TOX/2010/15

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

REGULATORY DEFINITION OF ENDOCRINE DISRUPTERS

Background

1. The prompt for this work was the introduction into the new European Plant Protection Products (PPP) Regulation (1107/2009) of an exclusion criterion for approval which explicitly indicates that any active substance, safener and synergist with endocrine disrupting properties that may cause adverse effects in humans cannot be approved for marketing and use unless the exposure of humans under realistic proposed conditions of use is negligible (see Appendix 1). Therefore, the consequence of identification of a substance as an endocrine disrupter (ED) is of great importance given the potential regulatory and commercial impact.

2. A similar approval exclusion criterion has been introduced in the proposed new EU “Biocidal Products Regulation”.

3. Substances with endocrine disrupting properties are also targeted within the REACH Regulation (1907/2006). Identification of substances as EDs may lead to their inclusion in the list of substances subject to the Authorisation requirements of REACH (see Appendix 1).

4. Despite these stipulations, at the present time there is no definition and/or set of criteria within these pieces of legislation, by which to identify EDs. The aim of this paper is to propose a definition and associated interpretative criteria that can be applied in a regulatory context.

5. In this document, the focus is on human health considerations only.

Argument

6. A number of definitions for EDs have been proposed (Kavlock, 1996; NRDC, 1998; Weybridge, 1996, WHO/IPCS, 2002 – see Appendix 2). Some of these definitions (e.g. Kavlock, 1996; NRDC, 1998) are ambiguous and, for regulatory purposes, are overly inclusive, in that they fail to discriminate between alterations of the endocrine system which fall within the physiological balance/homeostatic capabilities of the body, and adverse effects that disturb an organism’s endocrine system to an extent beyond that compatible with normal function. This has led to the development of more restrictive definitions (e.g. Weybridge, 1996, WHO/IPCS, 2002) that readily account for the fact that many alterations of the endocrine system can be regarded as adaptive, falling within a range for which compensation can occur readily, and which pose no threat to the normal functioning of the organism.

7. Still, even the more restrictive definitions remain quite general, which is acceptable as a working scientific definition for EDs but requires further development and elaboration for regulatory use and application.

8. The widely accepted scientific definition of ED by WHO/IPCS is proposed as a starting point for characterising an ED for regulatory purposes. This is a well-established and widely recognised definition produced by a global, authoritative organisation through a world-wide initiative of highly scientific rigour (WHO/IPCS, 2002). In addition, it is supported by a number of organisations and regulatory bodies around the world, including the US EPA, the Canadian Centre for Occupational Health and Safety (CCHOS) and the International Union of Pure and Applied Chemistry (IUPAC).

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations."

9. This definition embodies some key elements on which one can build criteria for identifying an ED of regulatory importance, as discussed below.

10. The key element in this definition is "adversity via endocrine perturbation". By definition, an endocrine disruptor perturbs the normal endocrine homeostasis, for instance, by changing the circulating levels of a particular hormone. However, such perturbation *in itself* is not considered to be an adverse effect, as the endocrine system is naturally dynamic and responsive to various stimuli as part of its normal functioning. In this context, endocrine perturbation is considered as a mechanism of action, potentially on a pathway to other outcomes, rather than a toxicological endpoint in itself. Crucially for regulatory purposes, any endocrine perturbation must result in adverse effects, such as pathology or functional impairment. This approach is entirely consistent with other areas of regulatory assessment. For instance, in hazard identification for classification and labelling purposes, chemicals are only classified where there is a clear induction of adverse effects; they are never classified simply because the substance acts via a particular mode of action known to have the potential to lead to an adverse effect; the effect must be demonstrated to occur.

11. Another important element in this definition is that the ED effect must be observed in an intact organism. This reinforces the requirement that an ED-mediated "whole-animal" adverse effect must be observed, rather than simply inferred from results obtained in a simpler test system designed to explore the possibility that a substance can express a property relating to potential endocrine disruption. For example, observations from screening tests in ovariectomised or castrated animals cannot be taken as evidence of real adverse effects as the integrity of the physiological homeostasis of the whole organism has been altered to maximise the test objective.

12. The WHO/IPCS definition is still a very broad description which does not have the power to discriminate between substances meriting genuine concern for their ability to disrupt the endocrine system and substances that merit little concern in relation to any endocrine-disrupting ability and for which regulatory action is not

justified or is of a low priority. Therefore, our aim is to use the WHO definition as the starting point to arrive at a regulatory definition of an ED by adding a number of criteria that need to be satisfied before an ED requiring regulatory action can be identified.

13. A tiered evaluation scheme to identify EDs of regulatory importance is described below (Figure 1). Appendix 4 contains examples of applying the scheme in two case studies. The scheme builds upon the WHO/IPCS definition but extends it with consideration of parameters of relevance from a regulatory perspective.

Relevant routes of exposure and acceptable studies

14. Endocrine disrupters can be identified in standard toxicology tests that are routinely performed to fulfil the requirements of various regulatory programmes. In particular, ED-mediated toxicity can be detected in repeated-dose, reproductive toxicity, and carcinogenicity studies, although more focussed ad-hoc studies, such as mechanistic studies, may be necessary to confirm unequivocally that the observed effects are due to ED. Given the wide ranging functions of the endocrine system, ED-mediated adverse effects could manifest in various organs and tissues and in different ways. Expert judgement is generally required to judge the toxicological significance of such changes.

15. The criteria for acceptability of any such studies follow general principles. The study must be conducted to an acceptable protocol and to good standards and be well reported. The studies should have used relevant routes of exposure (oral, dermal or inhalation). Studies using parenteral routes are not generally appropriate and should not override results from well performed studies using a more relevant route of exposure.

16. Additional *in vitro* mechanistic studies may provide extremely valuable information which sheds light on the specific mechanism of a substance, e.g. demonstrating binding to the androgen receptor. These should be evaluated on their merits on a case by case basis. However, it is noted that such studies demonstrate mechanism/mode of action and do not, on their own, provide conclusive proof of ED-induced adverse effects in an intact organism.

Most sensitive/lead effect: are the potential ED effects seen at doses in the same range or below dose levels producing other substantial toxic effects?

17. The most sensitive/lead toxic effect of a substance, that is a clear and significant toxic effect that occurs at a dose/exposure level lower than those producing other manifestations of toxicity, will generally drive the risk assessment and be used to determine appropriate risk mitigation measures. [The exception is genotoxicity leading to mutation and/or cancer, which might only be *observed* at higher doses but for which the threat, although not observable, might well persist at much lower doses; this concern drives risk management thinking]. It necessarily follows that risk management measures based on the lead toxic effect will also be intended to protect against any other toxic effects occurring at higher doses/exposure levels. Therefore, in the context of controlling the threat posed by a

substance, the most sensitive/lead effect is the manifestation of toxicity that is of most importance/relevance for regulatory purposes.

18. Hence it is proposed that a substance will be considered further, in relation to being an ED for regulatory purposes, where the (potential) ED-induced effect(s) are the lead toxic effect(s), seen at doses in the same range or below dose levels producing other substantial/major toxic effects. Where the potential ED-induced effects are not the lead toxic effect but are seen at dose levels significantly higher than those causing other toxic effects, the substance is not an ED of regulatory concern.

Relevant dose levels/potency considerations

19. In general terms toxic effects are only of regulatory relevance when they occur at dose/exposure levels that have some relevance to potential human contact with substances in general. If toxicity only occurs at excessively high dose/exposure levels then the toxicity is not realistically relevant to humans and is not used to drive regulatory action. This concept is applied in various regulatory approaches, such as in hazard classification and labelling where there are "cut-offs" (maximum upper limits) for classification for a number of toxicity endpoints (e.g. acute and repeated-dose toxicity); any effects occurring only at dose levels above these cut-off limits are not considered to be relevant to humans and so do not attract classification.

20. As for any other type of toxicity, where the (potential) ED-related effects are the lead toxic effect, the dose/exposure level causing those effects must be considered to determine if the effects occur at a relevant/reasonable dose level (as described above). It is proposed that the relevance of the dose level causing ED effects should be judged using the same well established approach used for classification.

[Note: This approach is based around the identification of overt toxicity, usually in standard regulatory tests conducted at relatively high doses. There are claims that at least some EDs show non-monotonic dose-response curves. Advocates suggest that EDs might cause effects at very low dose levels, in a manner that would not be detected by current testing approaches (Welshons et al., 2003). The effects may be of such a low magnitude that standard tests do not have the power to detect them, or the effects may be of a type that will not be detected by standard observations (e.g. epigenetic changes). At the moment this is still an area of research; it is surrounded by much controversy and inconsistency in reported findings (Ashby, 2003). It is premature to introduce these ideas into a regulatory approach. Further developments in this field will be monitored and the approach described in this paper should be modified if the balance of scientific opinion merits this].

21. The European Classification, Labelling and Packaging (CLP) Regulations, which implement the Globally Harmonised System for classification and labelling of chemicals (GHS), contains discriminatory dose thresholds for use in determining whether or not a wide range of expressions of toxicity seen in single and repeated exposure studies, collectively termed "Specific Target Organ Toxicity (STOT)", should be identified by hazard classification and be assigned appropriate labelling (this concept was also used in the predecessor to CLP, the Dangerous Substances Directive). It is proposed that these same dose thresholds (those for STOT Repeated Exposure-RE Category 2; the lowest category of classification for STOT,

capturing those substances of relatively lower potency) should be used to determine whether or not the hazardous property of “endocrine disruption” should be identified for regulatory purposes.

22. The guidance values (“cut-offs") for Category 2 STOT-RE are defined in CLP and GHS as:

For sub-acute and other short-term studies (e.g. dev.tox. studies):

Oral:	300 mg/kg bw/day
Dermal:	600 mg/kg bw/day
Inhalation (vapour):	3 mg/l/6h/day
Inhalation (dust/mist/fume):	0.6 mg/l/6h/day

For subchronic and other medium-term studies (e.g. 2-generation studies):

Oral:	100 mg/kg bw/day
Dermal:	200 mg/kg bw/day
Inhalation (vapour):	1 mg/l/6h/day
Inhalation (dust/mist/fume):	0.2 mg/l/6h/day

23. There are no guidance values in the CLP Regulations for chronic studies, but it is proposed here that they should be half the subchronic study values (by applying the subchronic to chronic extrapolation assessment factor of 2 recommended in the REACH guidance on information requirements and chemical safety assessment, chapter R8), ie:

Oral:	50 mg/kg bw/day
Dermal:	100 mg/kg bw/day
Inhalation (vapour):	0.5 mg/l/6h/day
Inhalation (dust/mist/fume):	0.1 mg/l/6h/day

24. These values are pragmatic but have been in place within the framework of the regulatory hazard classification system in Europe since 1967 and are well established and accepted. They are also widely accepted at a global level through GHS. Therefore, these guidance values are considered to be appropriate discriminatory values to identify those hazards for which a regulatory warning should be given.

25. It is proposed that, for an adverse effect potentially related to endocrine disruption, the effective dose at which a 10% change relative to controls is observed or would be predicted to occur (the “ed_{10}”) is derived and used as the dose-metric to be compared with the guidance values presented above. The “ed_{10}” should be estimated by linear interpolation (or extrapolation, as appropriate) of the lowest dose level causing a statistically or toxicologically significant effect. As a fixed level of effect, the “ed_{10}“ is considered a more representative parameter of the potency of the substance then the LOAEL, which is a chosen dose level affected by the dose spacing of the study. Then, only where the “ed_{10}” is at or below the discriminatory guidance dose levels for the application of Category 2 “STOT-RE” hazard

classification, should the substance remain under consideration as a potential ED for regulatory purposes.

Mode-of-action link to endocrine disruptive activity

26. In order to conclude that a substance is an ED there must be clear and robust evidence of a mechanistic link between the induced endocrine perturbation/activity and the adverse effects seen in the intact organism studies. Such evidence usually comes from a combination of findings from standard toxicity tests, which identify the adverse effect, and mechanistic studies, which provide supporting evidence. Such a mechanistic link could be established using information from the *in vitro* and *in vivo* screening assays (level 2 to 4) of the OECD conceptual framework for testing and assessment of EDs (Appendix 3) or from more ad-hoc studies. The OECD ED *in vitro* screening assays are capable of identifying binding to the oestrogen or androgen receptor, alterations in the synthesis of sex steroid hormones and inhibition of aromatase (the enzyme responsible for the conversion of androgens to oestrogens). The OECD ED *in vivo* assays can detect oestrogenic, androgenic, anti-androgenic and anti-thyroid activity and alterations in pubertal development via changes in gonadotrophins, prolactin or hypothalamic function. These screening assays are likely to provide varying degrees of evidence of endocrine disrupting activity of the substance which may explain the occurrence of the original adverse effects seen in the intact organism studies; they are less likely to provide a full sequence of biochemical and cellular events leading to the adverse effects, i.e. the mechanism of action of the substance. Therefore, where a more robust mechanistic link is sought, other, more specific/targeted investigations may be required to show this.

27. In relation to establishing that an endocrine-disrupting process applies to a particular toxic effect it is proposed that the IPCS mode of action framework (Boobis et al., 2008) is used to carry out a weight of evidence evaluation of the available information to reach a transparent and robust conclusion. Where a definitive conclusion cannot be reached then it cannot be concluded that the substance is an ED for regulatory purposes. The evaluation should have highlighted where additional studies may help provide the necessary clarification. The substance should proceed to the risk assessment stage of the evaluation and subsequent risk management process.

28. Where ED screening assays or other ad-hoc mechanistic studies are not available, then they should be requested. Under the PPPR, these additional investigations can be required by the regulatory authority performing the evaluation of the substance. Under REACH, additional studies can be requested within the context of “Substance Evaluation” by the Member State assessing the substance. A proposal for a testing approach is presented in Figure 2.

Relevance to humans

29. The adverse endocrine effects seen in intact animals must be relevant to humans for regulatory action to be justified.

30. It is proposed that the IPCS human relevance framework (Boobis et al., 2008) is used to analyse the available evidence to make sure that a robust and transparent conclusion is reached. If no information on the potential human relevance of the effects seen in animals is available, it must be assumed that the animal findings might be relevant to humans.

31. It is noted that even when effects are not relevant to humans, they could still be relevant to non-target species in the environment. This is of potential value to determining whether or not a substance merits consideration as an ED in relation to potential effects on other environmental species, but is outside of the scope of this paper.

Conclusion and proposal

32. In relation to potential human health concerns, it is proposed that a substance is regarded as an ED for regulatory purposes when it satisfies the following definition and associated criteria:

> It should be an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations.

33. And in doing so satisfies the following criteria (each of which is expanded on in the paper above):

- adverse effects to have been seen in one or more standard toxicity studies in which the substance was administered by a route relevant for human exposure.
- the adverse effect(s) believed to be related to endocrine disruption to be the lead toxic effect(s) in the study; or occurring at a dose level close to that at which the lead toxic effect was first seen.
- the adverse effect(s) believed to be related to endocrine disruption to have been produced at a dose at or below the relevant guidance value for the application of Category 2 “Specific Target Organ Toxicity-Repeated Exposure, STOT-RE” classification & labelling.
- a mode-of-action link between the toxic effects of concern and endocrine disruption to have been established.
- the effects seen in experimental animals to be judged to be of potential relevance to human health

Questions on which the views of the Committee are sought

34. The Committee is asked to discuss and agree the proposed definition and criteria.

HPA Secretariat
May 2010

References

Ashby, J (2003). Problems associated with the recognition and confirmation of low-dose endocrine toxicities. *Nonlinearity Biol Toxicol Med* 1(4): 439-453.

Boobis, AR, Doe, JE, Heinrich-Hirsch, B et al (2008). IPCS framework for analysing the relevance of a noncancer mode of action for humans. *Crit Rev Toxicol* 38(2), 87-96.

Kavlock, RJ, Daston GP, DeRosa, C et al (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA-sponsored workshop. *Enviro Health Perspect*. 104(suppl 4): 715-740.

National Resources Defence Council (1998). Endocrine disruptors. http://www.nrdc.org/health/effects/qendoc.asp

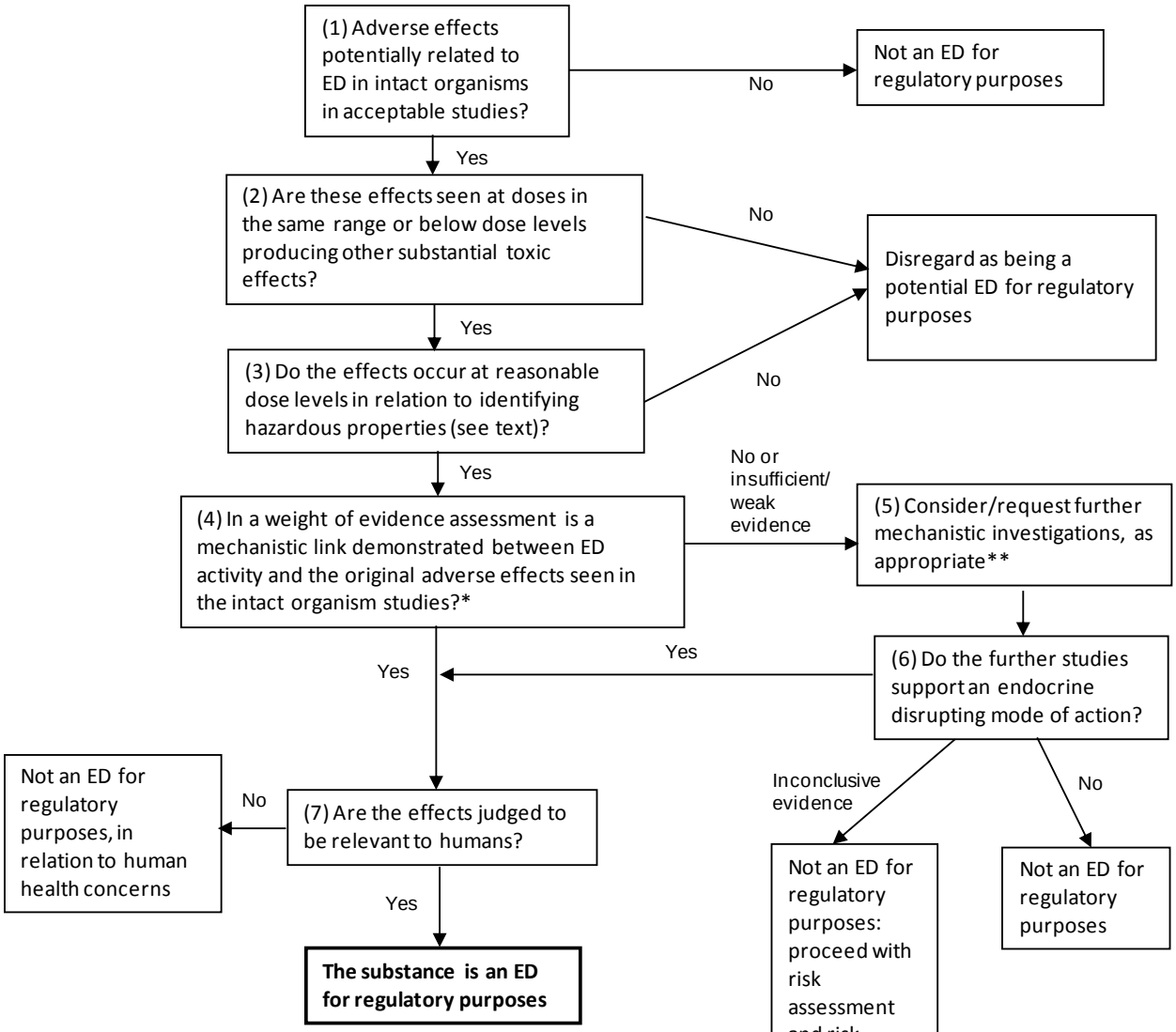
OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals.
http://www.oecd.org/document/58/0,3343,en_2649_34377_2348794_1_1_1_1,00.html

REACH Guidance on information requirements and chemical safety assessment, chapter R8.
http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r8_en.pdf?vers=20_08_08

Welshons, WV, Thayer, KA, Judy, BM, Taylor, JA, Curran, EM, and vom Saal, FS (2003). Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Enviro Health Perspect*. 111(8): 994-1004.

Weybridge (1996). European Workshop on the impact of endocrine disrupters on human health and wildlife. Report of Proceedings. EUR 17549.

WHO/IPCS (2002). Global assessment of the state-of-the-science of endocrine disruptors. Geneva, World Health Organisation.



*For instance using information from OECD Levels 2 - 4 *in vitro* and *in vivo* screening assays or ad-hoc studies.
**possibly including non-ED investigations to demonstrate alternative modes of action

Figure 1 – Flow diagram to determine whether or not a substance is an ED for regulatory purposes

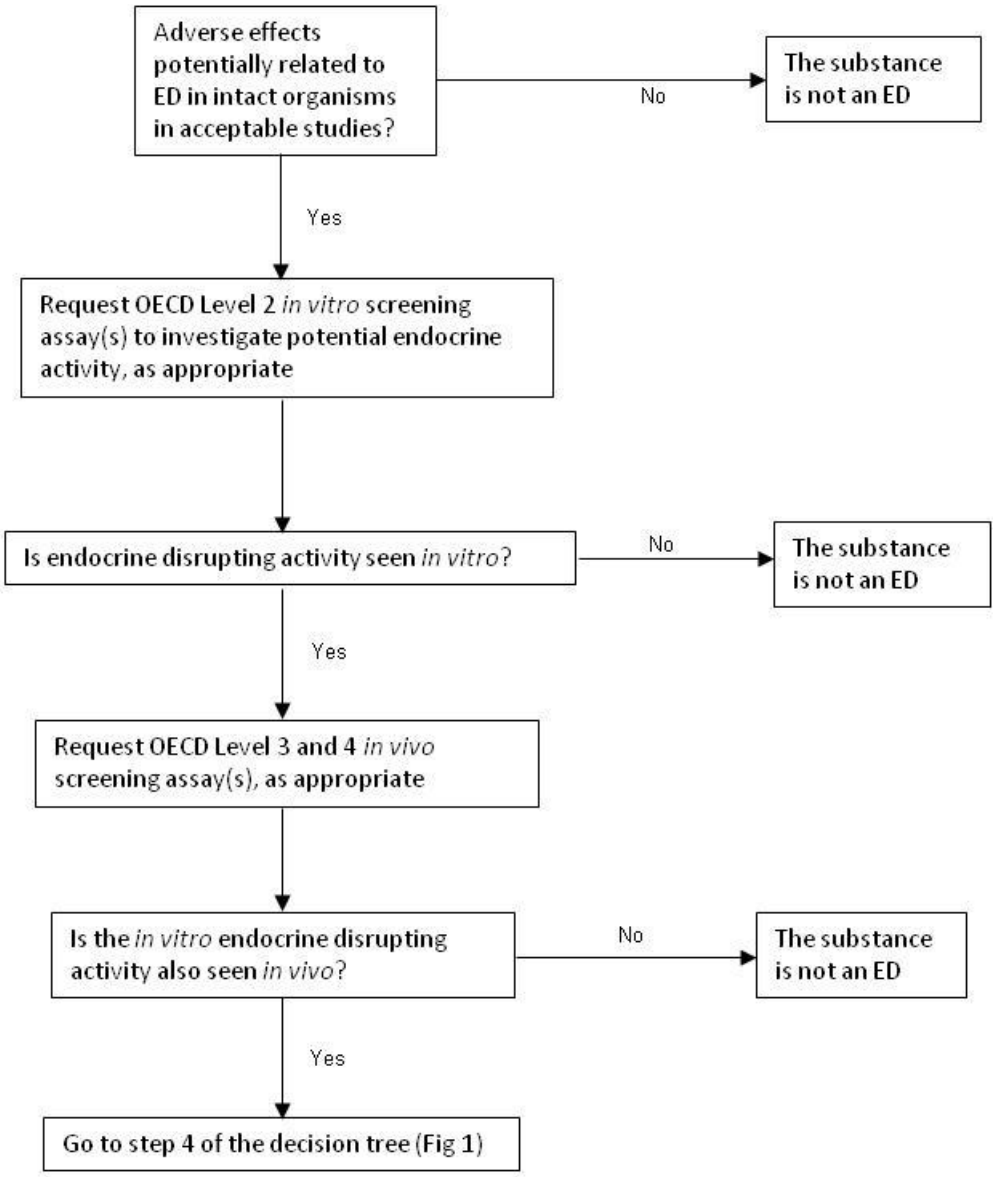


Figure 2 – Testing approach for the assessment of EDs

Regulation 1107/2009 for placing plant protection products on the market – substance approval criteria

Human health

3.6.5 An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, i.e. the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005.

Environment

3.8.2 An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible.

REACH (Regulation 1907/2006) – substances to be included in Annex XIV (substances subject to Authorisation)

Article 57 (f) : substances – such as those having endocrine disrupting properties or those having . . . . . - for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those other substances listed in points (a) to (e) and which are identified on a case-by-case basis in accordance with the procedure set out in Article 59

[points (a) to (e) cover category 1 and 2 carcinogens, mutagens, and/or substances toxic to reproduction; and/or (very) persistent, (very) bioaccumulative, toxic (PBT or vPvB) substances]

Definitions of EDs

Kavlock, 1996:

"An ED is an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes."

NRDC, 1998:

"EDs are synthetic chemicals that when absorbed into the body either mimic or block hormones and disrupt the body's normal functions through altering hormone levels, halting or stimulating the production of hormones, or changing the way hormones travel through the body."

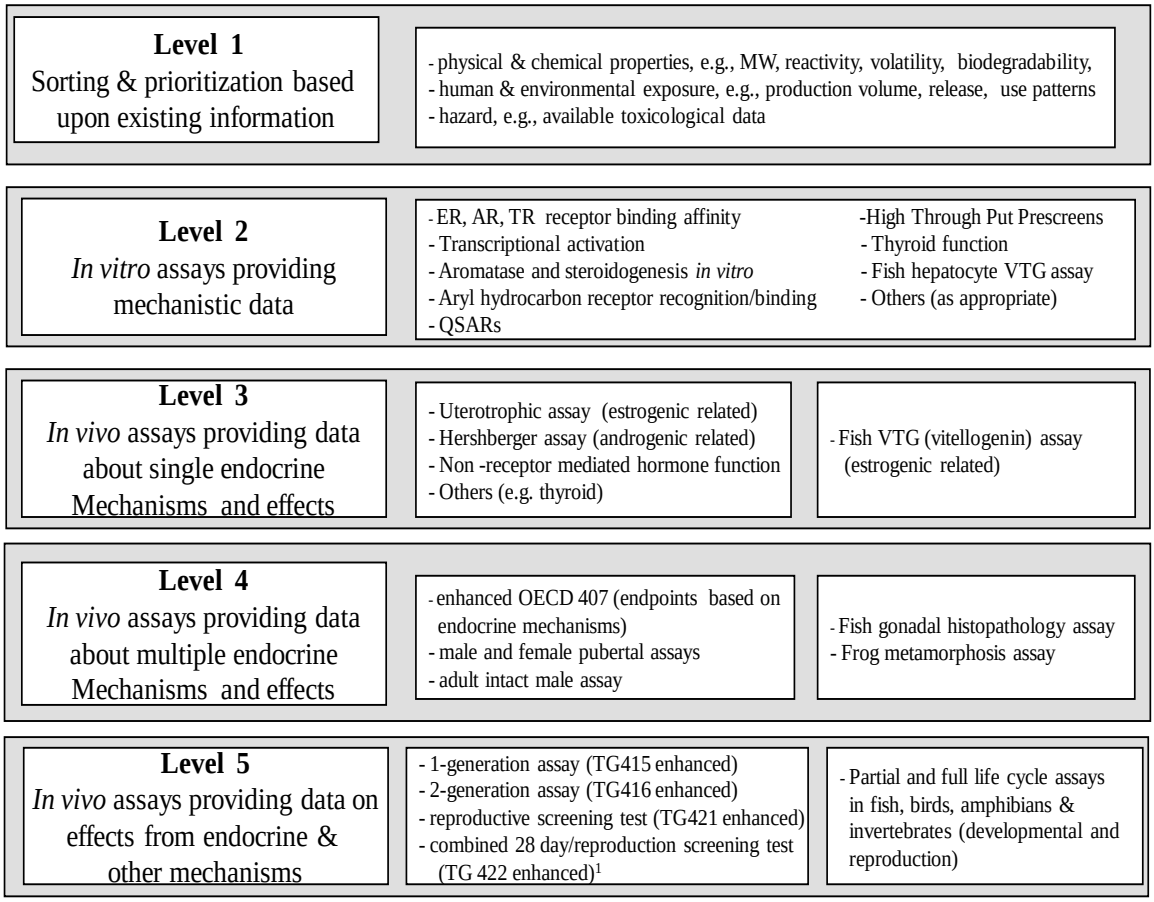
Weybridge, 1996:

"An ED is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function. A potential ED is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism."

WHO/IPCS, 2002:

"An ED is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations."

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals



Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

Notes to the Framework

Note 1: Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information needs for hazard and risk assessment purposes

Note 2: In level 5,ecotoxicology should include endpoints that indicate mechanisms of adverse effects, and potential population damage

Note 3: When a multimodal model covers several of the single endpoint assays, that model would replace the use of those single endpoint assays

Note 4: The assessment of each chemical should be based on a case by case basis, taking into account all available information, bearing in mind the function of the framework levels.

Note 5: The framework should not be considered as all inclusive at the present time. At levels 3,4 and 5 it includes assays that are either available or for which validation is under way. With respect to the latter, these are provisionally included. Once developed and validated, they will be formally added to the framework.

Note 6: Level 5 should not be considered as including definitive tests only. Tests included at that level are considered to contribute to general hazard and risk assessment.

ENDOCRINE DISRUPTER CASE STUDIES

Vinclozolin

Application of the decision tree (Fig 1)

(1) Adverse effects potentially related to ED in intact organisms in acceptable studies?

Vinclozolin causes Leydig cell tumours and atrophy of the accessory sex glands (including prostate and seminal vesicles) in adult rodents and and malformations in the male urogenital tract (including hypospadias, cleft phallus) and feminisation (nipple development, decreased ano-genital distance) of male rats, and benign and malignant uterine and ovary tumours in female rats. It is an androgen-receptor antagonist. These effects may be caused as a consequence of endocrine disruption. The mode of action analysis concentrates on the reproductive tract malformations – given the clear conclusion reached on endocrine disruption there is no need to consider the carcinogenicity in detail.

Possible ED effect

(2) Most sensitive/lead effect: are these effects seen at doses in the same range or below dose levels producing other substantial toxic effects?

Vinclozolin is of extremely low acute toxicity (oral LD_{50} > 5000 mg/kg). In repeated-dose studies the lead effects are those related to ED activity. There are no other significant toxic effects occurring at doses lower than those causing ED effects.

In a carcinogenicity study, the increased incidence of Leydig cell tumours and atrophy of the accessory sex glands was evident from 500 ppm and uterine and ovary tumours were only evident at the highest dose tested of 3000 ppm. A NOAEL of 50 ppm (around 2.7 mg/kg/day; the lowest dose tested) was determined for carcinogenicity.

The potential ED effects are the most sensitive effects

(3) Relevant dose levels/potency considerations: do the effects occur at reasonable dose levels in relation to identifying hazardous properties?

In a developmental toxicity study vinclozolin caused a 100% incidence of hypospadias in male pups from dams exposed to 100 mg/kg (the lowest dose tested; Gray *et al.*, 1994). The "ed_{10}" for this effect is 10 mg/kg. Another study by the same group investigated lower dose levels and showed an increase (40-45%) in hypospadias at 50 mg/kg, with no increase at 12.5 mg/kg (Gray *et al*. 1999). In this study there was also other evidence of ED-related effects, including dose-related increases in the incidence of retained nipples (0, 1, 2.6, 3.6, 5.4, 91 and 100% at 0,

3.125, 6.25, 12.5, 25, 50 and 100 mg/kg, respectively) and decreased ano-genital distance (100, 93.0, 95.8, 90.9, 85.3, 75.1, 61.7% of control at 0, 3.125, 6.25, 12.5, 25, 50 and 100 mg/kg, respectively). The "ed_{10}" for all of these effects was around 10 mg/kg.

In male pups dosed with vinclozolin from weaning (days 22 through 56) there was evidence of anti-androgenic effects, evidenced as increased serum luteinizing hormone, from 10 mg/kg (the lowest dose tested), although it was noted that in control animals this parameter fluctuated significantly over the developmental period covered in the study (Monosson *et al.*, 1999). The "ed_{10}" for this effect was around 1 mg/kg/day.

It is clear that the "ed_{10}" values for these ED-related effects are significantly below the CLP Regulation STOT RE Category 2 guidance value of 300 mg/kg/day for oral sub-acute exposure. Therefore, the potential ED effects of vinclozolin occur at reasonable dose levels.

The potential ED effects occur at relevant dose levels

(4) In a weight of evidence assessment is a mechanistic link demonstrated between ED activity and the original adverse effects seen in the intact organism studies?

Vinclozolin and its active metabolites, M1 and M2, bind competitively to human and rat androgen receptor (AR). M2 is the more potent, and is only 2 fold less potent than the pharmacological inhibitor of the AR, hydroxyflutamide. Additionally, M2 inhibits the binding of androgen-bound AR to the androgen response element.

Vinclozolin caused changes in androgen-dependent gene expression in the prostate and caused a decrease in AR bound to DNA, indicating anti-androgenic activity.

The evidence that the reproductive toxicity findings are due to endocrine disruption is strong. There is clear evidence that vinclozolin and its metabolites are AR antagonists and this affects the androgen-dependent gene expression. The pattern of reproductive toxicity findings is consistent with perturbation of androgen dependent development. In support of this the findings with vinclozolin are similar to those found with known AR antagonists such as flutamide.

The observed reproductive findings are highly consistent with vinclozolin acting as an AR antagonist

(5) *Consider further mechanistic investigations, as appropriate*

There is sufficient evidence available to allow a robust conclusion on vinclozolin.

No further testing is necessary

(6) Are the effects judged to be relevant to humans?

The toxico-kinetics and –dynamics of vinclozolin are expected to be similar in rats and humans.

The effects of vinclozolin in rats are considered to be relevant for humans

VINCLOZOLIN IS AN ED FOR REGULATORY PURPOSES, IN RELATION TO HUMAN HEALTH CONCERNS

References

Gray, L.E., Ostby, J.S. and Kelce, W.R. (1994) Developmental effects of an environmental antiandrogen. The fungicide vinclozolin alters sex differentiation of the male rat. *Tox.Appl.Pharm.*, 129: 46-52.

Gray, LE, Ostby, J., Monosson, E. and Kelce, W.R. (1999) Environmental antiandrogens - low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. *Tox.Ind.Health,* 15: 48-64.

IPCS Review of vinclozolin (www.inchem.org/documents/jmpr/jmpmono/v95pr18.htm).

Kavlock, R. and Cummings, A. (2005) Mode of action: Inhibition of androgen receptor function – Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, 35: 721-726.

Monosson, E., Kelce, W.R., Lambright, C., Ostby, J. and Gray, L.E. (1999) Peripubertal exposure to the antiandrogenic fungicide, vinclozolin, delays puberty, inhibits the development of androgen-dependent tissues, and alters androgen receptor function in the male rat. *Tox.Ind.Health*, 15, 65-79.

ENDOCRINE DISRUPTER CASE STUDIES

1,3-dinitrobenzene (1,3-DNB)

Application of the decision tree (Fig 1)

(1) Adverse effects potentially related to ED in intact organisms in acceptable studies?

1,3-DNB causes testicular toxicity in the rat. In 8-, 12- and 16-week repeated dose studies, testicular atrophy, decreased spermatogenesis, degeneration of the germinal epithelium and degeneration of Sertoli cells were observed. Similar effects were not seen in the mouse. These effects may be caused as a consequence of endocrine disruption.

Possible ED

(2) Most sensitive/lead effect: are these effects seen at doses in the same range or below dose levels producing other substantial toxic effects?

Depending on the duration of exposure, testicular toxicity starts to emerge from dose levels ranging from 1.5 to 4.7 mg/kg bw/day (Linder et al., 1986; Cody et al., 1981). Other toxic effects (haematological effects and decreases in body weight) occur at doses in the same dose range. Therefore, the potential ED effects of 1,3-DNB are considered to be among the most sensitive effects of 1,3-DNB.

The potential ED effects are among the most sensitive effects

(3) Relevant dose levels/potency considerations: do the effects occur at reasonable dose levels in relation to identifying hazardous properties?

A 61% decrease in testes weight (the most sensitive effect) was seen at 2.64 mg/kg bw/day in the 16-week repeated dose study (Cody et al., 1981). The "ed_{10}" for this effect is 0.43 mg/kg bw/day which is significantly below the CLP Regulation STOT-RE Category 2 guidance value of 100 mg/kg bw/day (oral, subchronic exposure). Therefore, the potential ED effects of 1,3-DNB occur at reasonable dose levels.

The potential ED effects occur at relevant dose levels

(4) In a weight of evidence assessment is a mechanistic link demonstrated between ED activity and the original adverse effects seen in the intact organism studies?

In the *in vitro* steroidogenesis assay, 1,3-DNB has no effects on testosterone secretion. Other ED screening assays are not available for 1,3-DNB; however, QSAR models predict that 1,3-DNB has no androgen or oestrogen receptor binding affinity. In the rat, no changes in serum LH, FSH and prolactin levels were found at dose levels similar to those at which testicular toxicity occurred, indicating that 1,3-

DNB exerts a direct effect on the testes and not through alterations in hypothalamic and pituitary control of gonadal function. The testicular effects occur at the same dose levels at which haematotoxicity is seen. It is possible that the observed testicular damage may be related to tissue hypoxia, which results from impaired oxygen transport as a consequence of methemoglobinemia. Overall, although the mechanism of action for the testicular toxicity of 1,3-DNB has not been elucidated, the weight of evidence suggests that it is not due to ED activity.

There is sufficient evidence of no mechanistic link between the observed adverse effects and ED activity in the rat. 1,3-DNB is not an ED for regulatory purposes

(5, 6) Consider further mechanistic investigations, as appropriate: do the further studies support an endocrine disrupting mode of action?

There is sufficient evidence available to allow a robust conclusion to be drawn.

No further testing is necessary

1,3-DNB IS NOT AN ED FOR REGULATORY PURPOSES

References

Cody et al (1981). 1,3-dinitrobenzene: toxic effects in vivo and in vitro. J Toxicol Environ Health, 7(5): 829-847.

Linder et al (1986). Testicular toxicity and infertility in male rats treated with 1,3-dinitrobenzene. J Toxicol Environ Health 19: 477-489.