CropLife International Position Paper

Endocrine Disruption: Regulatory Testing and Assessment of Crop Protection Products

Are Current Approaches Fit for Purpose?

November 2015

Executive Summary

Within the ongoing debate concerning the regulation of endocrine disruptors, increasingly questions are being raised regarding the current testing of chemicals and whether this is adequate for the assessment of potential endocrine-disrupting effects. This document describes the current testing approaches for crop protection products and outlines why the crop protection industry believes these are sufficiently robust for the evaluation of such effects. The document includes first a description of the tests typically employed in global data packages and then provides the crop protection industry's view on the criticisms most commonly made of these testing approaches.

Testing approaches: For human health, endocrine-mediated adverse effects are identified in the standard short term and longer term *in vivo* toxicological studies that are routinely performed to fulfil the current regulatory requirements for pesticide active substances. Endocrine-mediated toxicity may be detected in the repeated-dose, reproductive and developmental toxicity studies as well as carcinogenicity studies. However, supplementary and more focused mechanistic studies may be necessary to further investigate an endocrine mode of action.

For the environment, a range of ecotoxicological assays are performed across various animal species (mammals, birds, fish, aquatic and terrestrial invertebrates). Specific endocrine-related testing is typically triggered by an evaluation of the mechanistic data in the mammalian toxicology studies. While standard ecotoxicological studies often do not identify the mode of action, they capture adverse effects that may be endocrine-mediated.

Sensitive endocrine endpoints: While the existing internationally accepted test guidelines may not cover all potentially sensitive endocrine endpoints, this does not lead to an inability to detect an endocrine disruptor as suggested by some commentators. That's because endocrine disruptors produce a pleiotropic response with a wide range of diverse, adverse effects observed in one or more of the definitive, apical guideline studies. It is therefore highly unlikely that adverse effects resulting from endocrine disruption will not be detected in at least one or more of the mammalian toxicological and/or ecotoxicological studies mentioned above.

Sensitive windows of exposure and vulnerable populations: The current mandatory toxicological and ecotoxicological testing for crop protection products takes into account the potential for sensitive windows of exposure and vulnerable populations. This applies to the detection all adverse health effects, including those that may arise as a result of perturbation of the endocrine system. For human health, at least three studies are required, which are specifically designed to assess adverse effects that may occur as a result of exposure during sensitive time periods:[1] 1) the rodent two-generation reproduction toxicology study (Organisation for Economic Co-operation and Development/OECD Test No. 416) and 2) rodent and rabbit prenatal developmental toxicity studies (OECD Test No. 414). For environmental species, relevant ecotoxicological tests either address this directly by exposing all life stages (rat multi-generation reproduction studies, fish full lifecycle or invertebrate lifecycle studies) or operate by using known sensitive life stages that are predictive of effects on the whole lifecycle.

EATS and non-EATS modalities: Existing assays are currently focused on the estrogen, androgen, thyroid and steroidogenesis (EATS) modalities and it is recognized that

[1] Sensitive windows of exposure: exposures at conception and during fetal growth and development, pregnancy/*in utero*, development and growth throughout early life stages (neonatal and juvenile), and pubertal development and adolescence until early adulthood.

standardized mechanistic assays for non-EATS modalities[2] relevant to mammals, fish and other vertebrates are not yet available. However, most downstream effects of perturbation of the non-EATS pathways/modalities are detectable in existing standardized, apical definitive toxicity assays. Work is ongoing within the auspices of OECD[3] to develop methods covering the non-EATS modalities and the crop protection industry will continue to support these initiatives.

Human endocrine-related diseases and disorders: The animal models used in toxicological testing of crop protection products are capable of detecting adverse effects mediated by perturbation of all known mammalian endocrine modes of action as there are no endocrine modalities known to exist in humans that do not have relevant analogue(s) in animal models. These models are predictive of humans in that they can detect endocrine-mediated adverse effects that can be regulated appropriately. For example, mammary gland tumors and other hormonal cancers (e.g., prostate and testis cancer) are detectable in standard carcinogenicity studies (OECD Tests Nos. 451 and 453; United States Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention/USEPA OCSPP 870.4300).

There is still significant debate and research regarding the possible role of chemical exposures in the etiology of endocrine-related human diseases alongside other possible causative factors such as genetics, diet and lifestyle. A structured weight of evidence approach should be employed to weigh the existing scientific evidence on the potential role (if any) of chemical substances in these diseases, weighing both human epidemiological data and laboratory animal data. Such an approach would help assess the overall strength of the evidence and therefore, help inform on priorities for possible method development within the OECD test guideline program.

Environmental (wildlife) species: For the environment, it is acknowledged that assays for certain wildlife taxa (e.g., reptiles) and mode of action assays for others (e.g., invertebrates) are not yet available. However, adverse effects, regardless of the mode of toxicological action, are detected in the various longer term tests available for both vertebrates (mammals, fish and birds) and invertebrates. It is a well accepted principle of ecotoxicology to allow the extrapolation from a few standard representative test species to all species living in the environment (e.g., aquatic amphibians are covered by fish test species). It is not feasible, nor desirable due to ethical considerations regarding laboratory animal welfare, to test an exhaustive list of different environmental species.

Combined exposures to multiple substances (cocktail effects): The existing risk assessment and risk management approaches provide sufficient protection from combined exposures to low levels of crop protection products present in the environment or in food. Managing the risks from these substances individually will, in almost all cases, also ensure that combinations of substances do not present a concern for human health or the environment. This principle applies equally to all possible areas of concern, including potential endocrine-disrupting effects. The cumulative risk assessments undertaken by regulatory authorities in the European Union (EU) and United States have consistently confirmed that exposure to mixtures of pesticide residues present at low levels in food, are not of concern for human health.[4,5,6] Advanced methodologies for cumulative risk

[2] For example: Hypothalamus-pituitary-adrenocortical axis, peroxisome proliferator-activated receptor.
[3] Organisation for Economic Co-operation and Development. OECD work related to endocrine disrupters. http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm.
[4] European Food Safety Authority. March 2014. http://www.efsa.europa.eu/en/press/news/150312.htm.
[5] U.S. Department of Agriculture. 2013. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=stelprdc5098551.

assessment are under development and the crop protection industry will continue to provide expert input.

Low-dose effects and non-monotonic dose-responses **(*NMDRs):*** There is currently no consensus regarding the existence and/or relevance of low-dose effects and NMDRs) and these remain issues of considerable scientific debate. While NMDRs have been shown to exist, the current testing approaches do not fail to identify or establish appropriate No Observable Adverse Effect Levels (NOAELs) in the low-dose range of exposure. Studies claiming low-dose effects and/or NMDRs often suffer from methodological shortcomings, findings have not been reproduced consistently between different laboratories, and the toxicological significance of reported results is often questionable. Overall, the weight of currently available scientific evidence supports maintaining the current testing and risk assessment approaches and consequently, changes to test guidelines are not required in relation to NMDRs and low-dose effects.

Thresholds: There is nothing unique about endocrine disruption compared with other non-genotoxic forms of toxicity to justify adopting a default non-threshold approach for regulating potential endocrine-disrupting effects. Biological and mechanistic considerations confirm that thresholds of adversity exist and are the rule, not the exception, for all endpoints, including those arising from endocrine disruption.[7] The current toxicological and ecotoxicological test methods for crop protection products allow for the determination of thresholds of adversity and for the establishment of regulatory reference values for use in risk assessment and regulatory decision-making.

Conclusion

Overall, the crop protection industry believes that the scope and nature of the current testing approaches for crop protection products are scientifically robust and sufficient to:

- address adverse effects mediated through endocrine mode(s) of action
- characterize these adverse effects in terms of a dose response
- provide reference doses (safety levels) that can be used for human health and ecological risk assessment and regulatory decision-making.

These approaches are firmly founded on the extensive core and triggered data requirements that form global crop protection product registration packages. Ultimately, regulatory decisions should incorporate all scientific information in a weight of evidence approach and within a risk assessment framework considering both hazard and exposure.

Considerable strides have been made by the OECD and USEPA's Endocrine Disruptor Screening Program (EDSP) to develop standardized *in vitro* and *in vivo* mechanistic and *in vivo* apical assays for endocrine disruptors. The crop protection industry has significantly contributed to the development and validation of these assays – many of which form an integral part of the OECD Conceptual Framework. The industry will continue to provide expertise in developing new assays or modifying existing tests that may arise as a result of the continuing scientific debate around endocrine disruption.

Scientific understanding and technological progress will continue to advance across all areas of chemical regulation. Toxicological and ecotoxicological testing approaches will continue to evolve to reflect these developments and to address regulatory demands.

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[6] United States Environmental Protection Agency. 2001. Cumulative risk assessment: Developing the methods available, papers and where they may be located. June 21, 2001. http://www.epa.gov/pesticides/cumulative/Cum_Risk_AssessmentDTM.htm.

[7] Bogert CJ, Baker SP, Matthews JC. 2013. Potency matters: thresholds govern endocrine activity. *Regul Toxicol Pharmacol.* 67(1): 83-88.

Endocrine Disruption: Regulatory Testing and Assessment of crop Protection Products – Are the Current Approaches Adequate?

1. Background

Significant debate continues to take place principally within Europe and the United States in relation to the regulation of endocrine disruptors. Increasingly, questions are being raised regarding the adequacy of the current testing methods for regulated chemicals such as crop protection products, biocides and general chemicals. Some commentators suggest that the existing approaches may be ineffective at "detecting" endocrine-disrupting effects.

For example, the World Health Organization's United Nations Environment Progamme (WHO/UNEP) State of the Science Report on Endocrine Disrupting Chemicals - 2012[8] states that: *"There are currently many gaps in the available chemical test methods for screening chemicals for endocrine disrupting effects. Regulatory tests for many wildlife taxa are currently not developed and of the mammalian assays available, most do not cover endocrine endpoints adequately enough to detect the effects of endocrine disrupting chemicals … Perhaps most importantly, the exposure periods do not cover critical developmental windows of increased susceptibility now known to exist."*

The purpose of this document is to describe the current regulatory testing approaches for pesticide active substances and to outline why the crop protection industry believes these are scientifically robust for the evaluation of endocrine-disrupting effects. The document provides a description of the extensive battery of tests employed in global pesticide data packages and then provides the crop protection industry's view on the key criticisms most commonly made of these testing approaches.

2. Current Pesticide Testing Requirements and Approaches Related to Endocrine Disruption

The toxicological and ecotoxicological datasets developed for pesticide active substances are typically prepared to support the global registration and marketing of products. Core data packages therefore tend to be harmonized globally and may exceed the regulatory requirements of any one given region (e.g., the EU or United States).

Each active substance is tested in a battery of acute, sub-chronic and chronic assays according to internationally accepted regulatory test guidelines. Many of the tests are represented in the OECD's Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012).[9] This framework represents a toolbox of assays and information that can be used to evaluate chemicals for potential endocrine disruption as agreed by all OECD member countries at the international level. The framework includes five levels, from Level 1 (existing data and non-test information, e.g., on physical and chemical properties) up to Level 5 (*in vivo* studies providing comprehensive data on adverse effects on endocrine-relevant endpoints). The *in vitro* and *in vivo* screening assays that provide data on the ability of a substance to interact with selected endocrine endpoints (i.e., mechanistic or mode of action information) are contained in Levels 2 and 3.

[8] United Nations Environment Programme and World Health Organization. 2013. State of the Science of Endocrine Disrupting Chemicals.
[9] Organisation for Economic Co-operation and Development. Conceptual framework for testing and assessment of endocrine disrupters. http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm#CONCEPTUAL.

OECD Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption[10] provides support to practitioners and regulatory authorities using or evaluating tests in the Conceptual Framework.

Specific studies may also be generated under the USEPA's EDSP.[11] This is a two-tiered program initiated for the purposes of screening selected chemicals for their potential to interact with the endocrine system. The Tier 1 screening battery includes five *in vitro* and six short-term *in vivo* assays that test multiple species and life stages, capturing the EATS pathways. Based on a review of Tier 1 data and other submitted pesticide toxicity data (e.g., sub-chronic, chronic, developmental and reproduction studies) in a weight of evidence analysis, the Tier 2 assays may be required. Tier 2 testing assays include rat, fish and bird definitive, multi-generation reproduction studies and a growth and development assay in the frog. Further information regarding the EDSP is described in Attachment 2.

For human health, the current toxicology test methods for detecting endocrine-mediated adverse effects in mammals include the following OECD and USEPA OCSPP test guidelines:

- rodent and non-rodent repeat-dose toxicity studies (OECD Test Nos. 408, 409; OCSPP 870.3100 and 870.3150)
- rodent two-generation reproduction study (OECD Test No. 416; OCSPP 870.3800)
- rodent and rabbit developmental toxicity studies (OECD Test No. 414; OCSPP 870.3700)
- rodent (two species) chronic toxicity and carcinogenicity studies (OECD Test Nos. 451, 452, 453; OCSPP 870.4100, OCSPP 870.4200 and OCSPP 870.4300)
- rodent developmental neurotoxicity study (OECD Test No. 426 and OCSPP 870.6300).

These studies are able to identify adverse effects on the form and function of the test organism resulting from multiple biological processes, including a wide spectrum of sensitive endpoints that are vulnerable to endocrine perturbation. These tests represent the highest level of toxicological evaluation available and are included in Levels 4 and 5 of the OECD Conceptual Framework.

For the environment, a range of ecotoxicological assays are performed across various animal species (mammals, birds, fish, aquatic and terrestrial invertebrates). Specific endocrine testing in wildlife is typically triggered by an evaluation of the mechanistic data for vertebrates from the mammalian toxicology studies or by the pesticide mode of action. Indications from this evaluation and evidence from adverse apical effects in Level 4 tests from the Conceptual Framework (and Level 5 if available) form the basis for additional, targeted testing required to address the potential endocrine mechanism in ecotoxicological test species. For invertebrates, the fact that lifecycle tests are typically performed addresses adverse effects even if the specific biological mechanism is not clearly understood or necessarily known.

Attachment 1 describes the EU and U.S. data requirements relevant to the assessment of potential endocrine-disrupting effects, which are also represented in the OECD

[10] Organisation for Economic Co-operation and Development. Guidance document on standardized test guidelines for evaluating chemicals for endocrine disruption. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2012)22&doclanguage=en.
[11] United States Environmental Protection Agency Endocrine Disruptor Screening Program. http://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview.

Conceptual Framework. For the different levels of the framework, the following points should be highlighted:

- ***Level 1*** – A full and robust evaluation of the information listed in Level 1 will be available for all active substances.
- ***Level 2*** – The *in vitro* mechanistic assays listed in Level 2 are not routinely conducted in either the EU or United States. However, through the USEPA's EDSP, some Level 2 assays have been required for the pesticide active substances selected for Tier 1 screening based on multiple routes of exposure and the USEPA can request Tier 1 assays as part of the U.S. registration review process. EU regulatory authorities could request these data at first registration or re-registration if concerns for potential endocrine-disrupting effects are triggered by findings in core guideline (toxicology and ecotoxicology) studies (Levels 4 to 5). For active substances already on the market, much analogous data is available from the USEPA's ToxCast program[12,13,14,15] or from other screening exercises. While these *in vitro,* high-throughput screening assays have not yet undergone validation, they can still provide key supporting evidence, or lack thereof, as observed in Level 3, 4 and 5 studies.
- ***Level 3*** – These *in vivo* mechanistic assays are not routinely conducted in either the EU or United States. However, through the USEPA's EDSP, some Level 3 assays have been required for the crop protection products selected for Tier 1 screening based on multiple routes of exposure, and the USEPA can require Tier 1 assays for all products as part of the U.S. registration review process. EU regulatory authorities can and do request these data at first registration or re-registration if concerns for potential endocrine-disrupting effects are triggered by findings in core guideline (toxicology and ecotoxicology) studies.
- ***Level 4*** – Many of the tests at this level are core or conditional data requirements capturing adverse effects that *could* be from an endocrine mode of action. At least for vertebrates, these studies – in combination with mechanistic data (endocrine gland weights and histopathology, and hormonal measurements) from the toxicology package – would give an indication of likely endocrine activity in wildlife vertebrates.
- ***Level 5*** – For toxicology, the two-generation reproduction test (OECD Test No. 416, OCSPP 870.3800) or the alternative, extended one-generation reproduction test (OECD Test No. 443) is a mandatory requirement in the EU and United States. In contrast, for ecotoxicology, none of the tests at this level are core data requirements. Some will be performed if triggered by the properties of the active substance (e.g., toxicity, persistence and bioaccumulation potential) or based on the known mode(s) of action (e.g., insecticides). However, these tests could be requested at first registration or re-registration if concern for potential endocrine-disrupting effects was indicated in Levels 2 to 4 (toxicology and ecotoxicology).

In summary, the toxicological and ecotoxicological datasets developed for pesticide active substances provide a robust core (standard) package for the evaluation of

[12] Juberg DR, Borghoff SJ, Becker RA, Casey W, Hartung T, Hosapple MP, Marty MS, Mihaich EM, Van Der Kraak G, Wade MG, Willett CE, Andersen ME, Borgert CJ, Coady KK, Dourson ML, Fowl JR, Gray LE, Lamb JC, Ortego LS, Schug TT, Toole CM, Zorrill LM, Kroner OL, Patterson J, Rinckel LA, Jones BR. 2014. T4 workshop report – lesson learned, challenges, and opportunities: the U.S. Endocrine Disruptor Screening Program. *ALTEX.* 31(1):63-78.
[13] Reif DM *et al*. 2010. Endocrine profiling and prioritization of environmental chemicals using ToxCast data. *Environ Health Perspec.* 118(12):1714-1720.
[14] Rotroff DM *et al*. 2013. Using *in vitro* high throughput screening assays to identify potential endocrine-disrupting chemicals. *Environ Health Perspec.* 121:7-14.
[15] Rotroff DM *et al*. 2014. Predictive endocrine testing in the 21st century using *in vitro* assays of estrogen receptor signaling responses. *Environ Sci Technol.* 48:8706-8716.

potential endocrine-disrupting effects.[16,17,18] Many of the tests are represented in the OECD's Conceptual Framework for the testing and assessment of endocrine disruptors and specific assays may also be conducted under the USEPA's EDSP. The testing approaches typically employed for crop protection products will combine whole organism regulatory tests providing apical information on adverse effects, with information on potential mode(s) of action (endocrine activity).

Considerable strides have been made by the OECD and USEPA's EDSP to develop standardized *in vitro* and *in vivo* mechanistic and *in vivo* apical assays for endocrine disruptors. The crop protection industry has contributed significantly to the development and validation of these assays – many of which form an integral part of the OECD Conceptual Framework. The industry will continue to provide expertise in developing new assays or modifying existing tests that may arise from continued scientific research on endocrine disruption.

Risk assessment and regulatory decision-making

In reaching regulatory decisions on the potential endocrine-disrupting effects of a substance, all available scientific information should be assessed in a holistic weight of evidence approach considering consistency and reproducibility of effects, biological plausibility and coherence.[19] From the tests described above, mode of action (biological activity) information from *in vitro* screens or *in vivo* studies (if available) should be combined with (apical) *in vivo* studies providing data on the adverse effects resulting from the endocrine interaction. It is important to note that no single assay will provide all the information needed to fully evaluate a substance in relation to possible endocrine-disrupting effects, which is the conclusion reached by a number of expert scientific committees and panels.[20,21,20] Additional consideration may also be given to reliable and reproducible information available from the scientific, peer-reviewed literature. All information should be evaluated within a **risk assessment** framework, which combines both the **hazard** data described above with predicted or measured (relevant) human and environmental **exposures**.

While regulatory authorities remain committed to advancing the state of regulatory science, it is important to highlight that risk assessments are not intended to be research or academic exercises to explore a comprehensive list of potential effects. The objective of any regulatory testing strategy should be to address potential risks by identifying the most sensitive endpoints relevant to human health and the environment, not to create a hazard-based list of chemicals with all possible adverse effects. The fundamental purpose of any risk assessment is to inform risk management options and to support regulatory decision-making.

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[16] Bars R, Broeckaert F, Fegert I, Gross M, Hallmark N, Kedwards T, Lewis D, O'Hagan S, Panter GH, Weltje L, Weyers A, Wheeler JR, Galay Burgos M. 2011. Science based guidance for the assessment of endocrine disrupting properties of chemicals. *Regul Toxicol Pharm.* 59(1):37-46.

[17] Bars R, Broeckaert F, Fegert I, Gross M, Hallmark N, Kedwards T, Lewis D, O'Hagan S, Panter GH, Weltje L, Weyers A, Wheeler JR, Galay Burgos M. 2011. Corrigendum to "science based guidance for the assessment of endocrine disrupting properties of chemicals." *Regul Toxicol Pharm.* 59:37-46.

[18] Bars R, Fegert I, Gross M, Lewis D, Weltje L, Weyers A, Wheeler JR, Galay-Burgos M. 2012. Risk assessment of endocrine active chemicals: Identifying chemicals of regulatory concern. *Regul Toxicol Pharm. 64(1):143-154.*

[19] United States Environmental Protection Agency. FIFRA Scientific Advisory Panel. Review of EDSP weight of evidence. July 2013. http://www.epa.gov/scipoly/sap.

[20] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal* 11(3):3132.

[21] United States Environmental Protection Agency. July 1999. Review of the Endocrine Disruptor Screening Program by a Joint Subcommittee of the Science Advisory Board and Scientific Advisory Panel. EPA-SAB-EC-99-013. http://www.epa.gov/scipoly/sap.

Scientific understanding and technological progress will continue to advance across all areas of chemical regulation. Toxicological and ecotoxicological testing approaches will continue to evolve to reflect these developments and to address regulatory demands.

3. Major Criticisms of Current Testing Requirements and the View of the Crop Protection Industry

3.1. Is current pesticide testing adequate to detect endocrine disruptors?

Criticisms of the overall adequacy of current testing approaches are usually made broadly in relation to all regulated chemicals, including general chemicals, biocides as well as crop protection products. The basis for such statements is often the perceived lack of consideration of sensitive endocrine endpoints, failure to consider sensitive windows of exposure and vulnerable groups and/or failure to account for NMDRs and low-dose effects. These specific issues are discussed separately in sections 3.2 to 3.8 below. The crop protection industry's view on the overall adequacy of existing testing approaches is described below.

Crop protection industry view

The rigorous testing approaches typically used to develop global toxicological and ecotoxicological datasets for pesticide active substances (and subsequent USEPA EDSP screening and testing) are described in section 2 above. As mentioned, these include an extensive battery of assays conducted according to internationally accepted test guidelines and many are represented in the OECD's Conceptual Framework for Testing and Assessment of Endocrine Disrupters.

The crop protection industry believes that the scope and nature of the current testing is sufficient to detect adverse effects resulting from endocrine activity, characterize these adverse effects in terms of a dose response and provide reference doses that can be used in a risk assessment.

The validity and fitness for purpose of the current testing approach has recently been reviewed by the European Food Safety Authority (EFSA) Scientific Committee. It states in its Scientific Opinion published in March 2013: *"As for any other (eco)toxicological hazard, endocrine-mediated adverse effects may be identified in standard toxicological tests that are routinely performed to fulfil the requirements of various regulatory programmes. In particular, endocrine-mediated toxicity may be detected in repeated-dose, reproductive and developmental toxicity, and carcinogenicity studies."*[22]

The EFSA Scientific Committee also provided the following further important clarification: *"Despite the fact that the existing internationally standardised assays might miss some endocrine-sensitive endpoints, this should not necessarily lead to the non-identification of EDs. Given the complexity of the endocrine system with its multiple signalling pathways and cross-talks, an ED is expected to produce a pleiotropic response with a range of effects, some of which are likely to be observed in an appropriate guideline study."*[23]

[22] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 17).
[23] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 30).

For human health, the only circumstance in which additional testing may be required over and above the existing, extensive evaluation is to understand the potential endocrine mechanism for the adverse effect already identified in standard studies. Again, the EFSA Scientific Committee has provided a view on this point: "… *supplementary and more focussed studies, such as mechanistic studies investigating the potential for endocrine activity, may be necessary to decide whether a causal relationship between the observed adverse effects and an endocrine activity is biologically plausible."*[25]

These supplementary tests are fully described in the OECD Conceptual Framework and in the USEPA's EDSP Tier 1 screening battery. However, these should *not* be viewed as hazard identification tests but investigations conducted with the purpose of providing additional information on the potential mode of action.

For the ecotoxicological evaluation, a range of assays are performed across different animal species (mammals, birds, fish, aquatic and terrestrial invertebrates). Specific higher tier assays (e.g., full fish lifecycle toxicity test) may be triggered by toxicity or specific mode of action indications from the mammalian toxicology studies or by the pesticide mode of action. It is possible that specific information on the mode of action is missing in aspects of the ecotoxicological evaluation. For invertebrates, there are no widely accepted mechanistic endpoints. Our understanding of invertebrate endocrinology is limited[24] and therefore, there is a reliance on lifecycle studies. These measure apical endpoints that are population relevant (e.g., growth and reproduction), and any endocrine-specific toxicity should be adequately accounted for in the full life-cycle response (i.e., a risk assessment based on these data would be protective of any adverse effect that may result from an endocrine mode of action).

It is a common misconception that in order to fully protect human health and the environment, all hazards that may be potentially produced by a chemical need to be identified and described in experimental animal studies under all possible conditions of potential exposure. Paradoxically, this is particularly the case with endocrine disruption where the unique concern is driven mainly by the mode of action. Chemical regulation is, and should continue to be, founded on a safety-based approach that determines what should be known about a chemical to be reasonably certain it will not cause adverse health problems, rather than a hazard only approach which attempts to identify every possible problem that a chemical could cause[25] (i.e., without any exposure context or of other apical endpoints that may be equally protective of endocrine-related effects).

This conclusion was reached by the USEPA's 2013 state of the science evaluation on NMDRs,[26] which states: *"Chemicals that operate through endocrine modes of action (MOA) have multiple targets across organs, tissues, and cellular systems in various species, and across all life stages … No testing strategy is able to assess all potential adverse effects, for all biological systems, in all tissues, for all species, in all developmental time points … The goal of chemical testing is to identify the potential for hazard after exposure to the xenobiotic of concern, not to identify and describe 100% of all the possible biological effects."* Most contemporary thinking on the future of human

[24] DeFur PL, Crane M, Ingersoll C, Tattersfield L. 1999. Endocrine disruption in invertebrates: endocrinology, testing, and assessment. Pensacola, FL: SETAC Press. https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.books/abstract/175/displayColumns/1.
[25] National Academies Standing Committee on Use of Emerging Science for Environmental Health Decisions. 2011.
[26] United States Environmental Protection Agency. June 2013. State of the science evaluation: non-monotonic dose responses as they apply to estrogen, androgen, and thyroid pathways and EPA testing and assessment procedures. Draft. https://www.epa.gov/sites/production/files/2016-01/documents/nmdr.pdf.

health risk assessment actually recommends that the need to identify hazards should be driven by knowledge of exposure patterns and the extent of human exposure (e.g., RISK 21 project).

In summary, the crop protection industry believes that effects on the normal functioning of the endocrine system which are a concern for human health and the environment (i.e., those that produce adverse effects) will be identified in the extensive, standard suite of tests described above. The required studies have been repeatedly demonstrated to be comprehensive and scientifically robust. They have and will continue to address different durations and routes of exposure, sensitive life stages and critical (sensitive) windows of exposure, and differences across multiple species. The selection of the NOAEL for the most sensitive endpoint from the most sensitive test animal ensures that the risk assessment will be protective of apical endpoints from multiple mode(s) of action, including perturbation of the endocrine system.

3.2. Do current tests only cover the EATS modalities[27] and not the non-EATS modalities?

"Internationally agreed and validated test methods (OECD) for the identification of endocrine disrupters capture only a segment of the known range of endocrine disrupting effects, mainly focused on estrogenicity, (anti)androgenicity and thyroid disruption ('EAT'). Other aspects of the endocrine system(s) are not considered, although it is clear that the complexity of endocrine systems cannot be reduced to EAT. This introduces considerable uncertainties, and the likelihood of overlooking harmful effects in humans and wildlife is high." – The 2013 Berlaymont Declaration on Endocrine Disrupters[28]

Crop protection industry view

Within the OECD Conceptual Framework, a series of *in vitro* and *in vivo* screens have been validated internationally and are in use (e.g., USEPA's EDSP) providing mechanistic information for substances which may interact with the so-called "EATS modalities." It is recognized however, that in the case of the non-EATS modalities,[29] such assays do not yet exist.

In its Scientific Opinion, the EFSA Scientific Committee states that: *"A reasonably complete suite of standardised assays (for endocrine activity and for endocrine hazard identification and/or characterisation) is only available (or will soon be available) for the EATS modalities relevant for mammals and fish, with fewer tests available for birds and amphibians."*[30]

The EFSA Scientific Opinion also describes the possible extent of the not-EATS modalities: *"… it is possible that a range of additional endocrine modalities in vertebrates (including Hypothalamus-pituitary-adrenocortical axis (HPA) axis; somatotropic axis; retinoid pathway; vitamin D pathway; Peroxisome Proliferator-*

[27] A modality is an axis, pathway, signalling process or hormonal mechanism within the endocrine system.
[28] European Commission. The 2013 Berlaymont Declaration on Endocrine Disrupters. http://www.brunel.ac.uk/__data/assets/pdf_file/0005/300200/The_Berlaymont_Declaration_on_Endocrine_Disrupters.pdf.
[29] For example: Hypothalamus-pituitary-adrenocortical (HPA) axis; Peroxisome Proliferator-Activated Receptor (PPAR)
[30] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 36).

Activated Receptor (PPAR) signalling pathway; pancreatic signalling; renal signalling) may be susceptible to endocrine disruption …"[31]

While it is acknowledged that standardized assays are currently not available for the non-EATS modalities, the crop protection industry believes that most adverse effects which may occur as a result of disruption to the non-EATS pathways will be detected in the chronic apical assays for both vertebrates (mammals, fish and birds) and invertebrates. As recognized in 1998 by the Endocrine Disruptor Screening and Testing Advisory Committee, *"the lack of specificity of in vivo assays is a limitation if the goal is to only identify ER, AR and TR [estrogen, androgen and thyroid receptor] alterations. In contrast, the lack of specificity could be considered an advantage if a broader, more apical screening strategy is desired … results of even the most specific in vivo assays can be affected by endocrine mechanisms other than those directly related to ER, AR and TR action*."[32]

In 2012, the OECD published a detailed review paper (DRP) on *"novel in vitro and in vivo screening and testing methods and endpoints for evaluating endocrine disruptors"* (OECD DRP 178).[33] The paper provides a detailed overview of the endocrine pathways considered to be susceptible to endocrine disruption. It also describes the assays and endpoints that could be added to existing *in vivo* vertebrate apical assays and the new *in vitro* screens that could be developed and/or standardized. Work is ongoing under the auspices of OECD's Endocrine Disruptor Testing and Assessment advisory group to consider these developments to cover the non-EATS modalities. The crop protection industry will continue to provide expert input into these initiatives.

3.3. Does current testing for crop protection products adequately address sensitive windows of exposure and vulnerable groups?

"Early life exposures, i.e., during critical windows of foetal development, are not generally included in the tests required for regulatory purposes." – Watts/Pesticide Action Network Asia & the Pacific, 2013[34]

"There are currently many gaps in the available chemical test methods for screening chemicals for endocrine disrupting effects. Regulatory tests for many wildlife taxa are currently not developed and of the mammalian assays available, most do not cover endocrine endpoints adequately enough to detect the effects of endocrine disrupting chemicals … Perhaps most importantly, the exposure periods do not cover critical developmental windows of increased susceptibility now known to exist." – WHO-UNEP State of the Science Report on Endocrine Disrupting Chemicals - 2012[35]

Crop protection industry view

Sensitive windows of exposure

[31] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 29).
[32] Endocrine Disruptor Screening and Testing Advisory Committee. August 1998. Final report: volume I.
[33] Organisation for Economic Co-operation and Development. Series on testing and assessment: No 178: detailed review paper on the state of the science on novel *in vitro* and *in vivo* screening and testing methods and endpoints for evaluating endocrine disruptors. ENV/JM/MONO(2012)23.
[34] Watts M. Pesticide Action Network Asia & the Pacific. 2013. Poisoning our future: children and pesticides. http://www.panap.net/sites/default/files/Poisoning-Our-Future-Children-and-Pesticides.pdf.
[35] United Nations Environment Programme and World Health Organization. 2013. State of the science of endocrine disrupting chemicals - 2012. http://unep.org/pdf/9789241505031_eng.pdf.

The term "sensitive windows of exposure" is typically used to describe exposures at conception, during pregnancy, *in utero,* throughout early life stages (neonatal and juvenile) and during pubertal development and adolescence. The issue could be applied to all potential areas of concern for human health but is often mentioned in relation to endocrine disruptors.

The current regulatory testing approaches for pesticide active substances (generally performed in accordance with USEPA/OCSPP and OECD test guidelines) recognizes the potential for sensitive windows of exposure, hence the requirement for at least two studies specifically designed to assess any adverse effects that may occur as a result of exposure during these sensitive periods. These studies are as follows:

1. ***Two-generation reproduction toxicology study (OECD Test No. 416 and OCSPP 870.3800[36])***
   In this multigenerational study, young, sexually mature animals (usually rats) are exposed for 10 weeks and mated. Once offspring are born, their development is monitored until they are sexually mature. The cycle is repeated with offspring exposed and mated and so on to produce another generation. Exposure is continuous throughout and as a result, covers the following biological processes/life stages – all of which may be considered as sensitive windows of exposure:
   - Sexual development and release of gametes (sperm and eggs)
   - Mating and fertilization
   - Implantation of the conceptus (primitive embryo) in the uterus
   - Fetal growth and development (gestation/pregnancy)
   - Parturition (birth)
   - Lactation
   - Neonatal and juvenile development (pre- and post-weaning)
   - Growth and development to adulthood (including puberty)

   Exposure includes high-dose levels – many orders of magnitude greater than actual human exposure levels. An additional feature of the study design is that the very young offspring receive exposures several fold greater than the parents, so it can be argued that particular emphasis and attention is paid to what many would consider one of the most sensitive windows of exposure and vulnerable life stages. Extensive assessments are made in these studies of the normal behavior, sexual performance, morphology and development of several generations of animals.

2. ***Prenatal developmental toxicity studies (OECD Test No. 414 and OCSPP 870.3700[37])***
   In these studies, pregnant animals are exposed at high doses throughout gestation (pregnancy), after which exhaustive evaluations of *in utero* fetal development are conducted. These studies are conducted in two separate species, typically the rat and rabbit.

   When considered collectively, the two-generation reproduction and prenatal developmental toxicity studies can be considered a comprehensive evaluation for any potential effects of exposure during sensitive windows of exposure. Furthermore, the endpoints and biological processes (e.g., mating, implantation,

[36] United States Environmental Protection Agency.1996. OPPTS 870.3800 Reproduction and fertility effects [EPA 712–C–98–208]. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0018.
[37] United States Environmental Protection Agency. 1998. OCSPPS 870.3700 Prenatal Developmental Toxicity Study [EPA 712–C–98–207]. http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/epa/epa_870_3700.pdf.

pregnancy and neonatal development) evaluated in these studies are under extensive hormonal regulation, meaning that the studies are well suited to detect adverse effects resulting from perturbation of the endocrine system, i.e., endocrine disruption.

Despite this, it has been claimed that the current testing paradigm does not adequately predict adverse health outcomes that may manifest later in life as a result of exposures during these early, sensitive windows of exposure.[38] An often cited example is diethylstilbestrol (DES), a synthetic estrogen administered to pregnant women in the 1940s-70s to prevent pregnancy complications. It was later demonstrated that there was an increased risk of vaginal clear cell carcinoma in adult females that had been exposed to DES *in utero*. As the current regulatory testing approaches would not detect this type of adverse effect (carcinogenicity in an adult animal after *in utero* exposure), some believe they are insufficient for the protection of human health. This has led to a call for the introduction of a test guideline in which animals are exposed throughout sensitive windows of exposure (including *in utero*) and then either exposure continues to old age or exposure ceases and the animals continue to be monitored into old age.

For example, the EFSA Scientific Committee mentions that: *"In relation to mammals, a limitation of the current suite of test methods available for the identification of EDs (and therefore an area for further developing it) is the lack of a single study involving exposure through the complete life cycle of a mammal, from conception to old age or a single study involving developmental exposure with follow-up into old age."*[39]

It is acknowledged that this specific type of study does not currently exist. However, as discussed above, if the overall aim of regulatory testing is to ensure safety, then every potential hazard need not be identified for every possible exposure scenario and prescribed in animal studies.

If DES were tested under the current regulatory testing paradigm for crop protection products (without *a priori* knowledge of its mode of action or biological activity), then the overall conclusion regarding the hazard profile would be the same (potential carcinogenic and reproductive hazards) when integrating all available data from developmental, reproductive, and longer term, chronic cancer bioassays as if studies were also performed in animals exposed *in utero* and through to old age (see Attachment 3 for further details). That is, the current regime detects both the reproductive and carcinogenic effects of DES and so all relevant adverse effect "types" are addressed, including those that may be endocrine-mediated. Furthermore, based on the available data, there is no evidence that the inclusion of *in utero* to old age studies would identify lower, more health protective, NOAELs for use in regulatory risk assessment (i.e., there is no evidence that the transgenerational effect of DES occurs at lower doses than the other hazards). For example, based on the data from Gibson *et al.* (1967),[40] in which chronic toxicity and carcinogenicity in the rat were assessed following post-natal exposure to DES, the Lowest Adverse Effect Level (LOAEL) would be 0.02 mg/kg/day (the lowest dose tested), which is lower than the LOAEL of 0.1 mg/kg/day (the lowest dose tested) identified in the

[38] National Academies Standing Committee on Use of Emerging Science for Environmental Health Decisions. 2011.
[39] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 30).
[40] Gibson JP *et al.* 1967. Comparative chronic toxicity of three oral estrogens in rats. *Toxicol Appl Pharmacol*. 11:489-510.

study of Baggs *et al*. (1991),[41] in which carcinogenicity in the rat was assessed following pre-natal exposure. Therefore, from both the hazard classification and risk assessment perspectives, the current pesticide testing paradigm is fit for purpose.

Vulnerable groups

"Vulnerable groups" is a term often used to describe pregnant or nursing women, the very young or the very old. As described above, concerns over early life stage sensitivity are well covered by the current regulatory testing paradigm. Effects in aging/old animals are also comprehensively assessed in chronic toxicity and carcinogenicity studies in rats and mice (OECD Test Nos. 451, 452, 453 and OCSPP 870.4100, 870.4200 and 870.4300).

The U.S. Agency for Toxic Substances and Disease Registry of the U.S. Department of Health and Human Services has listed some general points on why certain groups may be considered more sensitive (i.e., vulnerable) to chemical exposures.[42] These points and the crop protection industry's response to each are summarized as follows:

- **Pregnant and nursing women**
  - *Exposures may affect the developing fetus:* As described above, the potential for fetal effects is comprehensively covered by the current testing approach.
  - *Potential for lactational transfer:* This is assessed in the two-generation reproduction toxicity study (OECD Test No. 416 and OCSPP 870.3800).

- **Children**
  - *Potential for higher exposure (e.g., by eating dirt, playing on the floor):* Toxicology studies are conducted at a series of dose levels, all considerably in excess of predicted human exposures; even when there is a potential for specific increased exposure, this is accounted for.
  - *Children have a more limited diet, so they may have more exposure to chemicals that are only in certain foods*: In dietary toxicology studies, animals are administered the test item at high doses in the same diet every day.
  - *Potential for developmental effects (e.g., during puberty)*: Effects are assessed in the two-generation reproduction toxicity study (OECD Test No. 416 and OCSPP 870.3800).

- **Older adults**
  - *Potential for higher exposures (reduced potential for avoidance):* Toxicology studies are conducted across a series of dose levels, all considerably in excess of predicted human exposures; even when there is a potential for specific increased exposure, this is accounted for.
  - *Increased sensitivity to adverse effects owing to reduced potential to respond to exposure:* The potential for increased sensitivity of geriatric animals is assessed in the chronic toxicity/carcinogenicity studies in rats and mice (OECD Test Nos. 451, 452, 453 and OCSPP 870.4100, 870.4200 and 870.4300).

An extra level of protection is also provided in the way in which risk assessments are conducted. An assumption is made that populations will contain vulnerable, sensitive subpopulations and an additional 10x (intra-species variability) safety factor is

[41] Baggs RB *et al.* 1991. Carcinogenicity of diethylstilbestrol in the Wistar rat: Effect of postnatal oral contraceptive steroids. *Cancer Res*. 51: 3311-3315.
[42] Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services. http://www.atsdr.cdc.gov/emes/public/docs/Sensitive%20Populations%20FS.pdfA.

applied to ensure these groups are adequately protected. This is in addition to the 10x (inter-species variability) safety factor applied when extrapolating results from test animal toxicity studies to humans. Therefore, in total, a 100x (100-fold) safety factor is applied in the risk assessment.

Note: In the U.S., the Food Quality Protection Act (FQPA), sets a more stringent safety standard for most crop protection products and offers particular protection to take into account the susceptibility of children. Under the FQPA, all pesticide tolerance decisions utilize an additional 10x (ten-fold) safety factor, as appropriate, in setting and reassessing tolerances. This is in addition to the 10x ***intra****-species and 10x* ***inter****-species safety factors described above. Together, a composite uncertainty factor would typically range between 100-1000x, with reference values being much lower than the experimental NOAEL/LOAEL of the most sensitive endpoint from the most sensitive species.*

While the term "vulnerable groups" is almost always applied to human health, it is worth mentioning how sensitive life stages are addressed in ecotoxicological testing. Firstly, it is important to highlight the differences between protection goals for human health and environmental risk assessments. For the environment (wildlife species), the protection goal is at the population level, not the individual level. Therefore, adverse effects, be they endocrine-mediated or not, are considered integrative of population-relevant responses, namely growth, development and reproduction. All relevant ecotoxicological tests either address this directly by exposing all life stages (rat multi-generation, fish full lifecycle or invertebrate lifecycle studies) or operate by using known sensitive life stages that are predictive of effects on the whole lifecycle. Importantly, the highest tier ecotoxicological tests (Level 5 in the OECD Conceptual Framework) are all single or multi-generational in design so consider all life stages *de facto*.

The EFSA Scientific Committee concluded that: *"In the OECD CF* [Conceptual Framework] *for testing and assessment of endocrine disrupting substances, some Level 4 and 5 tests do cover critical periods of development in utero and in later life stages. On the other hand, fish lifecycle and multi-generation tests cover all relevant windows of exposure and can be expected to reveal the longer-term effects of even short-term exposures at all stages of the lifecycle."*

With regard to ecological risk assessments, the fish early life-stage test guideline (OECD Test No. 210 or OCSPP 850.1400) is the most frequently used assay for predicting longer term, chronic fish toxicity and fish full lifecycle toxicity. The fish early life-stage test is also commonly used to support aquatic ecological risk assessment globally.[43] In addition, the USEPA's EDSP has developed additional Tier 2, longer term ecological toxicity tests: Japanese quail (bird), medaka (fish) and *Xenopus laevis* (frog) reproduction, growth and development studies. These studies can be used to augment the data from currently required studies, if triggered by the USEPA's Tier 1 screening weight of evidence determinations.

Summary: sensitive windows of exposure and vulnerable groups

In summary, the current regulatory testing and safety assessment of pesticide active substances addresses the potential for adverse effects from exposures during sensitive windows of exposure and to vulnerable groups. This applies to the detection all adverse

[43] Volz DC, Belanger S, Embry M, Padilla S, Sanderson H, Schirmer K, Schloz S, Villeneuve D. 2011. Adverse outcome pathways during early fish development: A conceptual framework for identification of chemical screening and prioritization strategies. *Toxicol Sci.* 123(2):349-358.

health effects, including those that may arise as a result of perturbation of the endocrine system.

3.4. Does current testing for crop protection products adequately assess human endocrine disorders associated with exposure to endocrine disruptors?

"It has also been suggested that a relevant weakness of current test methods is the limitation of some animal models in relation to certain human endocrine disorders in which EDs have been suggested to play a role, such as some mammary gland tumours and other hormonal cancers, endometriosis, metabolic syndrome and reproductive senescence …" – EFSA Scientific Committee[44]

"… several recent review reports concluded that current mammalian tests do not cover certain endpoints that might be induced by exposure during foetal or pubertal development but emerge later in life like certain cancers (breast, prostate, testis, ovarian and endometrial) and effects on reproductive senescence …" – EFSA Scientific Committee[45]

Crop protection industry view

The animal models used in the routine toxicological testing of pesticide active substances are capable of detecting adverse effects mediated by perturbation of all mammalian endocrine modes of action (EATS and beyond) as there are no endocrine modalities that exist in humans that do not exist in the animal models. Therefore, even if an observed, apical adverse effect may be different in humans compared with the test animal (e.g., rat, mouse, rabbit or dog), the animal models are still predictive of humans in that they can detect endocrine-mediated adverse effects and can be regulated appropriately. In most cases, the animal models are capable of detecting analogous effects to those that may occur in humans; for example, mammary gland tumors and other hormonal cancers (e.g., prostate and testis cancer) are detectable in the standard carcinogenicity studies (OECD Test Nos. 451 and 453; OCSPP 870.4300).

As discussed in section 3.3 above, it is acknowledged that a test guideline covering the lifespan of *in utero* exposure until old age currently does not exist. However, as illustrated with the DES case example, the current testing paradigm for crop protection products will identify endocrine-mediated adverse effects and correctly characterise these substances (e.g., carcinogenic or reproductive hazard).

Nevertheless, initiatives are ongoing under the auspices of OECD for the possible development of new assays or modifications to existing assays for the detection of effects considered to be predictive of endocrine-related human diseases. The OECD projects on Adverse Outcome Pathways (AOPs) are of particular interest. Specifically, the work on the AOP on the non-EATS modality, Peroxisome Proliferator-Activated Receptor γ pathway, could be informative in the design of *in vitro* and *in vivo* screening assays for predicting metabolic disorders and obesity. Other AOPs on relevant signalling pathways, such as the retinoid pathway, pancreatic signalling and renal signalling (relevant to the non-EATS modalities discussed above), could also be considered for further development within the OECD.

[44] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 30).
[45] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 37).

However, the most critical aspect in the development of such *in vitro* and *in vivo* screening assays is the demonstration of the causal link between the data obtained in these screens and adverse effects manifested in repeated-dose *in vivo* toxicity studies, in particular, in reproduction toxicity studies and chronic/cancer studies (OECD Test Nos. 451, 452, 453 and 416 and the corresponding OCSPP 870.4100, 870.4200, 870.4300 and 870.3800). The crop protection industry will continue to provide expert input into OECD and other initiatives and will support the development of alternative approaches to assess this issue, such as the AOP projects discussed above.

It should also be highlighted that there is still significant debate and ongoing research regarding the possible role of environmental exposures to chemicals in the etiology of endocrine-related human diseases: hormone-related cancers (prostate and breast cancer), male reproductive disorders (hypospadias, cryptorchidism, testicular cancer), female reproductive disorders, neurodevelopment disorders (autism, attention deficit hyperactivity disorder), obesity and diabetes. For many of the health endpoints, the available epidemiological evidence is contradictory or inconclusive and studies frequently suffer from methodological shortcomings, particularly relating to how exposure has been retrospectively assessed.[46] Drawing conclusions on the extent to which chemical exposures may or may not influence the incidence of these diseases is further compounded by the fact that the exact mechanism(s) for initiation and progression are unknown.

Significant concerns have been raised regarding the methodology employed and the scientific basis for the recent reviews undertaken on this topic, which cite both toxicological and epidemiological evidence (e.g., Kortenkamp *et al.* 2012,[47] WHO-UNEP).[48] For example, Lamb *et al.* (2014)[49] conclude that the WHO/UNEP 2012 report *"does not provide a balanced perspective, nor does it accurately reflect the state of the science on endocrine disruption."*

Similarly, the UK Hazardous Substances Advisory Committee's expert opinion[50] on the State of the Art Assessment of Endocrine Disrupters report (Kortenkamp *et al.* 2011) concluded that: *"This report, which focuses on the possible hazards posed by chemical-induced endocrine disruption, does not adequately reflect the current state of the science in this important and rapidly evolving area. Specifically … the approach taken precludes production of a fully up-to-date review and the search strategy adopted fails to ensure comprehensive coverage of the literature."*

The crop protection industry is of the firm view that the weight of scientific evidence does not support many of the statements and conclusions of these recent reviews. For example, the 2012 WHO-UNEP report states that: *"For prostate cancer, sufficient evidence exists for an association with exposures to mixtures of pesticides in agriculture and in pesticide manufacturing."* However, in its expert review for EFSA on epidemiological studies and

[46] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 77).
[47] European Commission. State of the Art Assessment of Endocrine Disrupters. http://ec.europa.eu/environment/chemicals/endocrine/pdf/sota_edc_final_report.pdf.
[48] World Health Organization and United Nations Environment Programme. State of the science of endocrine disrupting chemicals - 2012. http://www.who.int/ceh/publications/endocrine/en/.
[49] Lamb JC, Boffetta P, Warren FG, Goodman JE, Hentz KL, Rhomberg LR, Staveley J, Swaen G, Van Der Kraak G, Williams A. 2014. Critical comments on the WHO-UNEP State of the Science of Endocrine Disrupting Chemicals - 2012. *Regul Toxicol Pharmacol.* 69:22-40.
[50] UK Government's Hazardous Substances Advisory Committee. Comments on Kortenkamp *et al* (2012) "State of the art assessment of endocrine disrupters." https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/208643/comments-kortenkamp.pdf.

pesticide exposure, Ioannina Medical School concluded that: *"Overall, there is no evidence supporting an association between pesticide exposure and prostate cancer."*[51]

If crop protection products were significant causative factors in the various hormone-related cancers, one would logically expect to find higher incidences of such cancers among those populations with the most exposure to these products (i.e., farmers and agricultural workers). However, in fact, the most consistent finding across the large epidemiological studies undertaken on agricultural workers, which includes pesticide applicators, is that they are healthier than the general population.[52,53] The AGRICAN study in France[52] covering 180,000 people shows that agricultural workers have lower mortality rates and lower incident rates of almost all cancer types, including hormone related cancers. They are reported to have an 18 percent lower rate of prostate cancer and 29 percent lower rate of breast cancer.

Overall, therefore, it is likely to be premature to develop additional test guidelines or assays for predicting the human diseases under discussion until there is greater understanding of, and scientific consensus on, the current level of evidence. Well conducted epidemiology studies could provide reliable human data to support or refute the hypothesis that environmental exposures to chemicals are contributing to these diseases. As concluded by Lamb *et al.* (2014),[55] structured weight of evidence approaches should be employed to weigh the existing scientific evidence on the potential role (if any) of chemical substances in the etiology of human diseases (weighing both human epidemiological data and laboratory animal data). As noted by the USEPA (2010),[54] *"weight of evidence is a process where potentially relevant studies are judged in a professional manner for quality … it is not a process that simply involves tallying the number of positive and negative results … critical assessment of an entire body of available data is taken into account for consistency, coherence, and biological plausibility."* Such an approach would help assess the overall strength of the evidence and therefore, help inform on priorities for possible method development within the OECD test guideline program.

3.5. Does current testing for crop protection products adequately cover wildlife species?

"For invertebrates, relevant mechanistic assays are conspicuous by their absence from the OECD testing suite, mainly due to poor understanding of invertebrate endocrinology. Finally, a range of major taxa such as reptiles and echinoderms have not yet been considered by OECD for any endocrine assay development. It is unknown at present whether it will be possible to read-across to untested groups from tests with other taxa." – EFSA Scientific Committee[55]

Crop protection industry view

The assays commonly undertaken as part of the ecotoxicological evaluation for pesticide active substances (and from the USEPA EDSP Tier 1 and 2 testing) are described in section 2 above. The studies that are included in the OECD Conceptual Framework are listed in

[51] European Food Safety Authority. Literature review on epidemiological studies linking exposure to pesticides and health effects. http://www.efsa.europa.eu/en/supporting/doc/497e.pdf.
[52] Cancers & Préventions. AGRICAN. http://cancerspreventions.fr/.
[53] Agricultural Health Study. www.aghealth.nih.gov.
[54] United States Environmental Protection Agency. 2010. Weight of evidence guidance: evaluating results of EDSP tier 1 screening to identify candidate chemicals for tier 2 testing. http://www.epa.gov/endo.
[55] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132 (page 36).

Attachment 1. It is acknowledged that assays for certain taxa (e.g., reptiles) and mechanistic (mode of action) assays for others (e.g., invertebrates) are not yet available.

For the environment, the protection goal is at the **population level** and any adverse effects observed within ecotoxicological tests must be relevant to the population (i.e., effects on growth, development and reproduction). Adverse effects, regardless of the mechanism behind them, will be detected in the various long-term tests available for both vertebrates (mammals, fish and birds) and invertebrates. Specific higher tier assays (e.g., full fish lifecycle toxicity test) may be triggered by toxicity or specific mode of action indications from the mammalian toxicology studies. As discussed above, endocrine disruptors will not induce a single specific effect, but rather produce a pleiotropic response leading to a diverse range of adverse effects. These effects will be detected in the ecotoxicological apical assays performed and considered in the risk assessment despite the fact that specific information on the mode of action for the adverse effects may be lacking.

A well-accepted principle of ecotoxicology is to extrapolate from a few test species to the multiple species living in the environment (e.g., aquatic amphibians are covered by fish).[56] Also, surrogate or substitute organisms are used to represent a group of organisms. For example, the laboratory rat may be used to represent all mammalian species for the ecological risk assessment.[57] A series of assays are available in the OECD Conceptual Framework for mammals, bird, fish and invertebrates. It is simply not feasible to test an exhaustive list of different species, nor desirable to use many vertebrate animals due to ethical considerations regarding laboratory animal welfare. Potentially more sensitive wildlife species are accounted for in the risk assessment through the use of safety factors and the built-in conservatism in testing, e.g., continuous exposure of a sensitive life stage.

For invertebrates, there are no widely accepted mechanistic endpoints and our understanding of invertebrate endocrinology is rather limited. Therefore, there is a reliance on lifecycle methodologies which measure apical endpoints that are population relevant. Therefore, any endocrine-specific toxicity should be accounted for in the lifecycle response (e.g., a risk assessment based on these data would be protective of any adverse effect resulting from an endocrine mode of action). However, it is important to note that any effects observed cannot be considered diagnostic for endocrine disruption since they typically measure growth and reproductive effects only.

3.6. Does current testing for crop protection products take account of combined exposure to multiple substances (cocktail effects)?

"There is good evidence that several EDCs can work together to produce combined effects. Especially when exposure is to multiple chemicals simultaneously that are capable of affecting the same endpoint, combination effects can occur at doses where each chemical individually is without detectable effects." – State of the Art Assessment Report on Endocrine Disrupters (Kortenkamp *et al.* 2011)[58]

[56] Weltje L, Simpson P, Gross M, Crane M, Wheeler JR. 2013. Comparative acute and chronic sensitivity of fish and amphibians: A critical review of data. *Environ Toxicol Chem.* 32(5):984–994.
[57] United States Environmental Protection Agency. 2015. Technical overview of ecological risk assessment analysis phase: ecological effects characterization. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-0.
[58] Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Orton F, Rosivatz E. 2011. State-of-the-art assessment of endocrine disrupters. Final report of EU project contract 070307/2009/550687/SER/D3. http://ec.europa.eu/environment/chemicals/endocrine/pdf/sota_edc_final_report.pdf.

"Every day we are exposed to a mixture of man-made chemicals, via the air we breathe, the food we eat and the water we drink. And even when the exposure to individual chemicals is below the level where they cause an effect by themselves, new science is now showing that together they can 'add up' and cause a potentially dangerous 'cocktail effect' … The cocktail effect means that the current process by which governments decide on safe levels, i.e., via a 'risk assessment,' where single chemicals are considered separately, ignores the reality that people and wildlife are constantly exposed to many chemicals simultaneously. This process significantly underestimates the risk to our health from the real-life cocktail exposure". – World Wildlife Fund, Health and Environment Alliance, ChemTrust

Crop protection industry view

The potential for combined exposure to multiple substances is not unique to endocrine disruption and is equally applicable to substances with other modes of action. Initiatives to move this issue forward (e.g., those of the EFSA Panel on Plant Protection Products and their Residues/PPR) have therefore taken place in a broader context and several scientific committees have also provided expert opinions on the topic. In their opinion on the *"Toxicity and Assessment of Chemical Mixtures,"* the European Commission's Scientific Committees[59] concluded that:

- Chemicals with common modes of action can act jointly to produce a combination of effects that are larger than the effects of each mixture component applied singly. These effects can be described by *dose/concentration addition.*[60]
- For chemicals with different modes of action (*response addition*[61]), *"no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero effect levels.*"

These conclusions are consistent with those of the EFSA PPR Panel, which also published several scientific opinions[62]as part of the work to develop cumulative risk assessment methodology for pesticide residues in food in the setting of maximum residue levels (MRLs).[63] The PPR Panel is focused on assessing combined exposure of several substances affecting a common target organ or system, using the concept of *dose addition* to predict the toxicological outcome.

It is not possible, nor feasible, to predict or test the multitude of mixtures that may arise in the environment. It is therefore essential to develop tools to prioritize the chemical combinations to be assessed and identify those that may be of importance. The crop protection industry is monitoring the related initiative by the EFSA and will continue to provide expert input into this process. The industry does, however, believe that the existing risk assessment and management approaches such as those in the EU and United States do provide sufficient protection from exposure to the low levels of pesticide residues which may be present in the environment or food. The significant conservatism of this process ensures a high level of protection and high margins of safety are provided even in those cases of potential additive toxicity from substances with similar modes of action or that affect the same target organ or system. Managing the risks from these substances individually

[59] European Commission. Scientific Committee on Health and Environmental Risk, Scientific Committee on Consumer Safety, Scientific Committee on Emerging and Newly Identified Health Risks. 2012. Opinion on the toxicity and assessment of chemical mixtures.
[60] Dose-addition (similar action) occurs when chemicals in a mixture act in the same way, by the same mode of action and differ only in their potencies. The effect of exposure to a mixture of such compounds is equivalent to the effect of the sum of the doses of each component compound (corrected for their differing potencies).
[61] Response-addition (dissimilar action): occurs where the modes of action and possibly the sites of toxic effects (e.g.,, of exposure to such a mixture are the combination of the effects of each component compound.
[62] European Food Safety Authority Panel on Plant Protection Products and their Residues. http://www.efsa.europa.eu/en/panels/ppr.
[63] Regulation 396/2005 discusses the maximum residue levels of pesticides in or on food and feed of plant and animal origin. Regulation 1107/2009 concerns placing of plant protection products on the market.

(e.g., by continuing to ensure that individual pesticide residues are maintained well below their regulatory reference values) will, in almost all cases, also ensure that combinations of substances do not present a concern for human health or the environment.

The cumulative risk assessments already undertaken by regulatory authorities in the EU and United States have confirmed that exposure to mixtures of pesticide residues present at low levels in food are not of concern for human health. These include assessments on organophosphate (OP) insecticides alone (in the U.S.) or together with carbamates (in the UK, Denmark and Netherlands), triazines, chloroacetanilides, carbamates alone (in the U.S.), and all compounds (in Denmark). An additional evaluation on pyrethroids has also been recently completed by the USEPA.[64] The EFSA PPR Panel noted these activities in its scientific opinion and highlight that *"no assessment of actual cumulative exposure … conducted so far has indicated any significant risks from exposure to multiple chemicals belonging to a common assessment group where the individual compounds presented no unacceptable risks."*[65]

Similarly, following an extensive literature review, the European Centre for Ecotoxicology and Toxicology of Chemicals Technical Report 115,[66] "Effects of Chemical Co-exposures at Doses Relevant for Human Safety Assessments," concluded that: *"There was no convincing evidence of toxicity for combined exposures to substances present at concentrations that are acceptable for single chemicals … Only when single chemicals were at unacceptable concentrations did mixtures sometimes result in toxicity. The results of these studies are consistent with the finding of the other studies reviewed in this report … Based on our evaluation, there is no evidence that exposure to complex mixtures of components, each well regulated according to established risk assessment approaches, would pose a health risk to humans."*

Further research studies undertaken on groups of crop protection products specifically in relation to endocrine activity also confirm the above conclusions (i.e., that combined exposures of these products at environmental relevant concentrations pose little or no concern for human health). They include studies undertaken by the Danish Environmental Protection Agency[67,68] and The European Chemical Industry Council.[69]

Scientific evidence also shows that when several substances occur together in environmental mixtures, the toxicities of those mixtures are typically dominated only by one or a small number of compounds.[70] Even with a mixture of several crop protection products affecting the same target organ or system, the majority of substances might not contribute significantly to a given combination effect, either because exposure is very low and/or

[64] United States Environmental Protection Agency. Reevaluation: Review of Registered Pesticides. http://www.epa.gov/oppsrrd1/reevaluation/pyrethroids-pyrethrins.html#risk.

[65] Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. http://www.efsa.europa.eu/fr/efsajournal/doc/705.pdf.

[66] European Centre for Ecotoxicology and Toxicology of Chemicals. 2012. Technical Report 115. Effects of chemical co-exposures at doses relevant for human safety assessments. http://www.ecetoc.org/index.php?mact=MCSoap,cntnt01,details,0&cntnt01by_category=5&cntnt01template=display_list_v2&cntnt01order_by=Reference%20Desc&cntnt01display_template=display_details_v2&cntnt01document_id=6370&cntnt01returnid=89.

[67] Danish Environmental Protection Agency. 2012. Exposure of pregnant consumers to suspected endocrine disruptors. http://www2.mst.dk/Udgiv/publications/2012/04/978-87-92903-02-0.pdf.

[68] Jensen BH *et al.* 2013. Probabilistic assessment of the cumulative dietary exposure of the population of Denmark to endocrine disrupting pesticides. *Food Chem Toxicol.* 55:113-120.

[69] The European Chemical Industry Council. The capacity of the endocrine system to cope with combined exposure to exogenous endocrine active substances at environmentally relevant concentrations. http://cefic-lri.org/?s=EMSG56.

[70] Price PS, Han X. 2011. Maximum Cumulative Ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. *Int J Environ Heal R.* 8:2212-2225.

because potency in relation to the effect considered is weak. Instead, cumulative effects are typically driven by a few substances within the group. Therefore, continuing to control individual substances through the existing regulatory processes will also control the overall risk from such combined exposures.

3.7. Does current testing for crop protection products address low-dose effects and non-monotonic dose response curves?

"*We illustrate that non-monotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low-doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when non-monotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects at high doses. Thus fundamental changes in chemical testing and safety determination are needed to protect human health*."
– Vandenberg *et al.* 2012[71]

Crop protection industry view
Current testing guideline studies require multiple doses of a substance to be evaluated, usually in a significant number of laboratory animals per sex, per dose group and across three or more treatment groups. From these studies, dose-response curves are determined to show the relationship between a chemical's concentration and observed effects. Typical dose response curves are monotonic, meaning a greater response is observed as the dose increases (i.e., the dose makes the poison). However, some scientists (e.g., Vandenberg *et al.* 2012) have claimed that for endocrine disruptors, dose-response curves that are non-monotonic are common, meaning a response may be greater at lower doses than higher doses (i.e., NMDRs). These scientists have suggested that NMDRs are frequent to such an extent that current test guidelines would "miss" low-dose effects and risk assessments would not be deemed health protective.

Some scientists have also claimed that environmental exposure to chemicals can mimic hormones capable of causing effects in laboratory studies at low doses and that these effects may not be detected because standard toxicology studies are typically performed at higher doses. This is the "low-dose" hypothesis (i.e., that low doses of certain chemicals produce effects that are not observed at high doses). Although no scientific consensus has been reached in defining "low dose," the term is typically used to describe doses below the NOAEL or below a level that is environmentally relevant to humans.

The concepts of low-dose effects and NMDRs are not unique to endocrine disruption, but are generally relevant for all modes of action. If these concepts were accepted as general propositions, there would be profound consequences for the current testing approaches for chemicals, e.g., the ability to determine thresholds and to set regulatory reference values (safety limits). The crop protection industry's view on each of these issues described below.

Low-dose effects
The low-dose hypothesis suffers from many shortcomings and there are many reasons to question the assertion of effects at low doses:

[71] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 33:378-455.

(1) Studies of low-dose effects often suffer from various methodological deficiencies, including the use of small numbers of animals and single doses, the statistical analyses employed, and their experimental designs.[72,73]
(2) Reported low-dose findings are often of questionable toxicological relevance. For example, even in cases where biological responses are detected, not all observations at "low doses" are necessarily adverse or obligatory precursors to adverse effects in living organisms.[74]
(3) Subsequent, more robust studies, including those performed by the USEPA using three generational reproduction studies have been unable to reproduce the reported low-dose findings.[75]
(4) A peer reviewed commentary by Rhomberg and Goodman (2012)[76] analysing the recent publication by proponents of the low-dose hypothesis (Vandenberg *et al.*, 2012) concluded that the authors had overstated the scientific evidence on low-dose effects (and NMDRs). The scientific weaknesses identified in the review included:

- Selective citation of studies without examining whether the examples are consistent and coherent with other relevant information and selective dismissal of studies that do not show low-dose effects.
- Failure to evaluate all studies equally and the lack of uniformity in the evaluation of specific studies (i.e., studies with positive results are evaluated differently than those with null results).
- Lack of documentation as to whether exposures in studies are truly ''low-dose'' and relevant to humans.
- The inclusion of studies that do not address adverse effects, but rather, transient, adaptive responses.
- A failure to consider whether the doses examined in these studies are of any relevance to human exposure levels.

Non-monotonic dose responses
NMDRs have been the subject of several national and international workshops since 2000. If NMDRs occur for apical endpoints, they would present a significant challenge to human health testing and risk assessment because they do not follow the expected monotonic observation of an increasing dose leading to a proportionally increasing frequency or severity of effect. The 2012 review by Vandenberg *et al.* evaluated more than 800 scientific papers and the authors concluded that when NMDRs occur, the effects of low doses cannot be predicted by effects at high doses and therefore, in their view, fundamental changes in chemical testing and safety determination were considered necessary to protect human health.[77]

[72] European Food Safety Authority. 2010. EFSA scientific report of Endocrine Active Substances Task Force. *EFSA Journal.* 8(11):1932. [59 pp.] doi:10.2903/j.efsa.2010.1932. http://www.efsa.europa.eu/en/search/doc/1932.pdf.
[73] Lamb JC, Boffetta P, Warren FG, Goodman JE, Hentz KL, Rhomberg LR, Staveley J, Swaen G, Van Der Kraak G, Williams A. 2014. Critical comments on the WHO-UNEP State of the Science of Endocrine Disrupting Chemicals – 2012. *Regul Toxicol Pharmacol.* 69:22-40.
[74] European Food Safety Authority. Low-dose effects and endocrine active substances.http://www.efsa.europa.eu/en/faqs/faqlowdoseeffects.htm.
[75] Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM,Fail PA, Chang TY, Seely JC *et al.* 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci.* 68:121-146.
[76] Rhomberg, LR; Goodman, JE. 2012. "Low-dose effects and nonmonotonic dose-responses of endocrine disrupting chemicals: Has the case been made?" *Regul Toxicol Pharmacol.* 64(1):130-133.
[77] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 33:378-455.

In response to Vandenberg *et al.* (2012), the Danish Centre on Endocrine Disrupters (DTU) in its review[78] of the evidence concluded that while examples of NMDRs do exist, they are not as common as claimed by Vandenberg *et al.* (2012). The DTU also noted that the majority of the data supporting NMDRs were from *in vitro* studies and that Vandenberg *et al.* (2012) had inappropriately included findings where U-shaped or inverted U-shaped curves were the product of general toxicity. The lack of structured weight of evidence and clear criteria in reviewing and including studies was also noted in a review by Lamb *et al.* (2014), which supported more *"objective and systematic reviews that transparently capture the best available science and rely on explicit criteria for the evaluation of the evidence."*[79]

USEPA state of the science evaluation: The USEPA has initiated a comprehensive review of the state of the science on NMDRs focusing on endocrine disruption mode(s) of action and in particular estrogen, androgen and thyroid active chemicals. In 2012, the USEPA convened a working group of scientific experts from the USEPA, U.S. Food and Drug Administration, National Institute of Environmental Health Sciences and National Institute of Child Health and Human Development; together, they reviewed >2,000 scientific documents over the course of six months.

In 2013, the USEPA published its draft report "State of the Science Evaluation: Nonmonotonic Dose Responses as They Apply to Estrogen, Androgen, and Thyroid Pathways and EPA Testing and Assessment Procedures,"[80] which provided the following conclusions:

(1) NMDRs do occur, however, they are not commonly identified *in vivo* and are rarely seen in whole-organism studies after low-dose or long-term exposure.
(2) There is no reproducible evidence that the key biological events involved in the expression of NMDRs identified at low doses are predictive of adverse outcomes that may be seen in humans or wildlife populations (for estrogen, androgen or thyroid endpoints).
(3) For the estrogen, androgen or thyroid modes of action that provide adequate information to make an assessment, our evaluation shows that there is not sufficient evidence of NMDRs for adverse effects below the NOAELs or benchmark dose derived from the current testing strategies.
(4) While there are biological changes that may occur in a non-monotonic manner in the low-dose region, our review indicates that reproducible NMDRs for adverse effects occur in the high-dose region of the dose response curve. Thus, the current testing approaches do not fail to identify or establish appropriate NOAELs in the low-dose range of exposure, even if not all effects for every chemical are identified.
(5) The extensive evaluation conducted in the present review as well as almost two decades of experience with screening assays for hazard identification indicate that these assays do not fail to detect chemicals with endocrine activity for the estrogen or androgen hormone systems. Current testing strategies are "highly unlikely" to mischaracterize, as a consequence of NMDR, a chemical that has the potential for endocrine disruption (adverse perturbations of the estrogen, androgen or thyroid pathways).

[78] Danish Centre on Endocrine Disrupters. 2013. Input for the REACH review in 2013 on endocrine disrupters (tærskelværdi-projekt, j.nr. MST-621-00050): final report by Technical University of Denmark and the National Institute of Food, Division of Toxicology and Risk Assessment.
[79] Lamb JC, Boffetta P, Warren FG, Goodman JE, Hentz KL, Rhomberg LR, Staveley J, Swaen G, Van Der Kraak G, Williams A. 2014. Critical comments on the WHO-UNEP State of the Science of Endocrine Disrupting Chemicals - 2012. *Regul Toxicol Pharmacol.* 69:22-40.
[80] United States Environmental Protection Agency. 2013. State of the science evaluation: non-monotonic dose responses as they apply to estrogen, androgen, and thyroid pathways and EPA testing and assessment procedures. Draft. https://www.epa.gov/chemical-research/nonmonotonic-dose-responses-they-apply-estrogen-androgen-and-thyroid-pathways-and.

(6) NMDRs would be problematic only if a chemical with estrogen, androgen or thyroid activity produced an effect *in vivo* at a dose below those used in screening and the chemical had no effect on estrogen, androgen or thyroid related endpoints at the higher screening dosage levels.
(7) Although such NMDRs have been hypothesized, they have not been demonstrated reproducibly and none were found in the present evaluation.

U.S. National Academy of Science (NAS) review: The NAS Board on Environmental Studies and Toxicology reviewed the USEPA draft review paper to provide an expert peer review and provided its findings and recommendations to the USEPA in May 2014.[81] Across the many key recommendations was a clear need to perform a systematic review of the available literature and integration across multiple lines of biological evidence that could inform whether NMDR curves exist and, if so, to what extent they might impact the ability of regulatory risk assessments to be public health protective. The NAS review stated: "*An analytic plan should be developed and applied consistently to the evidence on the three hormone pathways. Important elements of the plan include predefining and documenting the literature-search strategies and their results, establishing criteria for selecting studies for analysis, establishing criteria for determining study quality, using templates for presenting evidence consistently in tabular and graphic form, and documenting approaches to integration of evidence."*

To that end, the USEPA commissioned the NAS to investigate the low-dose issue for endocrine-disrupting chemicals by applying the systematic review process to critically review the available data. This subsequent NAS final report on low-dose effects and its conclusions will be heavily considered by regulatory authorities prior to considering whether wholesale changes to the currently well established, regulatory testing paradigm are warranted.

Summary
A considerable body of scientific evidence does *not* support the low-dose hypothesis and claimed significance of NMDRs. Despite the recent scientific workshops, continued research and retrospective reviews of the available data, there is currently no scientific consensus regarding the existence and/or relevance of low-dose effects and NMDRs. These remain issues of considerable scientific debate both in relation to how to interpret the results of existing low-dose studies and what changes, if any, are required to current scientifically validated testing approaches.

The crop protection industry believes that the overall weight of currently available scientific evidence supports the existing regulatory testing and risk assessment approaches and changes to these are not justified in relation to NMDRs and low-dose effects. This is because of the reasons stated clearly by independent reviewers:

(1) Low-dose findings have not been reproduced consistently between different laboratories.
(2) Studies on low-dose effects often suffer from methodological shortcomings.
(3) The toxicological significance of reported low-dose effects is questionable, in particular regarding the relevance of such effects, if any, to adverse effects on human health
(4) The validity and interpretation of many of the low-dose and NMDR examples cited for example by Vandenburg *et al.* (2012) have been questioned by many independent scientists and subsequent expert reviews.[82]

[81] National Academy of Sciences. 2014. Review of the Environmental Protection Agency's state-of-the-science evaluation of nonmonotonic dose-response relationships as they apply to endocrine disruptors. http://www.nap.edu/read/18608/chapter/1.
[82] Rhomberg LR, Goodman JE. 2012. Low-dose effects and nonmonotonic dose-responses of endocrine disrupting chemicals: Has the case been made? *Regul Toxicol Pharmacol.* 64(1):130-133.

(5) As concluded in the USEPA's state of the science evaluation:[83] *"NMDRs do occur, however, they are not commonly identified in vivo, and are rarely seen in whole-organism studies after low-dose or long-term exposure. Significantly, the Agency considers that there is no reproducible evidence that the low-dose key biological events involved in NMDR expression are predictive of adverse outcomes in humans and, as such, the current testing strategies are highly unlikely to mischaracterize a chemical that has the potential for endocrine disruption."*

3.8. Is current testing for crop protection products adequate to allow the determination of thresholds for endocrine active substance with sufficient confidence?

"… the Swedish Chemicals Agency … is of the opinion that EDCs from a regulatory perspective as a default should be regarded as substances for which it in practice is not possible to determine safe threshold concentrations … This position is based on our view that identifying safe concentrations limits for all possible endpoints within the endocrine system that can be affected by EDCs is not possible with current test method guidelines … The following has been taken into account:

- *the complexity of the endocrine system*
- *the presence of sensitive developmental stages, prominently during foetal development, with a known risk for irreversible effects,*
- *the fact that the time between the exposure event and the appearance of the effect can be very long, in some cases not apparent until next generation,*
- *there is literally no threshold of effect for an endocrine disrupting compound when it is added to a hormone system that is already active,*
- *the scientific difficulties to establish a safe exposure level, especially for application to human and environmental populations,*
- *and the scientific uncertainty with regard to prediction of the effects and thereby the assessment of risks of EDCs."*[84]

Crop protection industry view

A threshold is a dose (level of exposure) above which adverse effects are observed in toxicological or ecotoxicological studies. No adverse effects are expected to be observed below this level; any changes seen are generally non-adverse or, even adaptive responses or for environmental species, viewed as not relevant at the population level. The existence of thresholds is a cornerstone of toxicology and ecotoxicology.

There is significant debate over the existence of thresholds and the ability to determine thresholds for endocrine disruptors. The difference of opinion has centred around the long accepted tenet of toxicology that "the dose makes the poison."[85] Some in the endocrine research community believe this principle does not apply to the endocrine system and they have suggested that based on the principles of endocrinology, it cannot be assumed that thresholds exist for the effects of endocrine disruptors. These scientists often do not specify what it is meant by threshold (e.g., absolute, biological or toxicological threshold) and many

[83] U.S. Environmental Protection Agency. 2013. State of the science evaluation: nonmonotonic dose responses as they apply to estrogen, androgen, and thyroid pathways and EPA testing and assessment procedures. Draft.
[84] Swedish Chemicals Agency. 2013. Position on the possibility to determine threshold levels for endocrine disrupters. (submission in relation to European Commission review under Article 138 (7) of the REACH regulation).
[85] Paracelsus. 1567. "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy" (i.e., the dose makes the poison and the higher the dose or exposure concentration, the greater the effect that occurs).

portray endocrine disruption as a "special" form of toxicity and endocrinology as a "special" form of biology. These views however, are not supported by the balance of scientific evidence. They conflict with decades of experience and repeatable observations in exposure-response relationships in pharmacology and toxicology and with the principles of homeostasis.[86]

In current regulatory practice, a non-threshold approach is reserved *only* for certain forms of mutagenicity and genotoxic carcinogenicity; all other endpoints (adverse effects) employ a threshold approach. The non-threshold or linear dose response approach for genotoxicity derives from the theory that even a single molecule of a genotoxic agent could produce a mutation in the DNA, leading to adverse consequences.

In contrast, endocrine disruption results from the interaction of a chemical with receptors, enzymes or other co-factors in a cell. Inactivation/activation of one single target by one molecule of the chemical is inconsequential as a minimum degree of interaction with the critical sites must be reached to elicit an effect; this minimum level constitutes a **biological threshold**. The amount of the chemical then needs to reach an even higher level to be able to counteract homeostatic mechanisms and other repair processes before an adverse effect can be induced; this higher level constitutes a **threshold of adversity**. Mechanistic and biological considerations therefore support that thresholds of adversity exist for endocrine disruptors and are the "rule," rather than the "exception."[87] It is inconceivable that a single molecule of any substance can, by itself, lead to adverse effects (i.e., endocrine disruption) in an organism or for ecotoxicological considerations, in a population (i.e., the mere presence of endocrine activity or change does not necessarily obligate the chemical to proceed down a path leading to an adverse endocrine health outcome).[88]

It is recognized that the existence of thresholds cannot be proven by experimentation.[89] It is also accepted that the numerical value of a "true" threshold (either biological or toxicological) cannot be determined experimentally as this would require an infinitely sensitive method with an infinitely large number of animals and an infinitely small dose, down to one molecule.[95] For any effect, including those occurring as a consequence of endocrine disruption, it is only the "experimental" threshold in a specified species that can be observed, i.e., the highest dose or concentration at which no adverse effects are observed within the confines of the experiment performed. The focus of regulatory risk assessment is therefore centred around the "experimental" or empirically driven (practical) threshold dose.[90]

The determination of this experimental threshold can be applied in the same way for endocrine disruption as any other form of toxicity. In regulatory risk assessment, the NOAEL is a practical value, determined by toxicological or ecotoxicological experimentation and used as a surrogate for the threshold of adversity. As discussed under section 3.3 above, safety factors are then applied to this value to account for intra-species (10x) and inter-species (10x) variability (i.e., with a composite safety factor of 100x below the experimental

[86] Rhomberg LR, Goodman JE. 2012. Low-dose effects and nonmonotonic dose-responses of endocrine disrupting chemicals: Has the case been made? *Regul Toxicol Pharmacol.* 64(1):130-133.
[87] Rhomberg LR, Goodman JE, Haber LT, Dourson M, Andersen ME, Klaunig JE, Meek B, Price PS, McClella RO, Cohen SM. 2011. Linear low-dose extrapolation for noncancer health effects is the exception, not the rule. *Crit Rev Toxicol.* 41(1):1-19.
[88] Bogert CJ, Baker SP, Matthews JC. 2013. Potency matters: thresholds govern endocrine activity. *Regul Toxicol Pharmacol.* 67(1):83-88.
[89] EFSA Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132.
[90] Lamb JC, Boffetta P, Warren FG, Goodman JE, Hentz KL, Rhomberg LR, Staveley J, Swaen G, Van Der Kraak G, Williams A. 2014. Critical comments on the WHO-UNEP state of the science of endocrine disrupting chemicals - 2012. *Regul Toxicol Pharmacol.* 69:22-40.

NOAEL) before setting regulatory reference values (e.g., the acute reference dose) which are used in risk assessment to ensure human and environmental safety.

The following points have frequently been used to argue for adopting a non-threshold regulatory approach for endocrine disruptors and for these substances presuming some degree of risk at any dose:

(1) *"Additivity-to-background"* argument: If a chemical enhances an already existing disease-causing process, then even small increases in exposure concentration and/or duration increase disease incidence in a linear manner.
(2) *"Infinite sensitivity of the population"* argument: There would always be at least one very sensitive individual in the population which will show an adverse response even to one molecule of a chemical.
(3) *Sensitive developmental stages*: It has been argued that in a developing organism, homeostatic mechanisms are not sufficiently developed so that a threshold of adversity cannot be assumed for endocrine disruptors acting during the developmental stages of the lifecycle of an organism (i.e., "sensitive windows of exposure").
(4) *Low-dose effects, NMDRs*: It has been argued that endocrine disruptors display "low-dose" effects and NMDRs, therefore, the NOAEL identified by conventional toxicity testing is incorrect.

(1) additivity-to-background: this argument does not preclude the existence of a threshold of adversity.[91] One single molecule adding to a process already active (e.g., hormone receptor agonism) cannot change by itself (or on its own) the normal/physiological response of that process into an adverse effect.

(2) infinite sensitivity of the population: this argument is an abstract concept, which has no corroboration from empirical observations as there are limits to intra-species variability. This potential variability is also currently accounted for in the 10x intra-species uncertainty factor which is then applied to a point of departure that is based on the most sensitive test animal and the most sensitive apical endpoint.

(3) sensitive developmental stages: this viewpoint is not supported by decades of observations and safety testing of developmental toxicants. Although the endocrine system in the embryo/foetus, is not fully functional and cannot ensure the homeostatic control of many vital processes of the organism to the same degree as adults, there are other homeostatic and repair mechanisms operating at the cellular level. In addition, there are hormonal homeostatic mechanisms operating in the maternal organism, which are able to counteract initial perturbation induced by the chemical agent before delivery to the embryo/foetus. A minimum level of interaction of the chemical substance with critical targets of the developing organism is therefore required to elicit a toxicologically relevant effect. This critical level of interaction (threshold of adversity) might be lower in the developing organism than in the adult, and the nature of the effect might be different (severe, permanent damage in the foetus versus a less severe effect in the adult), but a threshold of adversity must exist.

This sensitive window of exposure will be determined by the prenatal developmental toxicity in the rat and rabbit (OECD 414 and OCSPP 870.3700), and developmental neurotoxicity studies (OECD 426 and OCSPP 870.6300) described in section 2 and 3.3. To the extent that increased susceptibility is evident for the foetus, the risk assessment will be informed by the weight of evidence from prenatal developmental toxicity, developmental neurotoxicity

[91] Boobis AR, Daston GP, Preston RJ, Olin SS. 2009. Application of key events analysis to chemical carcinogens and noncarcinogens. *Crit Rev Food Sci Nutr.* 29.

and multigenerational reproduction (OECD 416 and OCSPP 870.3800) studies submitted in support of pesticide registrations.

(4) low-dose effects, NMDRs: as discussed under section 3.7 above, there is no consensus in the scientific community on the existence and relevance in toxicology of these issues. However, even if low-dose effects/NMDRs do occur, they do not preclude the existence of a threshold. It is therefore, premature to assume that low-dose effects/NMDRs are the rule and to justify the abandonment of the standard, threshold approach on this basis.

Overall, there is nothing unique about endocrine disruption compared with other non-genotoxic forms of toxicity to justify adopting a default non-threshold approach. Biological and mechanistic considerations support that thresholds of adversity exist and are the rule for all endpoints, including those arising from endocrine disruption. The presence of homeostatic and defence mechanisms mean that a minimum degree of interaction of a substance with the critical sites must be reached to produce a toxicologically relevant effect. Below this critical level of interaction (threshold of adversity), homeostatic mechanisms would be able to counteract perturbation produced by exposure to a substance, and no structural or functional changes would be observed.[92] The current toxicological and ecotoxicological test methods for crop protection products allow for the determination of thresholds of adversity and for the establishment of regulatory reference values (safety limits) used for risk assessment.

[92] European Food Safety Authority Scientific Committee. 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. *EFSA Journal.* 11(3):3132.

Attachment 1. Comparison of the OECD Conceptual Framework with EU and U.S. data requirements relevant to the assessment of potential endocrine-disrupting effects.

Mammalian and Non-Mammalian Toxicology		EU	USEPA
Level 1 Existing data and non-test information	Physical and chemical properties, e.g., molecular weight (MW), reactivity, volatility, biodegradability	Core to both regions	
	All available (eco)toxicological data from standardized or non-standardized tests	Core to both regions Will include acute eco/toxicological characterization in a range of bird, mammalian, fish, aquatic and terrestrial invertebrate species.	
	Read across, chemical categories, quantitative structure activity relationships (QSARs) and other *in silico* predictions and absorption, distribution, metabolism and excretion (ADME) model predictions		
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s)/ pathway(s) (mammalian and non-mammalian methods)	Estrogen or androgen receptor binding affinity (OCSPP 890.1250 or 890.1150)	May be requested by rapporteur Member State (RMS) following concerns raised by the standard evaluation	EDSP tier 1 or may be requested at registration review
	Estrogen receptor transactivation (OECD TG 455–457; OCSPP 890.1300)		
	Androgen or thyroid transactivation (If/when test guidelines/TGs are available)	No TGs available	
	Steroidogenesis *in vitro* (OECD TG 456; OCSPP 890.1550)	May be requested by RMS following concerns raised by the standard evaluation	EDSP tier 1 or may be requested at registration review
	MCF-7 cell proliferation assays (estrogen receptor ant/agonist)		Relevant for other scientifically relevant information (OSRI)
	Other assays as appropriate		

Mammalian and Non-Mammalian Toxicology		EU	USEPA
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s)/ pathway(s)	**Mammalian Toxicology**		
	Uterotrophic assay (OECD TG 440; OCSPP 890.1600)	May be requested by RMS following concerns raised by standard evaluation	EDSP tier 1 or may be requested at registration review
	Hershberger assay (OECD TG 441; OCSPP 890.1400)	May be requested by RMS following concerns raised by standard evaluation	EDSP tier 1 or may be requested at registration review
	Non-Mammalian Toxicology		
	Xenopus embryo thyroid signalling assay (when/if TG is available)	No TG available	
	Amphibian metamorphosis assay (OECD TG 231; OCSPP 890.1100)	May be requested by RMS following concerns raised by standard evaluation	EDSP tier 1 or may be requested at registration review
	Fish Reproductive Screening Assay (OECD TG 229; OCSPP 890.1350)		
	Fish Screening Assay (OECD TG 230)		Relevant for OSRI
	Androgenized female stickleback screen (GD 140)	No OECD TG available (OECD GD available	Relevant for OSRI

Mammalian and Non-Mammalian Toxicology		EU	USEPA
Level 4 *In vivo* assays providing data on adverse effects on endocrine-relevant endpoints	**Mammalian Toxicology**		
	Repeated dose 28-day study (OECD TG 407; OCSPP 870.3050)	Not a core requirement, but these studies are conducted for the vast majority of pesticide active ingredients as preliminary studies ahead of the repeated dose 90-day studies in rats, dogs and/or mice.	
	Repeated dose 90-day study (OECD TG 408; OCSPP 870.3100)	Core to both regions 2 species, typically rat and dog. In many cases, a 90-day study is also available for mice.	
	1-generation reproduction toxicity study (OECD TG 415)	The more comprehensive 2-generation reproductive toxicity study (OECD TG 416) is a core requirement in both regions (see below).	
	Male pubertal assay (see GD 150, Chapter C4.3; OCSPP 890.1500)	No OECD TG available	EDSP tier 1 or may be requested at registration review
	Female pubertal assay (see GD 150, Chapter C4.4; OCSPP 890.1450)	No OECD TG available	EDSP tier 1 or may be requested at registration review
	Intact adult male endocrine screening assay (see GD 150, Chapter Annex 2.5)	No OECD TG available	
	Prenatal developmental toxicity study (OECD TG 414; OCSPP 870.3700)	Core to both regions 2 species, typically rat and rabbit	
	Chronic toxicity and carcinogenicity studies (OECD TG 451, 452, 453; OCSPP 870.4100, 870.4200, 870.4300)	Core to both regions 2 rodent species, typically rat and mouse are evaluated for chronic toxicity and carcinogenicity in lifetime exposure studies (OECD TG 451/453). A non-rodent species, typically dog,* is evaluated for	

		chronic toxicity (OECD TG 452).	
	Reproductive screening test (OECD TG 421 if enhanced; OCSPP 870.3550)	The more comprehensive 2-generation reproductive toxicity study (OECD TG 416) is a core requirement in both regions (see below).	
	Combined 28-day/reproductive screening assay (OECD TG 422 if enhanced; OCSPP 870.3650)	The more comprehensive 2-generation reproductive toxicity study (OECD TG 416) and the repeated dose 90-day study (OECD TG 408) are core requirements in both regions (see below).	
	Developmental neurotoxicity (OECD TG 426; OCSPP 870.6300)	May be requested by RMS following concerns raised by the standard evaluation.	May be requested by USEPA following concerns raised by the standard evaluation.**

*Note: The crop protection industry's position is that the one-year dog is not required. This is also the conclusion of retrospective reviews from multiple countries with dog studies that demonstrated lack of value added from the one-year dog study in developing a chronic reference value or ADI.[93, 94]
**The USEPA may no longer request developmental neurotoxicity studies and may request alternatives (e.g., comparative cholinesterase assay for OPs and the developmental thyroid assay for other compounds).

[93] Kobel W, Fegert I, Billington R, Lewis R, Bentley K, Langran-Lerche C, Botham P, Sato M, Debruyne E, Strupp C, van Ravenzwaay B. 2014. Relevance of the 1-year dog study in assessing human health risks for registration of pesticides. An update to include pesticides registered in Japan. *Crit Rev Toxicol.* 44(10):842-8.
[94] Dellarco VL, Rowland J, May B. 2010. A retrospective analysis of toxicity studies in dogs and impact on the chronic reference dose for conventional pesticide chemicals. *Crit Rev Toxicol.* 40(1):16-23.

Non-Mammalian Toxicology		
Fish sexual development test (OECD TG 234)	May be requested by RMS following concerns raised by the standard evaluation	Relevant for OSRI
Fish Reproduction Partial Lifecycle Test (when/If TG is Available)	No TG available	
Larval Amphibian Growth & Development Assay (LAGDA) (OECD TG 241, OCSPP 890.2300)[95]	May be requested by RMS following concerns raised by the standard evaluation	EDSP Tier 2 assay
Avian Reproduction Assay (OECD TG 206; OCSPP 850.2300)	Core	2 species required in US
Mollusc Reproduction Assays (when TG is available)	Draft TGs available (*Lymnaea stagnalis*; *Potamopyrgus antipodarum*)	
Chironomid Toxicity Test (TG 218-219)	Conditional	Not required
Daphnia Reproduction Test (with male induction) (OECD TG 211)	Core*	
Earthworm Reproduction Test (OECD TG 222)	Core	Not required
Enchytraeid Reproduction Test (OECD TG 220)	Not required	
Sediment Water *Lumbriculus* Toxicity Test Using Spiked Sediment (OECD TG 225)	Conditional	Not required
Predatory mite reproduction test in soil (OECD TG 226)	Core	
Collembolan Reproduction Test in Soil (OECD TG 232)		

[95] United States Environmental Protection Agency. Public Draft Guidelines. Endocrine Disruptor Screening Program Tier 2 assay validation process. http://www.epa.gov/scipoly/oscpendo/index.htm.

Mammalian and Non-Mammalian Toxicology		EU	USEPA
Level 5 *In vivo* assays providing more comprehensive data on adverse effects on endocrine-relevant endpoints over more extensive parts of lifecycle of organism	**Mammalian Toxicology**		
	Extended one-generation reproductive toxicity study (OECD TG 443; alternative to OCSPP 870.3800)	Exists only as an alternative to the 2-generation reproduction toxicity study (OECD TG 416) which is a core requirement in both regions (see below). There are no apical endpoints in OECD TG 443 that would not be addressed in the current OECD TG 416; OCSPP 870.3800 and it is a validated EDSP Tier 2 assay.	
	2-Generation reproduction toxicity study (OECD TG 416 most recent update; OCSPP 870.3800)	Core to both regions	
	Non-Mammalian Toxicology		
	FLCTT (Fish Life Cycle Toxicity Test) (OCSPP 850.1500 but no OECD TG available)	Conditional**	
	Medaka Extended One-Generation Reproduction Test (MEOGRTTG) (OECD TG 240, OCSPP 890.2200)[96]	May be requested by RMS following concerns raised by standard evaluation	EDSP Tier 2 assay
	Avian 2 generation test in the Japanese Quail (no OECD TG available; OCSPP 890.2100[98])	Not required	EDSP Tier 2 assay
	Mysid Life Cycle Toxicity Test (no OECD TG available)	No OECD TG available	
	Copepod Reproduction and Development Test (GD 201)	No OECD TG available (OECD GD available)	

[96] United States Environmental Protection Agency. Public draft guidelines. Endocrine Disruptor Screening Program tier 2 assay validation process. http://www.epa.gov/scipoly/oscpendo/index.htm.

	Sediment Water Chironomid Life Cycle Toxicity Test (TG 233)	Conditional	Conditional US equivalent TG available (OCSPP 850.1790)

*Technically conditional in the EU (based on water persistence), however, in practice, always performed.
**Studies routinely performed despite the lack of validated test guideline.

Attachment 2: USEPA EDSP and Tiered Approach to Screening and Testing for Potential Endocrine Disruption

The USEPA developed a risk-based Endocrine Disruptor Screening Program (EDSP) in response to the statutory mandates in the Food Quality Protection Act of 1996 (FQPA) and 1996 Safe Drinking Water Act (SDWA). A Federal Advisory Committee Endocrine Disruptor Screening and Testing Advisory Council (EDSTAC) was chartered to determine how the agency would develop the scientific infrastructure to screen and test >10,000 compounds for potential endocrine effects. In 1998, the EDSTAC recommended a practical, incremental two-tiered process that would initiate the screening of chemicals for potential to interact with the endocrine system and only if the weight of evidence determination deemed necessary, subsequent Tier 2 testing with longer term studies conducted.

The EDSP Tier 1 screening battery includes five *in vitro* and six short-term *in vivo* assays that test multiple species and life stages, capturing the estrogen (E), androgen (A), thyroid (T), and steroidogenesis pathways. These 11 assays were extensively validated across multiple laboratories and results from the inter-laboratory validation efforts were scientifically peer-reviewed by an independent external peer review panel of experts on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) in 1999 and subsequently, in 2008.

More recently in 2013, the FIFRA SAP reviewed the Tier 1 battery for its collective demonstrated performance to detect potential effects across the E, A or T hormonal systems. At this review, the panel noted again that the battery of assays provides sufficient coverage for potential EATS pathways and no single assay in isolation can determine hormonal activity. The 2008 SAP pointed out that the strength of the *in vitro* assays is to provide "specificity and sensitivity … and do not provide complementary or redundant assessments of androgenic (hormonal) activities" and in the more current 2013 SAP evaluation, there were cases in which potential specific modes of action were not detected in the *in vitro* assays, but were detected in the *in vivo* responses from the Hershberger Assays, Rat Pubertal Assays and Fish Short-Term Reproduction Assays. The SAP panel further noted that while novel and unknown mode(s) of action may not be well represented across the Tier 1 battery, the *in vivo* studies provide "apical endpoints that may detect such effects even in the absence of specific understanding of the MOA."[97] This finding would be similarly true for the current, standard *in vivo* studies required for crop protection product registration listed in the U.S. Code of Federal Regulations (40CFR158).

The later observations emphasize the importance of these multi-parameter *in vivo* assays as a component of the battery to evaluate other known and unknown mode(s) of actions, hypothalamic pituitary gonadal alterations as well as potential differences between species. The level of complementarity and redundancy built into the Tier 1 battery is reflected in its ability to identify potential interactions with multiple endocrine mode(s) of action and pleiotropic effects associated with perturbation of the endocrine system.

[97] United States Environmental Protection Agency. FIFRA Scientific Advisory Panel review of the weight of evidence evaluations of tier 1 assays. July 2013. http://www.epa.gov/scipoly/sap.

Figure 1. EDSP Tier 1 Screening Battery of Assays Covering EATS modalities

					Steroid	Synthesis		
	E	**E-**	**A**	**A-**	**T**	**E**	**HPG**	**HPT**
In vitro								
ER Binding	X	X						
ER Transcriptional Activation	X							
AR Binding			X	X				
Steroidogenesis (H295R)					X	X		
Aromatase (Recombinant)						X		
In vivo								
Uterotrophic	X							
Hershberger			X	X				
Pubertal male			X	X	X		X	X
Pubertal female	X	X				X	X	X
Fish Reproductive Screen	X	X	X	X	X	X	X	
Amphibian Metamorphosis								X

* United States Environmental Protection Agency 2010. Weight of Evidence Guidance: Evaluating Results of EDSP Tier 1 Screening to Identify Candidate Chemicals for Tier 2 Testing. http://www.epa.gov/endo.

Based on the review of Tier 1 data and other submitted pesticide toxicity data (e.g., sub-chronic, chronic, developmental and reproduction studies) in a weight of evidence analysis, if the determination is made that the chemical has the potential to interact with the endocrine system, the Tier 2 assays would then be required. Tier 2 testing assays have been reviewed by the FIFRA SAP in 2013 as part of the validation process. Current Tier 2 cross-taxa assays include rat, fish, and bird definitive multi-generation reproduction studies and a study of growth and development assay in the frog; results from these studies would supplement the current pesticide toxicity submission data and be considered along with exposure information to support regulatory pesticide risk assessments. The specific assays for Tier 1 and Tier 2 screening and testing are listed below.

Table 1. EDSP Tier 1 and Tier 2 Assays

EDSP Tier 1 Assays		
OCSPP Guideline No.	**OECD Reference**	**Guideline Name**
890.1100	231	Amphibian Metamorphosis
890.1150		Androgen Receptor Binding (Rat Prostate Cytosol)
890.1200		Aromatase (Human Recombinant)

890.1250		Estrogen Receptor Binding (Rat Uterine Cytosol)
890.1300	455	Estrogen Receptor Transcriptional Activation (human cell line HeLa-9903)
890.1350	299	Fish Short-term Reproduction
890.1400	441	Hershberger (rat)
890.1450		Female Pubertal (rat)
890.1500		Male Pubertal (rat)
890.1550		Steroidogenesis (human cell line – H295R)
890.1600	440	Uterotrophic (rat)
EDSP Tier 2 Guidelines**		
870.3800	416, 443*	Rat Two-Generation Toxicity Test and alternative Extended One-Generation Reproduction Test* (EOGRT)
890.2100		Japanese Quail Two-Generation Toxicity Test
890.2200		Medaka Extended One-Generation Reproduction Test (MEOGRT)
890.2300		Larval Amphibian Growth and Development Assay (LAGDA)

http://www.epa.gov/ocspp/pubs/frs/home/testmeth.htm

**These guidelines are labelled as "public draft" as they are not yet available in final form. Although useful as a reference, please check with the appropriate office before using these draft guidelines to generate data for submission to the USEPA under the FIFRA; Federal Food, Drug, and Cosmetic Act; or Toxic Substances Control Act.

Since 2009, the USEPA has issued Tier 1 test orders for the initial List 1 chemicals (n=52) that included 50 pesticide active ingredients and 2 inert ingredients. Tier 1 data for List 1 chemicals have been submitted to the agency and are currently in the process of being reviewed using a weight of evidence approach. It is anticipated that once finalized, the weight of evidence analyses and data reviews will lead to determinations as to whether additional testing is warranted. For those chemicals determined through the weight of evidence analyses[98] to require additional testing, the USEPA may issue test orders for Tier 2 or other toxicological studies. Subsequent to List 1, the USEPA has finalized a second list (List 2) of 41 crop protection products and 66 commodity chemicals for EDSP Tier 1 screening based on the registration review schedule and potential exposure through a drinking water source, as stipulated under the 1996 Safe Drinking Water Act Amendment, section 1457.[99] The USEPA has indicated the second list of 107 EDSP chemicals is being prioritized for test order issuance using the Integrated Bioactivity and Exposure Ranking (IBER) methodology described below.

IBER: Computational Methods

Recognizing the current low throughput of the screening and testing of >10,000 chemicals for endocrine activity and adversity, the USEPA has been actively pursuing the application of computational toxicology and exposure methods to build increased efficiency in the

[98] United States Environmental Protection Agency. 2010. Weight of evidence guidance: evaluating results of EDSP tier 1 screening to identify candidate chemicals for tier 2 testing. http://www.epa.gov/endo

[99] United States Environmental Protection Agency. 2012. Agency information collection activities; proposed collection; comment request. EPA-HQ-OCSPP-2011-0966; FRL-9359-3.

screening program. Recent advances in the area of computational methods have initiated an "evolutionary turning point" for EDSP prioritization, screening and testing. However, before full incorporation of these new advanced tools, the agency must validate these tools and ensure they are "fit for purpose." To this end, several external, independent FIFRA SAP reviews have focused on application of these high-throughput (HTP) tools for prioritizing and screening the current universe of 10,341 EDSP chemicals.[100]

Previous SAP reviews in January 2013 and July and December 2014 have specifically focused on the HTP estrogen receptor assays, physical-chemical properties, HTP exposure predictions and HTP toxicokinetic (HTTK) methods to determine endocrine bioactivity and for chemicals with positive activity, to extrapolate *in vitro* doses to *in vivo* concentration for chemicals that have been run through a battery of HTP endocrine screening assays (e.g., ToxCast). HTTK provides a translational bridge between bioactivity measured in the HTP *in vitro* screening assays and exposure by predicting tissue concentrations from an administered dose or inferring administered doses that would be needed to cause tissue bioactive concentrations *in vivo*. In IBER, reverse toxicokinetics can be used to estimate the daily administered dose in mg/kg/bw necessary to produce a steady-state *in vivo* blood concentrations equivalent to concentrations showing biological activity in the *in vitro* HTP screening assays. The ultimate goal of using IBER is to more efficiently prioritize chemicals for Tier 1 screening based on biological activity and predicted exposure. An additional SAP is being planned to address the use of computational toxicology methods for predicting endocrine activity within the context of the Adverse Outcome Pathway framework.[101]

[100] United States Environmental Protection Agency. 2012. EDSP universe of chemicals inventory. www.epa.gov/endo.
[101] United States Environmental Protection Agency. 2014. Integrated bioactivity and exposure ranking: a computational approach for the prioritization and screening of chemicals in the Endocrine Disruptor Screening Program. FIFRA Scientific Advisory Panel. December 2-5, 2014. http://www.epa.gov/scipoly/sap.

Attachment 3: Relevant toxicology studies conducted with diethylstilbestrol (DES)

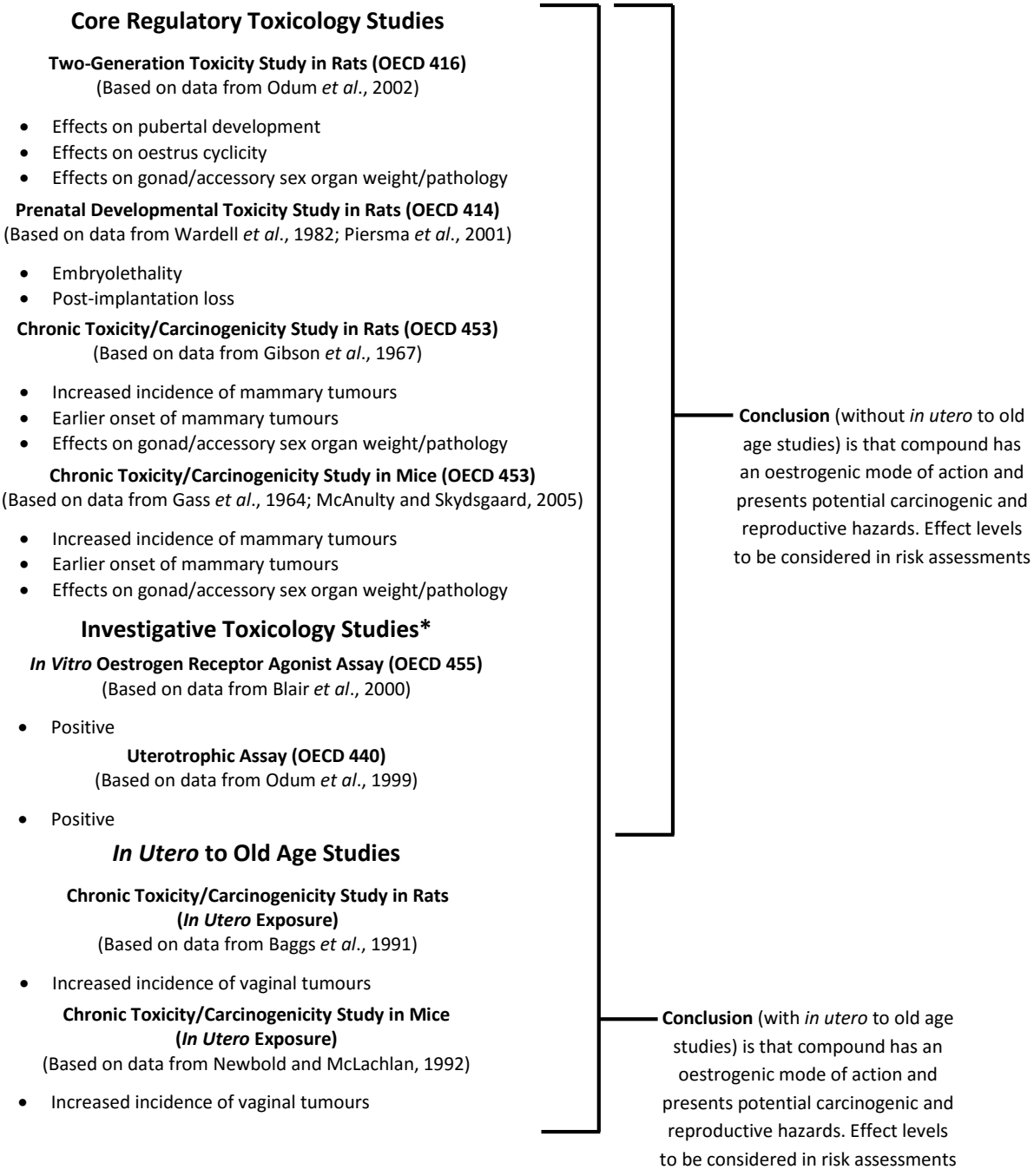

Figure 1. Summary of relevant toxicology studies conducted with diethylstilbestrol (DES)

* Investigative toxicology studies are those that are likely to have been initiated based on observations noted in the core regulatory toxicology assays.

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- Odum J, *et al*. 2002. Comparison of the developmental and reproductive toxicity of diethylstilbestrol administered to rats *in utero*, lactationally, preweaning, or postweaning. *Toxicol Sci.* 68:147-163.

- OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 414: Prenatal developmental toxicity study. http://www.oecd-ilibrary.org/environment/test-no-414-prenatal-development-toxicity-study_9789264070820-en.

- OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 416: Two-generation reproduction toxicology study. http://www.oecd-ilibrary.org/environment/test-no-416-two-generation-reproduction-toxicity_9789264070868-en.

- OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 440 : Uterotrophic assay. http://www.oecd-ilibrary.org/environment/test-no-440-uterotrophic-bioassay-in-rodents_9789264067417-en;jsessionid=e6uco392bhvc.x-oecd-live-02.

- OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 453: Combined chronic toxicity/carcinogenicity studies. http://www.oecd-ilibrary.org/environment/test-no-453-combined-chronic-toxicity-carcinogenicity-studies_9789264071223-en.

- OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 455: The stably transfected human estrogen receptor-alpha transcriptional activation assay for detection of estrogenic agonist-activity of chemicals. http://www.oecd-ilibrary.org/environment/test-no-455-the-stably-transfected-human-estrogen-receptor-alpha-transcriptional-activation-assay-for-detection-of-estrogenic-agonist-activity-of-chemicals_9789264076372-en.

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