been used in prenatal EDC exposure studies. Lipid oxidation products are commonly detected using a combination of LC or GC and MS.²⁴ F₂-isoprostanes are common markers of lipid peroxidation that are released into urine and plasma. They can serve as systemic markers of oxidative stress that can be measured with minimal invasiveness.²⁵ However, to prevent ex-vivo oxidation, BHT should be added to collected samples prior to freezing and storing at -80°C .²⁶ Elevated F₂-isoprostane levels, as measured by ELISA-based techniques, have been associated with metabolic dysregulation.²⁷ Elevated F₂-isoprostane levels have also been associated with direct exposure to EDCs such as polychlorinated biphenyls (PCBs) and organophosphate (OP) pesticides (Table 1).^{28,29} Investigations utilizing sensitive analytical techniques to measure F₂-isoprostanes in prenatal EDC exposure studies are needed to further elucidate how EDC exposures during critical periods in development may impact lipid peroxidation and health outcomes.

Oxidation products of nucleic acids—Multiple methods are used to measure DNA and RNA oxidation. To quantify DNA and RNA oxidation, HPLC combined with electrochemical detection (HPLC-ECD) is a common sensitive method used to measure oxidized guanine residues.³⁰ Urine, plasma and tissue samples should be stored at -20°C or -80°C immediately following collection, and prior to HPLC-ECD analysis. Specialized

technique with cold high salt guanidine thiocyanate, catalase and 2,2,6,6-tetramethylpiperidine-N-oxyl should be used for extraction DNA from the sample to prevent spurious oxidation.^{31,32}

Guanine residues are the most commonly measured oxidized nucleosides, because guanine has the lowest redox potential and is the most susceptible to a majority of oxidative processes, although thymine is the primary target of hydroxyl radicals.³⁰ Hence, 8-hydroxydeoxyguanosine (8-oxodG) is most commonly used to assess DNA oxidation and 8-hydroxyguanosine (8-oxoGuo) for RNA oxidation.³⁰ 8-oxodG and 8-oxoGuo in urine can be interpreted as measures of global oxidative stress, while in tissues, they represent local oxidative stress.³⁰ However, 8-oxodG and 8-oxoGuo levels in plasma are dictated more by kidney function than oxidative stress, and therefore cannot be used to make comparisons across individuals.³⁰

DNA and RNA oxidation measures are becoming increasingly more popular and can be useful in measuring oxidative stress associated with EDC exposure. For example, Ferguson et al. (2014)¹³ demonstrated that increased phthalate metabolites present in urine samples from a population of pregnant women were associated with increased urinary 8-oxodG. In addition, a recent study that used more sensitive measures for 8-oxodG found that it was associated with phthalate-induced toxicity in peripubertal rats, demonstrating its usefulness in EDC exposure studies (Table 1).¹⁷

Cellular redox potentials—Another method used to evaluate oxidative stress is the direct measurement of reactive thiol species. Reactive thiols, such as glutathione (GSH) and its oxidized form glutathione disulfide (GSSG), often govern the redox state of the cell and can be directly measured in cells, tissues, blood and other biological matrices using HPLC.³³ Because GSH may rapidly auto-oxidize upon contact with air, samples must be collected and stored in reducing buffer at -80°C .³⁴ Applying the Nernst equation to the measured concentrations of each redox pair provides an estimate of the cellular redox potential. The same redox HPLC method can be used to evaluate S-glutathionylation levels, protein-bound GSH, which is another indicator of oxidative stress.³³ These measurements are extremely sensitive and may detect early oxidative stress responses prior to lipid oxidation and other measures of oxidation-related outcomes.³³ Early data from current investigations in our group indicate that these measurements may be useful in detecting changes in oxidative stress due to prenatal BPA exposure (Marchlewicz et al. unpublished data).

Free Fatty Acids and Relationship with Oxidative Stress

Multiple studies have linked elevated plasma free fatty acids (FFA) levels with EDCs, metabolic syndrome, and insulin resistance, making FFAs an attractive measurement to use as an outcome measure.^{35,36} Free fatty acids (FFAs) have been demonstrated to increase the formation of oxidative species, such as hydrogen peroxide and hydroxide radicals, through activation of NAD(P)H oxidase, and thus can serve as a proxy for oxidative stress.³⁵ FFA levels are typically analyzed in plasma and tissues and can be quantified with GC.^{1,35} Since FFAs are not characterized by their oxidation state, there is no need for preventative measures against auto-oxidation, although collected tissues should be immediately frozen at

–80°C for storage.³⁷ Recently, we found that mice prenatally exposed to BPA had significant changes in FFAs; myristic acid was decreased and omega 6 γ -linolenic acid was increased relative to controls.¹ Other studies have found that FFA levels can be altered by exposure to OP pesticides and phthalates (Table 1).^{37,38} More research is needed to elucidate the role for FFAs in EDC-mediated toxicity.

EXPOSURES IN THE CONTEXT OF CRITICAL PERIODS AND SUSCEPTIBILITY DURING DEVELOPMENT

To assess human health risk to EDCs, it is imperative to evaluate exposure in the context of developmental stage. Critical periods of development are windows during prenatal and early postnatal life at which organ differentiation occurs. These periods are particularly susceptible to environmental insults due to the potential for inducing organizational changes that persist throughout the life course.³⁹ During fetal development, different organ systems begin to develop at different time periods. For instance, in humans, the nervous system begins developing at gestational day 18 to 19, while the reproductive system begins to develop at gestational day 27.⁴⁰ Thus, the susceptibility to EDC exposure and end outcome is dependent on the critical period for a given target organ system. Prenatal exposure to phthalates, for example, has been shown to alter Leydig cell differentiation, which occurs during sexual differentiation of the reproductive tract.⁴¹

It is important to recognize that critical periods differ among species. Some species are precocial, physiologically mature at birth, while others are altricial, physiologically immature at birth so developmental programming extends into early postnatal life.⁴² For example, humans and sheep are precocial and their ovaries develop entirely prenatally,⁴³ while rats and mice are altricial and their ovaries continue to develop after birth.⁴² These disparities in developmental timing among species should be considered in studies of EDC exposures.

Cellular redox environment and signaling is an important component of development and can be perturbed by EDC-induced oxidative stress.⁴⁴ EDC-induced oxidative stress during critical developmental windows would prevent the conceptus from properly responding and adapting to changes in redox state or increases in ROS.⁴⁴ For example, we found prenatal BPA exposure in humans during the first trimester, when several organ systems differentiate, induces oxidative stress in the mother and the fetus.¹

ASSESSING HUMAN-RELEVANT EDC EXPOSURE LEVELS AND TISSUES

Accurate characterization of human exposures to EDCs as well as utilization of human-relevant exposures with particular attention to dose levels, including low and non-monotonic dose effects,⁴⁵ in animal models is crucial for risk assessment. Of particular relevance here, some ROS can form from secondary effects rather than primary effects of EDCs, which can be dependent on dose.⁴⁶ EDCs and their metabolites are commonly measured in blood, plasma, and urine in order to estimate human exposures. Likewise, within mammalian toxicological studies, the choice of tissue of analysis for oxidative stress marker should reflect human biology, with multiple tissues assessed when possible. Our recent work

evaluating mouse dams exposed during gestation and lactation to BPA in the diet revealed tissue specific alterations in blood and liver analyzed for redox potentials.⁴⁷ Ultimately, a firmer understanding of EDC toxicokinetics in both animal models and humans is essential to accurately estimate exposure levels and estimate doses for testing in animal studies. In addition, because of the ubiquitous presence of many EDCs in the environment, care needs to be taken to use contaminant-free collection, storage, and analytical materials such as that employed in a recent BPA round robin study.⁴⁸ It is also important to utilize well-validated analytical methods for measurement purposes. Appropriate blanks need to be included both during collection and measurement.⁴⁹ Recoveries need to be verified by spiking known concentrations of the EDC of interest. Once accurate estimates of human exposure have been established, animal studies should use this information in choosing appropriate doses and dosing strategies of EDC for testing, which is crucial for assessing human health risk.

CROSS-SPECIES COMPARISON TO DETERMINE WEIGHT-OF-EVIDENCE ON ADVERSE HEALTH OUTCOMES

Because critical periods in development differ across species, it is imperative to interpret results within this context. Cross species comparisons enable increased evaluation of weight of evidence for risk assessment. A few investigations that have examined cross-species responses to EDCs have found distinct responses to EDCs across species. For example, Johnson et al.⁵⁰ demonstrated that in utero exposure to DEHP had different effects on Leydig cell hormone synthesis in the testes of rats, mice and humans; DEHP was found to inhibit hormone production in fetal Leydig cells in testes of rats, but not in mice. Another recent *in vitro* study that examined the direct effects of six different EDCs (mono-(2-ethylhexyl) phthalate (MEHP), cadmium, depleted uranium, diethylstilbestrol (DES), BPA, and metformin) on gametogenesis and steroidogenesis in rat, mouse, and human testes cells found that many of the compounds had species-specific effects.⁵¹ Thresholds of oxidative stress have also been shown to be different across species. For example, Hassan et al.⁵² found that rats, mice, guinea pigs, and hamsters had different sensitivities to endrin-mediated lipid peroxidation. Therefore, cross-species studies are essential for providing accurate risk assessment and help translate findings in animals to humans.

CONCLUSIONS

Our recent study, Veiga-Lopez et al.¹ follows the outlined criteria for assessing human health risk to BPA, a well-known EDC. We examined oxidative stress as a mediator of adverse health outcomes, studied BPA exposure in the context of the prenatal period when organizational effects are documented, assessed BPA levels in humans using the validated methodology, applied relevant human exposure and dose levels in animal studies, and evaluated four species, including humans. In following these criteria, our study demonstrated that prenatal exposure to BPA leads to oxidative stress, a risk factor for development of cardiovascular disease and diabetes in adulthood, in offspring of three species. Our study was not without limitations, however. The samples sizes used in this study are small, limiting the generalizability of the study. Additional large-scale studies are needed to expand these observations.

Given the growing body of evidence linking oxidative stress to EDCs and the necessity to interpret results in the context of species being studied, investigations using a cross-species approach are needed for evaluating risk from developmental exposures to EDC. The recent abundance of sensitive analytical methods that can be applied to measure oxidative stress will now allow for further elucidation of toxicity mechanisms for these compounds, thus advancing our understanding of how these chemicals contribute to human health.

Acknowledgments

This work was supported by NIH grants R01 R01ES01654, R01 ES017005, R01 ES017524, P01 ES02284401, P30 ES017885, as well as U.S. Environmental Protection Agency (US EPA) grant RD83543601. Support for KN and EHM was provided by NIH Institutional Training Grants T32 ES007062 and T32 HD079342, respectively. The contents of this publication are solely the responsibility of the grantee and do not necessarily represent the official views of the US EPA or the NIH. Further, the US EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

REFERENCES

1. Veiga-Lopez A, Pennathur S, Kannan K, Patisaul HB, Dolinoy DC, Zeng L, Padmanabhan V. Impact of gestational bisphenol a on oxidative stress and free Fatty acids: human association and interspecies animal testing studies. *Endocrinology* [Internet]. 2015; 156:911–922. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25603046>.
2. De Coster S, Van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. *J Environ Public Health*. 2012 2012.
3. Barker DJ. The origins of the developmental origins theory. *J Intern Med*. 2007; 261:412–417. [PubMed: 17444880]
4. Zoeller RT, Bergman Å, Becher G, Bjerregaard P, Bornman R, Brandt I, Iguchi T, Jobling S, Kidd KA, Kortenkamp A, et al. A path forward in the debate over health impacts of endocrine disrupting chemicals. *Environ Heal*. 2014:1–11.
5. Bondia-Pons I, Ryan L, Martinez JA. Oxidative stress and inflammation interactions in human obesity. *J Physiol Biochem*. 2012; 68:701–711. [PubMed: 22351038]
6. Burdet J, Paula A, Rubio D, Inés A, Laura M, Ibarra C. Inflammation, infection and preterm birth. *Curr Pharm Des*. 2014; 20:4741–4748. [PubMed: 24588830]
7. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem Biol Interact* [Internet]. 2014; 224:164–175. Available from: <http://dx.doi.org/10.1016/j.cbi.2014.10.016>.
8. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *JAMA*. 2008; 300:1303–1310. [PubMed: 18799442]
9. Bonomini F, Rodella LF, Rezzani R. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis*. 2015; 6:109–120. [PubMed: 25821639]
10. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*. 2006; 440:944–948. [PubMed: 16612386]
11. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A. Bisphenol a exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. *Environ Health Perspect*. 2010; 118:1243–1250. [PubMed: 20488778]
12. Peter Stein T, Scholl TO, Schluter MD, Leskiw MJ, Chen X, Spur BW, Rodriguez A. Oxidative stress early in pregnancy and pregnancy outcome. *Free Radic Res* [Internet]. 2008; 42:841–848. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18985484>.
13. Ferguson KK, Cantonwine DE, Rivera-González LO, Loch-Carus R, Mukherjee B, Anzalota Del Toro L V, Jiménez-Vélez B, Calafat AM, Ye X, Alshwabkeh AN, et al. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. *Environ Sci Technol*. 2014

14. Badham HJ, Renaud SJ, Wan J, Winn LM. Benzene-initiated oxidative stress: Effects on embryonic signaling pathways. *Chem Biol Interact* [Internet]. 2010; 184:218–221. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19913523>.
15. Sulkowski ZL, Chen T, Midha S, Zavacki aM, Sajdel-Sulkowska EM. Maternal thimerosal exposure results in aberrant cerebellar oxidative stress, thyroid hormone metabolism, and motor behavior in rat pups; sex- and strain-dependent effects. *Cerebellum* [Internet]. 2012; 11:575–586. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22015705>.
16. Watkins DJ, Ferguson KK, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. *Int J Hyg Environ Health* [Internet]. 2015; 218:212–219. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S143846391400114X>.
17. Shono T, Taguchi T. Short-time exposure to mono-n-butyl phthalate (MBP)-induced oxidative stress associated with DNA damage and the atrophy of the testis in pubertal rats. *Environ Sci Pollut Res Int* [Internet]. 2014; 21:3187–3190. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24310901>.
18. Vivekanandan-Giri A, Byun J, Pennathur S. Quantitative analysis of amino acid oxidation markers by tandem mass spectrometry. *Methods Enzymol*. 2011; 491:73–89.
19. Wu G-R, Cheserek M, Shi Y-H, Shen L-Y, Yu J, Le G-W. Elevated plasma dityrosine in patients with hyperlipidemia compared to healthy individuals. *Ann Nutr Metab* [Internet]. 2015; 66:44–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25531053>.
20. Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an oxidative pathway for generating dysfunctional HDL. *Chem Res Toxicol*. 2010; 23:447–454. [PubMed: 20043647]
21. Daugherty A, Dunn JL, Rateri DL, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest*. 1994; 94:437–444. [PubMed: 8040285]
22. Groenendaal F, Lammers H, Smit D, Nikkels PGJ. Nitrotyrosine in brain tissue of neonates after perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed*. 2006; 91:F429–F433. [PubMed: 16835259]
23. Manrique C, Lastra G, Habibi J, Mugerfeld I, Garro M, Sowers JR. Loss of estrogen receptor a signaling leads to insulin resistance and obesity in young and adult female mice. *Cardiorenal Med* [Internet]. 2012; 2:200–210. Available from: <http://www.karger.com/doi/10.1159/000339563>.
24. Spickett, CM.; Pitt, AR. Oxidative lipidomics coming of age: advances in analysis of oxidized phospholipids in physiology and pathology; *Antioxid Redox Signal* [Internet]. 2015. p. 1-50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25694038>
25. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev*. 2000; 32:377–385. [PubMed: 11139135]
26. Milne GL, Gao B, Terry ES, Zackbert WE, Sanchez SC. Measurement of F2-isoprostanes in isofurans using gas chromatography-mass spectrometry. *Free Radic Biol Med*. 2013; 59:36–44. [PubMed: 23044261]
27. Tian Y-F, Hsia T-L, Hsieh C-H, Huang D-W, Chen C-H, Hsieh P-S. The importance of cyclooxygenase 2-mediated oxidative stress in obesity-induced muscular insulin resistance in high-fat-fed rats. *Life Sci* [Internet]. 2011; 89:107–114. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21640730>.
28. Newsome BJ, Petriello MC, Gu Han S, O’Murphy M, Eske KE, Sunkara M, Morris AJ, Hennig B. Green tea diet decreases PCB 126-induced oxidative stress in mice by upregulating antioxidant enzymes. *J Nutr Biochem*. 2014; 25:126–135. [PubMed: 24378064]
29. López-Granero C, Cañadas F, Cardona D, Yu Y, Giménez E, Lozano R, Avila DS, Aschner M, Sánchez-Santed F. Chlorpyrifos-, diisopropylphosphorofluoridate-, and parathion-induced behavioral and oxidative stress effects: Are they mediated by analogous mechanisms of action? *Toxicol Sci*. 2013; 131:206–216. [PubMed: 22986948]
30. Poulsen HE, Nadal LL, Broedbaek K, Nielsen PE, Weimann A. Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. *Biochim Biophys Acta - Gen Subj*. 2014; 1840:801–808.

31. Pilger A, Rüdiger HW. 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int Arch Occup Environ Health*. 2006; 80:1–15. [PubMed: 16685565]
32. Bhattacharjee S, Deterding LJ, Chatterjee S, Jiang J, Ehrenshaft M, Lardinois O, Ramirez DC, Tomer KB, Mason RP. Site-specific radical formation in DNA induced by Cu(II)-H₂O₂ oxidizing system, using ESR, Immuno-spin trapping, LC/MS and MS/MS. *Free Radic Biol Med*. 2011; 50:1536–1545. [PubMed: 21382477]
33. Hansen JM, Harris C. Redox control of teratogenesis. *Reprod Toxicol*. 2013; 35:165–179. [PubMed: 23089153]
34. Harris, C.; Hansen, JM. Oxidative Stress, thiols, and redox profiles. In: Walker, JM., editor. *Methods in Molecular Biology*. 2012. p. 325-346.
35. Wu Y, Zhou H, Wu K, Lee S, Li R, Liu X. PTEN phosphorylation and nuclear export mediate free fatty acid-induced oxidative stress. *Antioxid Redox Signal* [Internet]. 2014; 20:1382–1395. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3936505&tool=pmcentrez&rendertype=abstract>.
36. Chen JQ, Brown TR, Russo J. Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. *Biochim Biophys Acta - Mol Cell Res*. 2009; 1793:1128–1143.
37. Xu Y, Agrawal S, Cook TJ, Knipp GT. Di-(2-ethylhexyl)-phthalate affects lipid profiling in fetal rat brain upon maternal exposure. *Arch Toxicol*. 2007; 81:57–62. [PubMed: 16951938]
38. Suzuki H, Ito Y, Noro Y, Koketsu M, Kamijima M, Tomizawa M. Organophosphate agents induce plasma hypertriglyceridemia in mouse via single or dual inhibition of the endocannabinoid hydrolyzing enzyme(s). *Toxicol Lett* [Internet]. 2014; 225:153–157. Available from: <http://dx.doi.org/10.1016/j.toxlet.2013.12.004>.
39. Thornton J, Zehr JL, Loose MD. Effects of prenatal androgens on rhesus monkeys: a model system to explore the organizational hypothesis in primates. *Changes*. 2009; 29:997–1003.
40. DeSesso, JM. Comparative gestational milestones in vertebrate development. In: Hood, RD., editor. *Developmental and Reproductive Toxicology: A Practical Approach*. 2012. p. 100-131.
41. Beverly BEJ, Lambright CS, Furr JR, Sampson H, Wilson VS, McIntyre BS, Foster PMD, Travlos G, Gray LE. Simvastatin and dipentyl phthalate lower ex vivo testicular testosterone production and exhibit additive effects on testicular testosterone and gene expression via distinct mechanistic pathways in the fetal rat. *Toxicol Sci* [Internet]. 2014; 141:524–537. Available from: <http://www.toxsci.oxfordjournals.org/cgi/doi/10.1093/toxsci/kfu149>.
42. Zelditch ML, Lundrigan BL, Sheets HD, Garland T. Do precocial mammals develop at a faster rate? A comparison of rates of skull development in *Sigmodon fulviventer* and *Mus musculus domesticus*. *J Evol Biol*. 2003; 16:708–720. [PubMed: 14632234]
43. Padmanabhan V, Veiga-Lopez A. Sheep models of polycystic ovary syndrome phenotype. *Mol Cell Endocrinol*. 2013; 373:8–20. [PubMed: 23084976]
44. Hansen JM. Oxidative stress as a mechanism of teratogenesis. *Birth Defects Res Part C - Embryo Today Rev*. 2006; 78:293–307.
45. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee D-H, Shioda T, Soto AM, vom Saal FS, Welshons WV, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. 2012; 33:378–455.
46. Meyer JN, Leung MCK, Rooney JP, Sandoel A, Hengartner MO, Kisby GE, Bess AS. Mitochondria as a target of environmental toxicants. *Toxicol Sci*. 2013; 134:1–17. [PubMed: 23629515]
47. Neier, K.; Marchlewicz, EH.; Harris, C.; Dolinoy, DC. Society of Toxicology Annual Meeting. San Diego, CA: 2015. Hepatic oxidative stress in pregnant and nursing female mice exposed to bisphenol A and high-fat diets (abstract #2590).
48. Vandenberg LN, Gerona RR, Kannan K, Taylor Ja, van Breemen RB, Dickenson Ca, Liao C, Yuan Y, Newbold RR, Padmanabhan V, et al. A round robin approach to the analysis of bisphenol A (BPA) in human blood samples. *Environ Heal* [Internet]. 2014; 13:25. Available from: <http://www.ehjournal.net/content/13/1/25>.

49. Ye X, Zhou X, Hennings R, Kramer J, Calafat AM. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: An elusive laboratory challenge. *Environ Health Perspect*. 2013; 121:283–286. [PubMed: 23458838]
50. Johnson KJ, Heger NE, Boekelheide K. Of mice and men (and rats): phthalate-induced fetal testis endocrine disruption is species-dependent. *Toxicol Sci* [Internet]. 2012; 129:235–248. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3491958&tool=pmcentrez&rendertype=abstract>.
51. Habert R, Muczynski V, Grisin T, Moison D, Messiaen S, Frydman R, Benachi A, Delbes G, Lambrot R, Lehraiki A, et al. Concerns about the widespread use of rodent models for human risk assessments of endocrine disruptors. *Reproduction* [Internet]. 2014; 147:R119–R129. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3959776&tool=pmcentrez&rendertype=abstract>.
52. Hassan MQ, Numan IT, al-Nasiri N, Stohs SJ. Endrin-induced histopathological changes and lipid peroxidation in livers and kidneys of rats, mice, guinea pigs and hamsters. *Toxicol Pathol*. 1991; 19:108–114. [PubMed: 1771364]
53. Bravo CF, Curtis LR, Myers MS, Meador JP, Johnson LL, Buzitis J, Collier TK, Morrow JD, Laetz Ca, Loge FJ, et al. Biomarker responses and disease susceptibility in juvenile rainbow trout *Oncorhynchus mykiss* fed a high molecular weight PAH mixture. *Environ Toxicol Chem*. 2011; 30:704–714. [PubMed: 21298713]
54. Rattner, Ba; Lazarus, RS.; Heinz, GH.; Karouna-Renier, NK.; Schultz, SL.; Hale, RC. Comparative embryotoxicity of a pentabrominated diphenyl ether mixture to common terns (*Sterna hirundo*) and American kestrels (*Falco sparverius*). *Chemosphere* [Internet]. 2013; 93:441–447. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0045653513007650>.
55. Kuang D, Zhang W, Deng Q, Zhang X, Huang K, Guan L, Hu D, Wu T, Guo H. Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. *Environ Sci Technol*. 2013; 47:7446–7456. [PubMed: 23745771]
56. Yi B, Kasai H, Lee HS, Kang Y, Park JY, Yang M. Inhibition by wheat sprout (*Triticum aestivum*) juice of bisphenol A-induced oxidative stress in young women. *Mutat Res - Genet Toxicol Environ Mutagen* [Internet]. 2011; 724:64–68. Available from: <http://dx.doi.org/10.1016/j.mrgentox.2011.06.007>.
57. Cho SH, Man HC, Oh SK, Lee WY, Bong CC. Metabolic significance of bisphenol A-induced oxidative stress in rat urine measured by liquid chromatography-mass spectrometry. *J Appl Toxicol*. 2009; 29:110–117. [PubMed: 18980270]

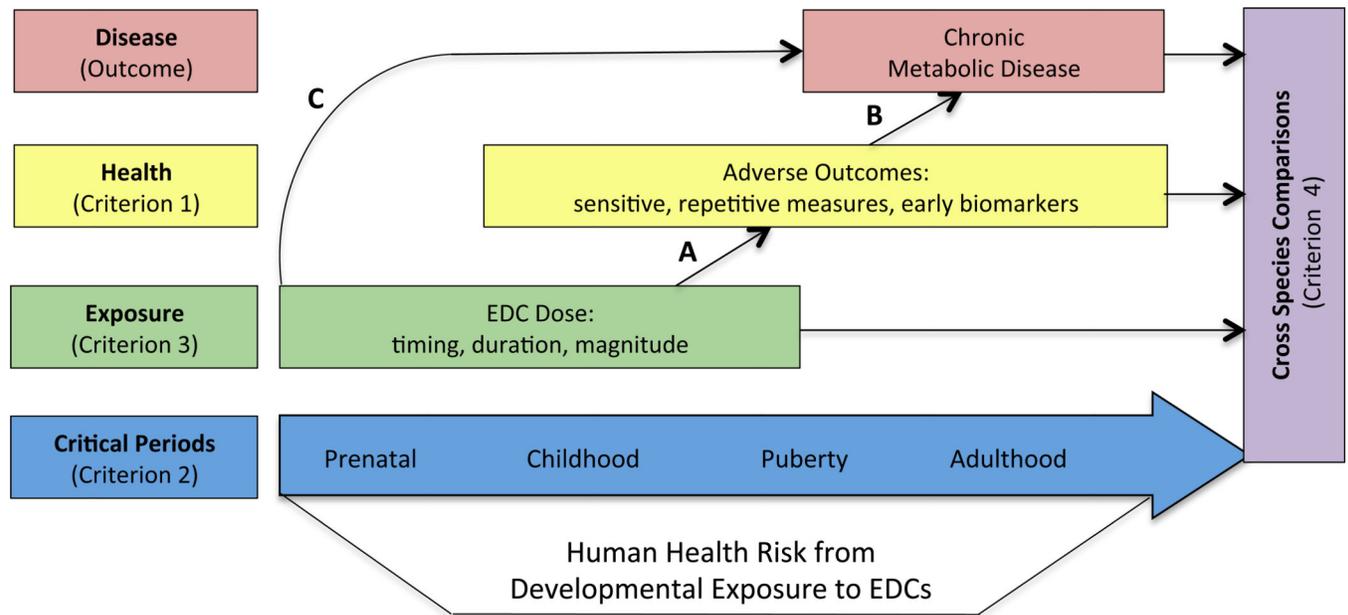


Figure 1. Criteria to Establish Human Risk from Developmental EDC Exposures

Cross species comparisons are necessary at each stage of experimental design from critical periods of exposure to dose to adverse outcome measurements to establish weight of evidence for translation to human health and determination of human risk of disease. Using animal models that have similar, measurable adverse health outcomes, such as sensitive measure of oxidative stress, in the pathway to chronic disease development are important for understanding disease development and progression. Mediation of oxidative stress is shown via arrows A and B. In order for oxidative stress to be a mediator, it must be significantly associated with both EDC dose and chronic disease outcome and must be in the pathway from exposure to disease.

Table 1

Sensitive Markers of Oxidative Stress and Their Use in Studies on Representative EDCs

Measure of Oxidative Stress	Target Biomolecule	Sensitive Analytical Methods*	Sample Matrix	EDCs Studied - Direct Exposure	EDCs Studied - Fetal Exposure
o,o-dityrosine (DiY), 3-chlorotyrosine (ClY), and 3-nitrotyrosine (NY)	Amino acids	LC-MS/MS; GC-MS/MS	Plasma, serum, urine, tissues	NS [^]	BPA ¹
F2-isoprostanates	Lipids	LC-MS; GC-MS	Plasma, urine	PCBs, ²⁸ OP pesticides, ²⁹ PAHs ⁵³	NS [^]
Free Fatty Acids (FFA)	Lipids	GC; GC-MS	Plasma, tissues	OP pesticides ³⁸	BPA, ¹ phthalates ³⁷
8-oxodG	DNA	HPLC-ECD	Urine, tissues	phthalates, ¹⁷ BPA, ⁵⁴ PAHs ⁵⁵	BPA ^{36,57}
8-oxoGuo	RNA	HPLC-ECD	Urine, tissues	NS [^]	NS [^]
Cellular redox potentials	Cell	HPLC	Plasma, serum, blood, tissue	NS [^]	NS [^]

* Only the most sensitive analytical methods reported

[^] Not yet studied in published literature

Representative EDCs considered for this table included bisphenol a (BPA), phthalates, polychlorinated biphenyls (PCBs), lead, organophosphate (OP) and organochlorine (OC) pesticides, organotins, flame retardants, and polycyclic aromatic hydrocarbons (PAHs)