

## **Criteria for the determination of endocrine disrupting properties.**

### **0. Introduction**

By the early 1990's, it was established that the traditional toxicological testing protocols used to determine chemical safety had completely missed vast numbers of chemicals that penetrate the womb and interfere with the construction and programming of developing animals, including humans. Since then, more evidence has accumulated indicating that the presence of infinitesimally small quantities of certain chemicals during the continuously changing stages of development can alter one's inherited phenotype e.g., the ability to learn, love, bond reproduce and function even to maintain normal body weight. The endocrine system is so fine tuned that it depends upon changes in hormones in quantities as little as a tenth of a trillion of a gram to control the womb environment.

Recent advances in research confirm that endocrine disruptors can interfere with the gene-controlled, normal signalling systems that determine every aspect of embryonic and foetal development. Over the past decade it has been demonstrated that there are endless ways endocrine disruptors can interfere with gene expression. They can interfere with how genes are programmed in the developing tissues of the unborn, thus changing how a teen or an adult would ordinarily respond to the normal chemical signals that control function as they mature. Disorders that have increased in prevalence in recent years such as abnormal male gonadal development, infertility, ADHD, autism, intellectual impairment, diabetes, thyroid disorders, and childhood and/or adult cancers are now being linked to foetal exposure. Despite the accumulated research chemicals are still not regulated due to their endocrine disrupting properties. EU Regulation 1107/2009 provides for the elements to do so.

In Regulation 1107/2009 endocrine disrupting properties are seen as unwanted properties and pesticides having these properties will not be allowed on the market (unless in exceptional cases such as use in closed systems). The criteria for determining such properties still need to be defined. By 14<sup>th</sup> December 2013, the Commission shall present measures concerning specific scientific criteria for the determination of endocrine disrupting (ED) properties (Regulation 1107/2009). These criteria will likely be used in the implementation of other regulations, and as such will cover 'horizontal' legislation, including that relating to cosmetics, biocides, pharmaceuticals, and industrial chemicals at large. While December 2013 is still somewhat in the future,

discussions have already started and several stakeholders and some EU Member States have already put forward their ideas on the criteria. In this paper environmental NGO's present their proposals and conditions for setting these criteria.

## **1. Legal procedure for controlling chemicals with ED properties.**

We foresee a 3 step procedure for regulating chemicals with endocrine disrupting properties:

- Ensure that the toxicity data generated are sufficient to identify chemicals with endocrine disrupting properties.
- Set out criteria to apply to the toxicity data to identify which chemicals are considered to have endocrine disrupting properties.
- Regulate the chemical with endocrine disrupting properties according to the relevant legislation in force (e.g. the 'cut-off' regime of the Pesticide Regulation<sup>1</sup>, or, for industrial chemicals, the authorisation regime of REACH<sup>2</sup> (or the restrictions regime).

## **2. Abandoning traditional risk assessment.**

There can be no doubt that EU policy makers, Council and Parliament, have chosen and agreed on a hazard, not a risk-based, approach for endocrine disrupting properties in Regulation 1107/2009. Specific inherent effects of pesticides (carcinogenic, mutagenic, reprotoxic), specific chemical qualities (persistence, bioaccumulation) and endocrine disrupting properties (apart from specific cases such as closed systems) are identified as sufficient to prevent chemicals with such properties from entering the European market. Also under REACH, regulation of CMR chemicals is based on hazard.

This new element of hazard approach -thereby disregarding the traditional practice of using risk assessment methodologies- needs to be emphasized from the beginning in the development of the criteria. Given the past situation, where risk assessment and the prevailing culture of ways of evaluating chemicals led to inaction and did not provide an adequate level of protection, there was political motivation to move to a more hazard based approach, and this was reflected in the agreed final text of the pesticide legislation. Therefore, it must be made very clear from the start that any discussion of for instance 'thresholds of no concern', "relevance for humans' and 'acceptable exposure levels' is unwanted and unacceptable. A chemical hazard approach differs utterly from traditional risk assessment of chemicals.

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<sup>1</sup> 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, that is, 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.

<sup>2</sup> By the criteria listed in Art. 57(f) of REACH, substances displaying endocrine disrupting properties explicitly qualify for authorisation, as follows:

"...substances such as those having endocrine disrupting properties - 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 of other substances listed in points (a) to (e) and which are identified on a case-by-case basis..."

### **3. A discussion on a definition of endocrine disruptors is futile.**

The European Commission must put forward a proposal for “criteria for endocrine disrupting properties”, not for an “endocrine disruptor”. This means a discussion about a definition of an “endocrine disrupting chemical” is useless because a definition is no part of the legislation on chemicals with endocrine disrupting properties. Mentioning of definitions like the one of WHO/IPCS is unwanted and using these kind of definitions in the development of criteria for endocrine disrupting properties of chemicals is simply changing democratically agreed policy. Some definitions also introduce new elements like “consequently” which are no part of EU legislation and which should not be introduced and used in any way as part of the criteria, because it means a change of the rule and an extra barrier for regulators trying to regulate chemicals with endocrine disrupting properties.

### **4. Why chemicals with endocrine disrupting properties need a special test system to be uncovered as such.**

In fact, endocrine disruption overturns the very concept that risk assessment is based on, for the simple reason that hormone signals (or their disruption) are designed to be more potent at low than at high dose, in fact receptor production often shuts off and the signal stops above a very low dose. In contrast, traditional risk assessment, for reasons of cost, chronic toxicity tests use almost-poisonous doses and extrapolate downward (instead of testing). The solution is to require testing of far greater part of the area of the dose/response curve, especially the dose we experience.

All tests should dose animals during development, as that is biochemically far more complex than adulthood and therefore very vulnerable. Critically also, animals shouldn't be killed at human equivalent of 60 years old, or one will find only part of the diseases caused by the chemicals as shown by tests recently conducted by Ramazzini Foundation<sup>3</sup>.

- Available data requirements and testing procedures will miss the effects of chemicals with endocrine disrupting properties.

Standard toxicity testing of chemicals is focussed on well-known effects like mutagenicity and carcinogenicity. Mental disorders and effects on metabolic processes (which might lead to obesity and diabetes) (particularly as a result of exposure during developing life) are among the most obvious endocrine disruption related endpoints which are missing in current traditional toxicity testing. But the endocrine system requires much more endpoints like negative effects of the thyroid system, effects manifesting in old age, diabetes, obesity, etc. to fully include the potential effects of disturbing the endocrine system. A full mapping of the known elements of the endocrine system, hormone production, windows of vulnerability and potential endpoints of adverse effects is a necessary starting point for drawing up data requirements and determining necessary tests. Recent findings like the role of placenta hormone serotonin in neurodevelopment of the foetus and the role of bone hormone osteocalcin in fertility need to be taken into account. Additionally many of these signalling pathways interconnect with one another (creating “cross talk”), which is another element to be taken into account in testing. Data requirements need to be developed to fully and

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<sup>3</sup> James Huff, Michael F. Jacobson and Devra Lee Davis, The Limits of Two-Year Bioassay Exposure Regimens for Identifying Chemical Carcinogens, Environmental Health Perspectives • VOLUME 116 | NUMBER 11 | November 2008

effectively protect citizens and the environment. Any delay like calls for international harmonisation of test requirements by for example OECD should be prevented.

- Need to modernise the test system.

A new testing system for chemicals with endocrine disrupting properties should start by learning from independent scientists how effects of endocrine disruption can be discovered in studies. Taking into account exposure during a special window of vulnerability (which may be different for different chemicals and different developing biochemical processes) is one element. Doing tests at low doses is another. Choosing the right test animal and the right strain also count. Many chemicals with endocrine disrupting properties act at (very) low doses. The traditional idea of relying on the threshold linear dose response curve does not work for many chemicals with endocrine disrupting properties. In several cases chemicals with endocrine disrupting properties have been proven to act according to a non-monotonic, inverted U-shaped dose-response curve<sup>4</sup>.

Some examples of such a non-linear dose-response curve are: High neonatal doses of the anti-miscarriage drug DES cause weight loss in newborn mice, while low doses caused obesity in later life. Rat experiments on DEHP, a phthalate found in plastics, show that low doses suppress an enzyme needed for proper development of the male brain, while high doses stimulate the enzyme. The well-known drug tamoxifen, given to treat certain breast cancers, is known to have opposite effects at different levels in the body.

- Need to involve independent scientists active in the field of endocrine disruption.

Since testing of chemicals with endocrine disrupting properties comes close to a paradigm change, those (academic) scientists who developed the field by publishing research should be given a leading position in developing tests, instructing other scientists how to perform tests and play a role in assessing the outcome. These scientists should have no connection whatsoever to commercial interests. Samples of tissue of tests performed by applicants of chemicals should be stored for scrutiny by (these) independent scientists.

- Mixture effects following combined exposure.

Chemicals with endocrine disrupting properties require special treatment which should be based on elimination of exposure wherever possible because it has been shown that chemicals with endocrine disrupting properties which act on the same target organs can have additive effects. A single substance risk assessment approach, which did not take into account the 'mixture effect' would not result in the protection of the public or the environment

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<sup>4</sup> John Peterson Myers, R. Thomas Zoeller, and Frederick S. vom Saal, A Clash of Old and New Scientific Concepts in Toxicity, with Important Implications for Public Health, volume 117 | number 11 | November 2009 • Environmental Health Perspectives

Hundreds of studies of independent scientists in academic laboratories show how a broad selection of chemicals can interfere with the normal development of offspring at extremely low levels of exposure (Bisphenol A, Chlorpyrifos, Mancozeb, etc.). These studies were done with the knowledge that the embryo and foetus develop under the control of hormones at parts per billion and parts per trillion, and that as the baby matures hormone concentrations are regulated by sensitive, thermostat-like feedback control systems in the brain.

Theo Colborn, author of *Our Stolen Future* and founder and president of TEDX has called for a radical change of the testing battery, doing tests at very low doses, checking all organs and tissues and systems that make up the endocrine system. She has further highlighted that expert scientists, working at the cutting edge of research into endocrine disruption should be given the opportunity and wherewithal to design a couple of comprehensive multi-organ assays to detect the most sensitive alterations in embryonic and foetal development and function, and that the various tissues from such a test should be sent to known experts in that field. We suggest Commission takes a look at this approach and considers adoption in EU-practice. Especially the “critical window of development” approach needs consideration.

## 5. Conditions and criteria for testing for endocrine disrupting properties.

- Any comprehensive test battery to identify chemicals with endocrine disrupting properties should include test methods for ALL hormonal systems, including not only the sex hormone disruptors, but also for example, chemicals that disrupt thyroid, adrenal, pituitary and hypothalamic hormones and insulin, steroid as well as non-steroid hormones. Interfering with gene expression or the regulation of expression should get a main emphasis. This test battery should be developed with independent actively publishing scientists in the field and should make use of sensitive animal strains. The Joint Research Centre (JRC) of the EC could take the lead in establishing a forum for developing tests;
- Low-dose testing needs to be performed in all testing assays;
- In particular, test methods need to be devised and implemented to identify irreversible later life (behavioural) effects due to early life (prenatal) exposures; the “critical window of development” approach. This must be a central element in developing tests. The data gathered in the TEDX website<sup>5</sup> should also be taken as a basis for developing tests;
- Existing financially-independent peer-reviewed literature should be collected on the chemical in question at the start of the hazard assessment and be used to determine the hazard (all adverse effects found in published literature need to be considered); different methodologies and different routes of exposure in the tests should in no way prevent data from being used in the assessment or resulting in them being disregarded. Because publication is science’s best method of assuring the reliability of knowledge; in case of conflicting outcomes, the peer-reviewed literature in independent journals should be properly considered and valued over non peer-reviewed studies

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<sup>5</sup> <http://www.endocrinedisruption.com/prenatal.criticalwindows.overview.php>

- Financial interests of sponsors of scientific studies will disqualify them from being used in the agent's hazard assessment.
- Non-published studies or studies not disclosed to the public will not be taken into account for the assessment of endocrine disrupting properties as scrutiny by independent scientists and the general public is not possible;
- Epidemiology studies available in open literature should always be taken into account and statistical relations between a chemical with endocrine disrupting properties and a negative health effect considered as an 'adverse' effect, particularly when these give weight to other studies, including in-vitro or in vivo studies; however, given that epidemiology is a blunt tool, and that there are many confounders (– not least the number of chemicals to which we are exposed –) it should not be necessary for associations with specific chemicals to be reported in such studies before action is taken.
- It should be possible to classify a chemical as having endocrine disrupting properties on the basis of a positive in-vitro study (thereby reducing animal testing), unless within a specific set time-period industry has come up with in-vivo data which shows without any doubt the in-vitro result to have been a false positive;
- Any animal (GLP) test performed by applicants on endocrine disrupting properties must ensure that tissues and organs are stored and made available for an examination by independent experts trained in that particular field;
- If data generated by the applicant and/or published by industry-sponsored studies differ to a great extent from the data generated by independent scientists, the data from independent scientists will prevail. Overall, independent research, published in quality journals, must be recognized as the best quality data. If industry wants to challenge the tests of independent scientists, they need to try to replicate it in an independent laboratory paid for by a levy by industry. If a controversy remains, the chemical cannot enter the market based on the precautionary principle (beyond reasonable doubt);
- Statistical re-examination or re-interpretation by industry or by industry-supported scientists or consultancies of studies of independent scientists, will be disregarded in the identification process of chemicals with endocrine disrupting properties. These kinds of statistical re-examination are generally biased and on a large scale used as a lobby tool in registration of chemicals. Industry of course has every right to do these calculations and interpretations, but they should not play any role in a scientific assessment.

## **6. Criterion for classifying a chemical as having endocrine disrupting properties.**

For assessing if a chemical has endocrine disrupting properties, all effects on the endocrine system should be considered, including those that are secondary to another effect. Effects on the endocrine system that occur either directly or indirectly, should classify a chemical as having endocrine disrupting properties. For this classification a known mechanism of action should not be necessary. Regulation should not require mechanisms of actions to be known, because elucidating mechanisms could delay regulating some chemicals with ED properties for decades. Moreover, knowledge about the precise mechanism of action might alter over time as more science became available, which could lead to the undesirable outcome that a chemical could be proposed for

market again and industry could take legal action against the Commission for the costs they had incurred. Endocrine disrupting properties should therefore be taken as embracing any alteration in hormone levels, or any effect on the endocrine system. For defining 'adverse effect' we propose to take 'any significant biochemical alterations, following dosing with the chemical during key development stages that is above background or averages in testing'. Biochemical alterations during key development stages above background may lead to serious, but subtle pathology later in life or in subsequent generations. In the cases of subtle but discernable biochemical alterations, a precautionary approach is required, as human and ecosystem health is at stake. Small changes to reproduction or functioning may have a strong long term effect on the survival of populations, even a species. Cumulative effects of chemicals acting on the same endpoint are another reason for choosing a precautionary approach.

The regulatory text should be the basis for the decision taken. For example, in the pesticides legislation, it effectively states that chemicals with endocrine disrupting properties cannot get access to the market if they "may" cause adverse effects. In this case the precautionary principle is embedded in the Regulation, saying in case of doubt a conservative approach needs to be followed and no market access granted.

## **7. Considerations on the outcome of tests.**

- A discussion on the mechanism of action of endocrine disruption is scientifically interesting (and can add some certainty) but it should play no role in the identification and regulation of a chemical with endocrine disrupting properties. It will delay the required evaluation and put citizens at preventable harm for yet longer. It is far too biochemically complex to require a mechanism of action in order to define and adverse effect.
- An evaluation on the 'relevance' of the outcome of tests for humans (route of exposure, doses, different response by species, etc.) should not be allowed. The outcome in test animals should by default be considered relevant for humans. For the pesticide regime any such 'relevance' consideration cannot be part of the assessment –as the regulation requires a "hazard" regime. However for the regulation of other chemicals an evaluation of relevance is also highly unwanted. Chemicals with endocrine disrupting properties are able to do harm at low doses and at vulnerable phases in life with irreversible effects. In this case precaution is warranted and no 'relevance' criteria used which are based generally on assumptions of risk assessment bodies.
- No threshold approach for endocrine disrupting properties.  
A traditional approach of doing tests at high doses and extrapolating to an alleged no effect level is already highly questionable in general (it postulates a level without effects) and this may not be true for endocrine disruption given the non-monotonic dose-response curves.  
Additional arguments for abandoning the idea of a no-effect level for chemicals with endocrine disrupting properties are:  
Hormonally active chemicals act in concert with natural hormones, so the assumption of a threshold dose below which there are no effects is an unlikely one. Also, within populations there are varying levels of natural hormones—so infinitesimal doses of exogenous endocrine disrupting substances can tip

susceptible individuals over the threshold for effects and cause a substantial overall effect;

Cumulative effects of chemicals with endocrine disrupting properties are possible and clearly demonstrated in robust scientific studies; exposure to mixture of endocrine active chemicals are an everyday reality and it would be inappropriate to use thresholds while these cumulative effects are not accounted for<sup>6</sup>

Susceptibility is likely during sensitive windows of exposure, at levels below those usually administered during routine testing, possibly leading to effects manifesting some considerable period of time later; a safe level -if it exists at all- is not easy to assess in these cases.

And –most importantly- a threshold approach is in full contradiction to the ‘hazard’ approach in the EU legislation. Threshold doses have no place in the regulation of chemicals with endocrine disrupting properties.

- Any endocrine disruption observed should be used for a positive identification of having endocrine disrupting properties. The effects of the disruption are often masked by other toxic effects but this is no reason to disregard this special unwanted effect. EU regulations are now in place to deal with such endocrine disrupting effects and this should certainly not be undone because other effects occur at the same time. Proposals to talk about a ‘lead’ toxic effect and disregard other effects in the regulation of the chemical should be denied. This ‘lead’ effect is scientifically not justified in that it could lead to a chemical with ED properties – that contributed to additive effects – not being regulated;
- Any discussion and general assumptions on the ability of a human body to compensate for a certain amount of endocrine disruption are based on scarce scientific evidence. Adaptivity is used in traditional risk assessment in an unscientific way to create an inappropriate sense of safety, and moreover is in contradiction to the need to apply precaution.. For the hazard approach that is required for chemicals with endocrine disrupting properties adaptivity can play no role at all because a discussion on safe levels or harmless exposure is not scientifically justified.
- If there is doubt about the adverse effects of chemicals with endocrine disrupting properties, e.g. studies are contradicting each other, the precautionary principle must be used and a chemical taken from the market until further (independent) studies are evaluated.

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<sup>6</sup> Nissanka Rajapakse, Elisabete Silva, and Andreas Kortenkamp, Combining Xenoestrogens at Levels below Individual No-Observed-Effect Concentrations Dramatically Enhances Steroid Hormone Action, Environmental Health Perspectives • VOLUME 110 | NUMBER 9 | September 2002.