Superior Health Council

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Endocrine disrupters: low dose effects, non-monotonic dose responses and critical windows of sensitivity

In this scientific policy advisory report the Superior Health Council of Belgium provides an expert opinion on endocrine disrupting chemicals, more specifically concerning the recognition of low dose effects and non-monotonic dose responses and concerning the existence of critical windows of sensitivity.

The Superior Health Council recognizes the existence of low dose effects, concludes that the non-monotonic dose response relation deserves further consideration, and recognizes the sensitivity during critical stages of development of humans.

3 July 2013

1. INTRODUCTION AND ISSUES

On 13 February 2013 the Superior Health Council (SHC) received a request for an advisory report concerning the issue of endocrine disrupters in the light of the on-going discussions on the European level in order to regulate these substances in several legislative frameworks such as REACH, biocides, cosmetics and toys.

The first part of the request asked for the opinion of the SHC on the WHO definition of endocrine disrupters, and on the use or non-use of the criteria “adversity”, “mode of action”, “relevance”, “potency”, “lead toxicity”, “severity”, “specificity” and “irreversibility” for the identification or the characterisation of the danger. The answer was given by the Council on the 8th of May (SHC, 2013), forwarded to the Minister of Social Affairs and Public Health, and added in annex to this advisory report.

The second question asks whether according to the SHC the scientific elements are sufficient to recognise low dose effects and non-monotonic dose responses of endocrine disrupters, and which are, following the SHC, the critical windows of sensitivity. The answer to these questions was requested for June. This advisory report refers to the adverse health effects of endocrine disrupters. It deals with fundamental and methodological aspects of the discussion on Endocrine Disrupting Chemicals (EDC). It should be read in combination with the recent advice No. 8732 on “Bisphenol A – dietary and non-dietary routes of exposure” which provides specific examples for the concepts dealt with in this advice (SHC, 2012).

To answer this question an ad hoc working group consisting of experts in endocrinology, toxicology and environmental health was established.

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2. CONCLUSIONS

The SHC recognizes the existence of low dose effects because even if all the available data (epidemiological, *in vivo* and *in vitro* studies) are not always strictly confluent, there is no scientific doubt that some pollutants interfering with the endocrine system cause effects at low doses of exposure.

The SHC concludes that the non-monotonic dose response relation deserves further consideration both in research and from a regulatory perspective. This advice discusses aspects showing that some endocrine disruptors exert effects that are not consistent with the classical pattern of dose-response relationship of toxicology. Although scientific consensus is (still) lacking on this issue, sufficient evidence exists stating that the findings impact the evaluation and the management of EDCs. As a consequence, the strategy of defining "safe" and "threshold" doses is no longer applicable to all EDCs.

Taking into account scientific uncertainty on particular (e.g. experimental) aspects, the SHC concludes there is ample evidence for the exquisite sensitivity of the developing organism to chemical exposures that may interfere with normal hormone action during critical stages of development. These stages include gestation (embryonic and fetal life), lactation and adolescence, but also senescence.

In view of this scientific basis the Council advocates adopting a testing policy which involves all chemicals for which indications of endocrine disturbance exist, and strategies and tests which bring on board the endocrinological findings, even when classical toxicological paradigms are challenged.

Keywords

Keywords	**Mesh terms***	**Sleutelwoorden**	**Mots clés**	**Stichwörter**
Endocrine disruptors	"Endocrine disruptors"	Hormoonverstoorders	Perturbateurs endocriniens	Endokriner Disruptor
Low dose effects	Low "dose response relationship, drug"	Lage dosis effecten	Effets des faibles doses	Low-Dose-Effekte
Non-monotonic dose responses	Non-monotonic "dose response relationship"	Niet-lineaire dosis-respons relaties	Relations dose-effet non-linéaires	Nicht lineare Dosis – Wirkungsbeziehungen
Windows of sensitivity	None	Perioden van verhoogde gevoeligheid	Fenêtres de susceptibilité	Sensitive Zeitfenster
Public health	"Public health"	Volksgezondheid	Santé publique	Volksgesundheit
European legislation	European "legislation"	Europese regelgeving	Législation européenne	Europäische Gesetzgebung

** MeSH (Medical Subject Headings) is the NLM controlled vocabulary thesaurus used for indexing articles for PubMed.*

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3. FURTHER DETAILS AND ARGUMENTATION

List of abbreviations

DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
EC	European Commission
EDCs	Endocrine disrupting chemicals
EFSA	European Food Safety Authority
IQ	Intelligence Quotient
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
SHC	Superior Health Council
TCDD	2,3,7,8-tetrachloordibenzo-p-dioxine
WHO/IPCS	World Health Organization/ International Programme on Chemical Safety

3.1 Methodology

The advisory report is based on a consultation of the existing scientific literature, the grey literature and on the expertise of specialists in endocrinology, toxicology and environmental health.

3.2 Details

3.2.1 Low dose effects

Low-dose effects of EDCs are defined as any biological alteration occurring at doses lower than those used in classical testing protocols for toxicology assessment or occurring in the range of what general human population is exposed to. For some chemicals, low doses can be (e.g. for dioxin-like compounds) or could potentially be in the nanogram per kilogram range. However, for a large amount of chemicals, doses in the micro to milligram per kilogram range could be considered as low doses, because in most of the traditional toxicological trials, quantities below the milligram per kilogram dose range were not examined.

From an endocrinological perspective, low doses are those acting in physiological conditions because they interact with receptors. They are usually defined using concentrations in biological fluids, which vary in a wide range (from picomolar level - 10^{-12}M - to nanomolar level -10^{-9}M). Effects do appear at such low concentrations as a result of the hormone-receptor interaction including non-linear or non-monotonic dose-response curves which favor the effects at low doses. Some compounds act either as an agonist or as an antagonist, depending on the dose. In addition, endogenous factors act as co-regulators and influence the response to hormones or EDCs in *in vivo*. The feedback mechanisms, involving the hypothalamus-pituitary systems, provide examples of such regulations. Finally, for the same receptors (e.g. estrogen receptors), large differences may occur in binding affinity between the EDC and the nuclear or other membrane receptor. For all these reasons, small changes in hormone concentrations can produce major differences in the resulting effects. (Vandenberg et al., 2012)

The hormones are released either into the bloodstream or locally in the tissues by endocrine glands or cells and they act on cells or organs either far from their synthesis site or in its vicinity.

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The presence of hormones all over the body is not a key to their action as this latter depends on the binding to specific high affinity receptors. This is in contrast to the EDCs which bind in a less specific way than the hormone-receptor interaction. Endocrine disruptors, however, can operate at very low levels because they interact with membrane receptors, activated nuclear receptors, binding proteins and metabolizing enzymes.

Small concentrations of EDCs may interfere with any aspect of the endocrine system. They can produce direct effects, through binding to hormonal receptors, or indirect effects, through impairment of production, release, metabolism, transport or recapture of the natural hormones. For direct effects, when EDCs and hormones share a synergistic action on a cellular receptor, a significant final effect can be achieved even if the binding affinity of the EDC to hormonal receptors is low. This is explained by the shape of the dose-response curve, but also because the EDC can modify the proportion of the free fraction of the natural hormones in the serum. For example, 17β-estradiol, the main human estrogen, is mainly bound to serum proteins. The free fraction is a hundred times lower than the bound fraction. Only the free fraction can interact with receptors and produce an effect (such as regulation of the estrogen cycle). If exogenous chemicals have enough affinity for the binding site on the serum transport proteins, even low concentrations of EDCs will be able to displace the bound fraction of 17β-estradiol, and increase of the free (active) fraction. (Vandenberg et al., 2012)

Low dose effects may occur at levels below those tested in classical toxicology trials. In these experiments, toxicological risk assessment is performed at high levels to estimate endpoints such as death, weight loss, change in organ weight and limited histo-pathological analyses. A "No Observed Adverse Effect Level" (NOAEL) - the greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure (Nordberg et al., 2004) - is calculated. The endpoints of this approach, however, do not allow identifying subtle molecular and structural low-dose effects, even if some *in vitro* cell models can provide information regarding possible non-monotonic dose-response curves. Furthermore, the period of testing is critical. The endocrine system plays a crucial role in establishing/timing the physiological adaptation to environmental conditions during fetal life. Therefore, the fetus is particularly vulnerable. EDCs can cross from the maternal to the fetal blood circulation through the placenta. This aspect is even more challenging since detrimental health consequences of fetal (upcoming epigenetic effects in the developing organism) and neonatal exposure to EDCs may appear during adolescence and adulthood and could be trans-generational. (Vandenberg et al., 2013)

Low dose effects can occur at current environmental exposure levels of the general population. Epidemiological studies suggest an association between hormonal diseases (cancer, diabetes) and exposure to doses below the NOAEL. This challenges the NOAEL and NOEL (No Observed Effect Level) concepts. Either they do not apply to a number of endocrine disruptors, or they are difficult to establish. In any case these findings urge for dealing with uncertainty and applying the precautionary principle. Moreover, the human population is exposed simultaneously to mixtures of hundreds of chemicals, with potential additive effects. An increasing number of experimental *in vitro* studies assume that a mixture with agonists on the same receptor, each at a concentration below the NOAEL, will result in a response (Kortenkamp, 2008). Low-dose effects have been demonstrated *in vitro*, *in vivo* but also in epidemiological studies for many EDCs: *bisphenol A, atrazine, chlordane, chlorothalonil, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT), heptachlor, hexachlorobenzene, maneb, parathion, tributyltin oxide, vinclozolin, dioxin, methylparaben, nonylphenol, octylphenol, polychlorobiphenyl, polybrominated diphenyl ethers, perchlorate, 4-methylbenzylidene*, etc. among others (Vandenberg et al., 2012; vom Saal and Hughes, 2005; Palanza et al., 1999; Eustache et al., 2009).

This summary illustrates that different issues are interconnected when addressing EDC effects. Low dose effects are related with aspects such as vulnerable periods (with the fetal stage having the highest sensitivity) and mixtures. The discussion is even more complex as it is equally related with the assessment of adversity of the effects. This issue was discussed in a previous advice (SHC, 2013) and is therefore not revisited in this document. Low dose effects are an extra dimension brought up by endocrinology to toxicology. A consequence is that an increasing number of researchers point to the challenge for defining a safe level of exposure for these chemicals. In the field of endocrine disruption, the classical toxicological principle" *the dose makes the poison"* definitely needs a revision. Others argue that existing testing procedures and methods may be extended detecting the adverse effects of EDCs.

3.2.2 Non-monotonic dose responses

This section of the advice addresses the following questions:

- Does sufficient scientific evidence exists to recognize non monotonic dose-responses of endocrine disrupters?
- Are the relevant hypotheses extendable to all endocrine disrupters?
- Are the effects reported in the literature adverse?
- What is the consequence on determining thresholds?

Vandenberg et al. (2012) define a non-monotonic dose-response curve as following: "*a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined*", resulting in U-shaped, bell-shaped or even more complex multiphasic curves (figure 1C).

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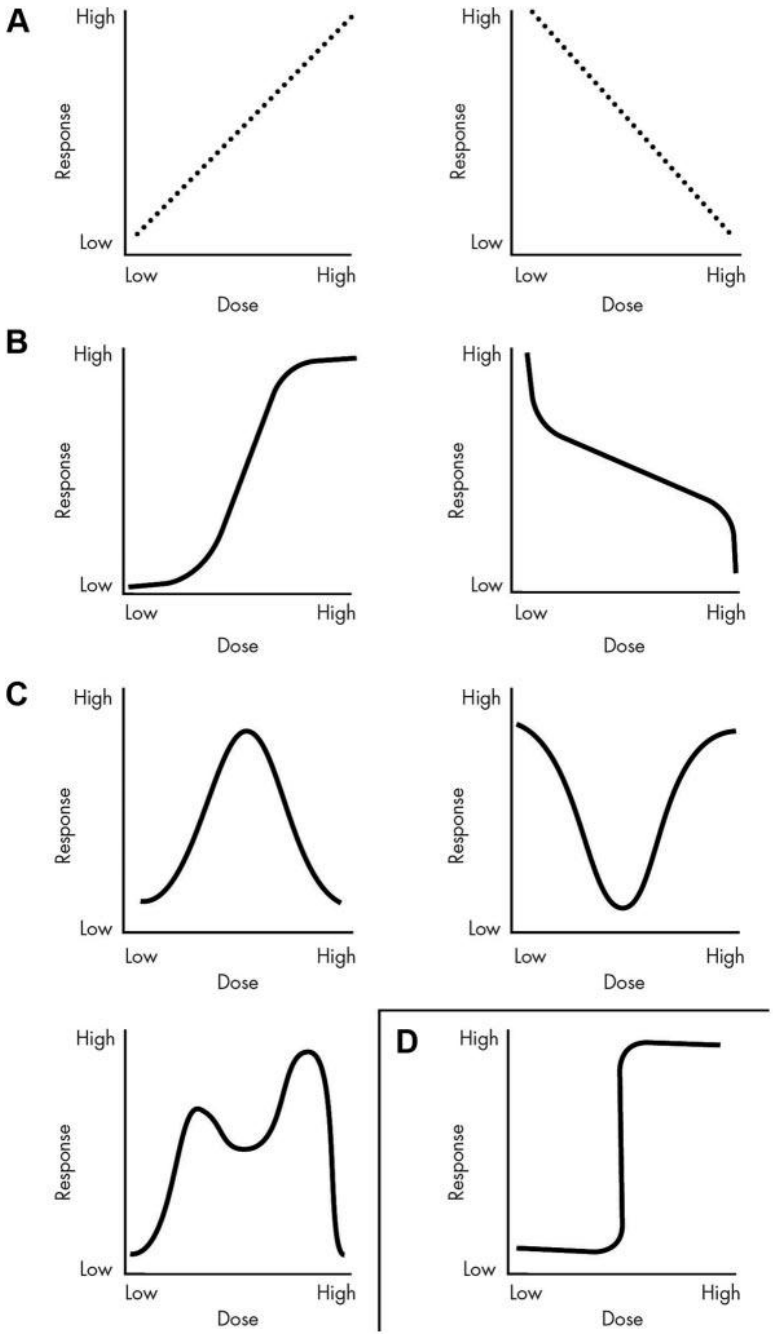

Figure 1: Examples of dose-response curves. A. Linear responses. B. Examples of monotonic, nonlinear responses. C. Examples of non-monotonic dose-response curves (inverse U-shaped curve, U-shaped curve, multiphasic curve). D. Binary response. (Vandenberg et al., 2012).

These non-monotonic effects impair using the classical toxicological paradigm, which assumes that the dose-response relationship is monotonic (linear (figure 1A) or nonlinear (figure 1B)), but without a change in the sign of the slope.

Always following Vandenberg et al. (2012), "*the consequence of non-monotonic dose-response curve for toxicity testing is that a safe dose determined from high doses does not guarantee safety at lower, untested doses that may be closer to current human exposures*" (figure 2).

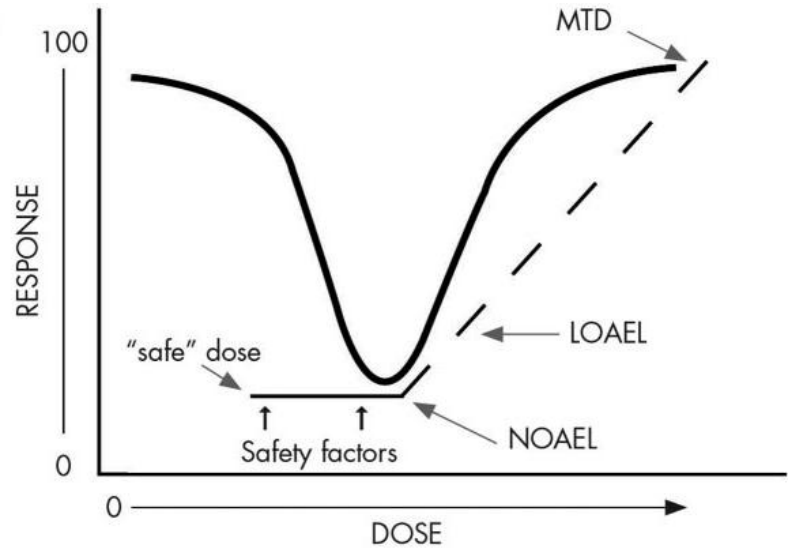

Figure 2: Illustration of the non-relevance of a traditional toxicology testing (NOAEL, LOAEL (Lowest Observed Adverse Effect Level), and calculation of a safe reference dose) when applied to a substance displaying a U-shaped dose-response curve. (Vandenberg et al., 2012).

The authors (Vandenberg et al., 2012) mention that possible mechanisms causing non-monotonic responses in cells, tissues, and animals include cytotoxicity, cell- and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative feedback loops.

The paper of Vandenberg et al (2012) refers to a wealth of scientific references showing non monotonic dose-response curves observed mainly *in vitro* (in cell culture experiments) but also *in vivo*, in laboratory animal experiments, and in humans (through epidemiological studies). A large number of compounds seem to be able producing such effects. The chemicals include natural hormones, drugs, environmental pollutants, plastic components, phytoestrogens, and pesticides.

However, other authors (for example, Rhomberg and Goodman (2012), but also others cited in European Food Safety Authority (EFSA, 2013)) are not convinced by the importance of non-monotonic dose-response curves. They argue that the experimental data are insufficiently strong from a scientific point of view (for example, lack of assessment of reproducibility). Along the same line, the EFSA (2013) noted "*the lack of consensus in the scientific community as to the existence and/or relevance of low-dose effects and non-monotonic dose-response curves in (eco)toxicology in relation to endocrine disruption, or other endpoints/modes of actions*", and recommended " *to clarify… non-monotonic dose response curves*".

A recent paper (Vandenberg et al., 2013) responded, in a well-documented way, to the criticisms of Rhomberg and Goodman (2012). The authors have answered, using the weight-of-evidence approach, to the following questions: Why should the study of EDCs rely on endocrine principles? Is there consensus on whether "low dose effects" exist? Are non-monotonic dose-response curves reproducible? What is the frequency of non-monotonic dose response curves?

The definition of an adverse effect is still controversial. This was already discussed in the recent letter from the SHC to the Minister of Social Affairs and Public Health, responding to a request for an urgent advisory report on endocrine disrupters (SHC, 2013). The definition proposed by the World Health Organization / International Programme on Chemical Safety (WHO/IPCS, 2009)

seems to be unanimously admitted: "*A change in the morphology, physiology, growth, reproduction, development or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of capacity to compensate for additional stress or an increase in susceptibility to other influences*". The EFSA (2013) notes "*that in line with protection goals embedded in EU legislation, adverse effects are addressed at the level of the individual(s) for human health and at the level of the (sub)population for wildlife".*

Endocrine disrupting chemicals may cause biochemical and molecular effects in the organism, which are not automatically/necessarily adverse. The difficulty is to determine a threshold at which the endocrine modulation becomes an adverse effect.

In its recent opinion, the EFSA (2013) recommends to use "*expert judgment to assess on a case-by-case basis the (eco)toxicological relevance of such changes and when the biological threshold between endocrine modulation and adverse effect has been crossed*". It should be stressed that adversity may have a different meaning at the individual and at the population levels. For instance, a loss of 5 IQ (Intelligence Quotient) points at a population level has a significance that cannot compare with the consequence for a single individual.

In summary, the evidence that some endocrine disruptors exert effects that are not consistent with the classical pattern of dose-response relationship of toxicology is not generally accepted by the scientific community but sufficiently substantiated to impact the evaluation and the management of EDCs. As a consequence, the strategy of defining "safe" and "threshold" doses does no longer apply to all EDCs. The non-monotonic dose response relation deserves further consideration both in research and regulation.

3.2.3 Critical windows of sensitivity

Endocrine systems of the body are essential for both the short- and long-term regulation of metabolic processes. Nutritional, behavioral, and reproductive processes are intricately regulated by endocrine systems, including growth (including bone growth/remodeling), gut, cardiovascular, and kidney function and responses to stress. Disorders of any of the endocrine systems, involving both overactive and underactive hormone secretion, result inevitably in disease, the effects of which may extend to many different organs and functions and are often debilitating or life-threatening. In view of this general perspective, the threat from environmental chemicals with endocrine activity (either agonistic or antagonistic) is potentially serious.

Hormones are key factors in the proper development and functioning of a multiplicity of organ systems and tissues, with those of the reproductive tract, the brain and the neuro-endocrine system being the most prominent ones. Accordingly, there is ample evidence for the exquisite sensitivity of the developing organism to chemical exposures that may interfere with normal hormone action during critical stages of development (WHO, http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/index.html).

These stages include gestation (embryonic and fetal life), lactation and adolescence, but also senescence. The endocrine system, through a developmentally regulated pattern of functions including hormone production, transport, metabolism and receptor expression, controls the pathways which are essential for cell proliferation, differentiation, and organ development. Therefore it is not surprising that perturbations of the endocrine system during critical windows of sensitivity are associated with the highest risk for adverse health effects. In many cases, the impact of the interfering chemicals is irreversible and remains almost unchanged during the lifetime of the affected organism. Exposure to EDCs during the period when "programming" of the endocrine system is in progress may result in a permanent change of function or sensitivity to stimulatory/inhibitory signals. Often a considerable latency exists between the exposure and the

moment when effects become (clinically) manifest. The timing of exposure is key to human disease because there are critical developmental periods during which there is an increased susceptibility to environmental endocrine disruptors. In those cases in which the programming of a function is disrupted or changed, e.g., reproductive health, this may interfere with early life organization, followed by a latency period, after which the function becomes activated and the dysfunction becomes obvious. Exposure to the same level of an endocrine signal during different life history stages may produce different effects.

Some prominent examples include (Kortenkamp et al., 2012):

1) The action of chemicals capable of interfering with androgen action during the male programming window in fetal life; this includes androgen receptor antagonists such as certain dicarboximide, imidazole and azole pesticides, and certain phthalates. Some read-outs of diminished androgen action in experimental animals only become apparent in adult life; this includes disorders of fertility and neoplasia. The effects are largely irreversible.
2) Epidemiological studies show that exposures to dioxin (TCDD (2,3,7,8-tetrachloordibenzo-p-dioxine)) in perinatal life have a negative impact on semen quality, while exposure during adulthood has no influence on semen quality. For reproductive function in both humans and animals, fetal life is most vulnerable because rapid structural and functional events exist.
3) Estradiol and estrogenic chemicals can interfere with the KiSS peptide system in rodents in neonatal life, with influences the timing of puberty.
4) The acquisition of the female reproductive capacity (ovulation) is programmed *in utero* and can be disrupted at this stage by undue signaling from chemicals such as DES (diethylstilbestrol), with multiple and irreversible consequences. Early postnatal life is also a time when maturation is still rapid (e.g., the central nervous system develops fast during this period, including the hypothalamus which controls reproduction). The organization of the neuro-endocrine control of reproduction is not completed at birth and remains sensitive to the interaction of EDCs during the neonatal period, which has been shown for the control of ovulation in rodents. Breast or formuled feeding could be of particular significance due to the capacity of human milk to concentrate EDCs and the potential high concentrations of phytoestrogens in soy milk and/or plasticizers in formula-containing cans. Many hormonal cancers, including breast, prostate, testis, ovarian and endometrial cancer are thought originating partly during fetal and pubertal life. During these life stages, increased sensitivity to chemicals implicated in these cancers was described.
5) The action of thyroid hormones during development in the womb is essential for many developmental landmarks, including the development of the brain and the neuro-endocrine system. Disruption of thyroid action by chemical exposure at this stage of development may have detrimental and irreversible effects on cognitive abilities.

It is important to note that some potential latent effects that may result from short-term exposures during critical development windows are very difficult to identify, as some EDC effects are trans-generational.

Exposure of an adult to an EDC may have consequences which differ from those exposing a developing fetus or infant. Exposure in adulthood may be compensated for by normal homeostatic mechanisms and may therefore not result in any significant or detectable effect. Nevertheless, in the elderly, exposure to polychlorinated biphenyls has been identified as a risk factor for the development of type 2 diabetes. (Lee et al., 2011) In fact, the field of endocrine disruption has embraced the terminology "*the fetal basis of adult disease*" to describe observations that the environment of a developing organism, which includes the maternal environment (in eutherian mammals), the egg (other vertebrates), and the external environment,

interacts with the genes and the epigenome determining the propensity of that individual to develop a disease or dysfunction later on in life. This concept can be extended beyond the fetal period to the early postnatal developmental period when organs continue undergoing substantial development, referred to as the "*the developmental basis of adult disease*".

In summary, taking into account scientific uncertainty on particular (e.g. experimental) aspects, the SHC concludes there is ample evidence for the exquisite sensitivity of the developing organism to chemical exposures that may interfere with normal hormone action during critical stages of development. These stages include gestation (embryonic and fetal life), lactation and adolescence, but also senescence.

4. FURTHER RESEARCH AND POLICY (Nicolopoulou-Stamati et al., 2001)

4.1 This advice of the SHC should be read in conjunction with the previous one (SHC, 2013). Both advices point to the biological complexity of EDCs in their nature and effect mechanisms. Consequently a health policy on these substances necessitates covering this wide array of aspects. Therefore it is necessary that policy opens the possibility researching and assessing the endocrine disrupting effects of a wide range of chemicals for which indications exist that they show one or more of the discussed characteristics.

4.2 Endocrine disrupting chemicals challenge well established paradigms of classical toxicology testing. The issue is not that these existing methods should be abandoned (classical toxicology identified indeed a number of EDCs, some of which have been retracted from the EU-markets, but many of them are still present in the environment and continue causing exposure); rather they should be complemented with a set of methods addressing the issues of non-monotonic dose-responses and critical windows of exposure. Also the consequences for risk assessment of chemicals should be addressed. For some EDCs the concepts of NOEL, NOAEL, and related values should be revisited in the context of the endocrine disturbance data. Particular aspects of these more extended assessments (e.g. the dose-effect relationships) can be addressed on a short term basis. Other aspects (e.g. trans-generational effects, effects of "cocktail" exposure) will necessitate more research over longer periods. In such instances of uncertainty, a precautionary principle based attitude, aiming at limiting the exposure of the population consequently has to be adopted.

4.3 The Council shares the opinion that currently sufficient scientific evidence exists to implement more stringent measures reducing the exposure of the population to EDCs. Current European legislation, such as the REACH Regulation (EC/1907/2006), the biocidal products Regulation (EU/528/2012), the cosmetic products Regulation (EC/1223/2009) and the toys safety Directive (2009/48/EC), is a help for this end. In particular the REACH Regulation which takes into account complexity, uncertainty, and the precautionary principle might be helpful in this respect. Nevertheless in a context of the widespread distribution of EDCs in the environment, these initiatives cover only fragments of the problem. A more comprehensive policy covering the totality of the problem in all its new aspects is mandatory.

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6. APPENDIX

Superior Health Council

YOUR LETTER DATED 12 February 2013
YOUR REF. MIRR/MIR/2013/01

OUR REF. 8314
DATE 8 May 2013

ENCLOSURE(S): -

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Ms. Laurette Onkelinx
Minister of Social Affairs and Public Health

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SUBJECT: Request for an urgent advisory report on endocrine disrupters.

Dear Minister,

On 13 February 2013 the Superior Health Council (SHC) received your request for an advisory report concerning the issue of endocrine disrupters in the light of the on-going discussions on the European level in order to regulate these substances in several legislative frameworks such as REACH, biocides, cosmetics and toys.

The first part of the request asks for the opinion of the SHC on the WHO definition of endocrine disrupters, and on the use or non-use of the criteria "adversity", "mode of action", "relevance", "potency", "lead toxicity", "severity", specificity" and "irreversibility" for the identification or the characterisation of the danger. The answer is expected for April.

The second question asks whether according to the SHC the scientific elements are sufficient to recognise low dose effects and nonmonotonic dose responses of endocrine disrupters, and which are, following the SHC, the critical windows of sensitivity. The answer to these questions is requested for June.

The SHC wants to express its disappointment about the way in which its opinion is asked. Why was the SHC not consulted in an earlier phase of the process? Discussions on endocrine disrupters are going on already for 15 years or longer and the SHC is consulted only in the final phase of the process, an urgent advisory report being requested now. This is a global, very broad and sensitive issue on which a lot of research has been done and ample international literature exists. Therefore an advisory report of the SHC needs a careful and thorough analysis and consideration of the issue and an in depth examination and summary of the literature. The Council is most willing to contribute, within the bounds

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of its possibilities, to the Belgian activities in a constructive way, but in view of the very limited timing the only possibility is to formulate initial comments instead of a constructive and founded support to a Belgian position. So the SHC has the feeling being consulted only "pro forma" and insists to be involved in the future during an earlier phase of the process.

The first question was treated by experts in endocrinology and discussed in the permanent working group of the SHC on chemicals. This advisory report refers to the adverse health effects of endocrine disrupters.

In general the Council focuses on the limiting interpretation of the definition of "endocrine disruptors" as provided in the documents at stake. The Council understands that stakeholders as industry aim limiting tests at a level which is financially acceptable. On the other hand, from a scientific and a public health point of view, it is advisable widening the scope of endocrine disruptors to substances and mixtures for which scientifically sound laboratory evidence has been developed. The Council wants to avoid that a too limiting definition excludes in beforehand groups of products or pollutants which might offer a hazard to public health.

The present comments refer to a recent EU report (*extracts in italics*) on the protection of public health from endocrine disruptors (2012/2066(INI)) that was voted by the European Parliament on 13 March 2013.

Definition from the WHO/IPCS (2002) report: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." A potential endocrine disruptor is "an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations." (http://www.who.int/ipcs/publications/en/ch1.pdf).

> (-)The **endocrine system** includes any cell in the body producing and releasing chemical mediators (the hormones) for action on other cells through specific receptors. Originally, the hormones were thought to be only produced by endocrine glands and to be released into the circulation to act away from their sites of origin. Now, it is recognized that hormone-producing cells are not only located in endocrine glands but also in other tissues (e.g. the stomach and the adipose tissue) and that their action can also take place in the vicinity of their site of origin. It is important that the endocrine system is viewed in such a broad sense in the perspective of endocrine disruption.
> (-)The definition mentions not only individual substances but also **mixtures**. This is most relevant from an environmental point of view because, except accidental toxic spills exposing to high doses of a single chemical ("Seveso"), humans are most commonly exposed to low doses of several chemicals together. Different environmental media (e.g. sludge of different types) and mixtures of pollutants (e.g. pesticides) were shown having endocrine disrupting properties. The issue is rather complex since as experience from the past (e.g. on biocides or vegetation protection substances) showed, difficulties in developing strategies analyzing the hazards of these mixtures exist. More importantly, the approach for testing by industry and legislating by authorities has been so far oriented towards individual chemicals.

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(-)The definition equally refers to **intact organisms**. With regard to this approach the Council wants to stress that *in vitro* testing including the use of micro-organisms with reporter genes is useful but complementary to the *in vivo* approach. While evidence of effects *in vitro* is highly suggestive of endocrine disruption, absence of effects does not exclude endocrine disruption because several parameters such as critical windows of sensitivity and interaction with endogenous endocrine system are not included. Although it is recommended to limit animal testing, and in that case the advice of the ethical council dealing with animal maltreatment is most indicated, it can be inadequate to rely on only one approach.

"6. Stresses that it is essential to base the criteria to determine endocrine disrupting properties on a comprehensive hazard assessment carried out on the basis of state of the art science, taking into account potential combination effects as well as long-term effects and effects during critical windows of development; the hazard assessment should then be utilized in the risk assessment and risk management procedures as prescribed in various relevant legislation;"

Importantly, according to the above WHO definition, a **potential endocrine disruptor** is an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action. When such interference leads to hazard, the chemical becomes effectively an **endocrine disruptor** requiring risk assessment. Thus the conditions of screening for hazard identification are critical including the selected endpoints, the age window of exposure, the latency between exposure and effects, the frequency of exposure, the dose of chemicals and the possible mixture effects, among other parameters. Therefore the word *comprehensive* hazard assessment is crucial.

"8. Takes the view that the criteria for defining endocrine disruptors should be based on criteria for defining 'adverse effect' and 'endocrine mode of action'; the WHO/IPCS definition being the appropriate basis for that purpose; considers that both 'adverse effect' and 'endocrine mode of action' must be examined and weighed up in parallel in a comprehensive assessment; considers that observed effects should be assumed to be harmful if there is scientific data to indicate this; stresses that any possible combination effects such as mixtures or cocktail effects should be taken into consideration;"

(-)**Adversity** is linked with harm caused by an endocrine disruptor as an outcome of interference with hormone action that is the **mode of action.** While adversity of effects is a component of the definition, the endocrine mode of action provides weight of evidence of endocrine disruption. **Adversity** is meant as any non-physiological observation i.e. that is beyond the "normal" variations within a population. Depending on the conditions (e.g. sample size, cross-sectional versus longitudinal studies) and the endpoint, the normal limits can be adjusted; e.g.: abnormal adiposity can be defined as a BMI > 90th centile (overweight) or > 97th centile (obesity). In an animal study, it will be defined as adiposity different from a control group. Adversity should not involve any reference to severity because an apparently benign effect occurring first in life can increase the likelihood of other more severe effects occurring subsequently; e.g.: a reduced ano-genital distance in males at birth is a non-severe adverse effect that can be associated with severe effects later in life such as reduced sperm count and testicular cancer.
In relation to endocrine disruptors specific attention should be given to intrauterine

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exposure.
Moreover adversity in relation to health effects is value loaded. Over the past decades it proved hardly possible determining objective criteria which define "adverse" health characteristics. In relation to endocrine disruptors specific attention should be given to pre-symptomatic effects providing an indication for the likelihood of disease.
(-)On the basis of the present understanding and experimental evidence all chemicals that bind specifically to cell receptors could in some way contribute to endocrine disruption. This implies that data on *in vitro* binding to receptors followed by activation or blocking are certainly relevant. All chemicals binding to receptors at relevant concentrations should be considered potential endocrine disruptors. The **endocrine mode of action**, however, implies that endocrine disruption follows the *in vivo* principles taken from fundamental endocrinology with complex mosaic effects produced through simultaneous actions or a cascade of effects in different tissues with changing sensitivity throughout the life cycle. Moreover these complex events occur at very low concentrations (parts pro billion – ppb); often no threshold dose can be determined. It is very important that scientific studies on the modes of action of endocrine disruptors are pursued. However, while evidence on the endocrine mode of action adds to the demonstration that a chemical is an endocrine disruptor, it is not required according to the definition but useful for characterization. Furthermore, emphasis has been put in the past on sex steroids and thyroid hormones in characterizing the endocrine mode of action of endocrine disruptors. It is now recognized that other endocrine systems or mechanisms can be involved as well. Of major importance is that endocrine disruptors not only act through the classical nuclear receptors, but also through membrane-bound receptors, through estrogen-like receptors, through cytoplasmic interactions, through cross-talk between genomic and non-genomic pathways, and that effects of activation of receptors differ in function of the ligand.

"25. Stresses that current science does not give sufficient ground for setting a limit value below which adverse effects do not occur and therefore endocrine disruptors should be regarded as 'non-threshold' substances, and that any exposure to such substances may entail a risk, unless the manufacturer can show scientific proof that a threshold can be identified, taking into account increased sensitivities during critical windows of development, and the effects of mixtures;"

Low-dose effects and **non-monotonic dose responses** have been proven for some endocrine disruptors and should be considered as a possibility for any potential endocrine disruptor until evidence of no such effects is provided using low to very low doses (usually in the range of human exposure). This issue is challenging the classical concepts of toxicology based on thresholds below which exposure should not cause any adverse effect. This is also challenging the screening programs since they tend towards reduced number of tested doses in a single condition that could not be in the **window of highest sensitivity** for endocrine disruptors. Evidence is accumulating that prenatal and early postnatal periods are crucial for the fetus establishing regulation of essential body functions. This organizing phase will determine homeostasis of processes such as control of energy balance and reproduction for the entire life under the influence of physiological environmental factors including nutrition and stress as well as non-physiological factors such as endocrine disruptors. Women before and during

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pregnancy and young children are thus a priority concern. The issues of low dose effects and critical windows of sensitivity cannot be addressed separately since they are central in the demonstration that a chemical is or is not an endocrine disruptor.

Sincerely yours,

On behalf of the Superior Health Council,

André Pauwels,
CSS Coordinator

The following experts were involved in drawing up the advice:

- Mr. ADANG Dirk (environmental health - UCL)
- Mr. BOURGUIGNON Jean-Pierre (pediatric endocrinology - ULg)
- Ms. CHARLIER Corinne (toxicology - ULg)
- Mr. DEMOULIN Vincent (ecotoxicology - ULg)
- Ms. DIRINCK Eveline (endocrinology – UZA)
- Mr. HENS Luc (human ecology - VITO)
- Mr. STEURBAUT Walter (human exposure - UGent)
- Mr. VAN GAAL Luc (endocrinology – UZA)
- Ms. VANHAECKE Tamara (toxicology - VUB)
- Mr. VAN LAREBEKE-ARSCHODT Nicolas (toxicology, cancer - UGent)
- Mr. VERSTEGEN Geert (toxicology - Antigifcentrum)

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7. COMPOSITION OF THE WORKING GROUP

All experts joined the working group ***in a private capacity.*** The names of the members and experts of the Superior Health Council are indicated with an asterisk*.

The following experts were involved in drawing up the advice:

BOURGUIGNON Jean-Pierre	Pediatric endocrinology	ULg
CHARLIER Corinne	Toxicology	ULg
DEWALQUE Lucas	Toxicology	ULg
DIRINCK Eveline	Endocrinology	UZA
HENS Luc*	Human ecology	VITO
MAGHUIN-ROGISTER Guy*	Food analysis	ULg
PUSSEMIER Luc*	Residues and contaminants, chemical risks	CODA
SCIPPO Marie-Louise*	Food analysis	ULg
VAN DEN BERG Martin	Toxicology	UUtrecht
VAN GAAL Luc*	Endocrinology	UZA
VAN LAREBEKE-ARSCHODT Nicolas*	Toxicology, cancer	UGent

The following expert was heard:

DUVERGER VAN BOGAERT Martine*	Toxicology	IPH

The lead of the working group was taken by Corinne CHARLIER, Eveline DIRINCK and Marie-Louise SCIPPO, the scientific secretary was Marleen VAN DEN BRANDE.

About the Superior Health Council (SHC)

The Superior Health Council is a federal body that is part of the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC takes no decisions on the policies to follow, nor does it implement them. It does, however, aim at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, members of scientific institutions), 200 of whom are appointed experts of the Council. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a referring committee) and 4) the final endorsement of the advisory reports by the Board (ultimate decision-making body). This coherent set of procedures aims at allowing the SHC to issue advisory reports based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

The advisory reports drawn up by the working groups are submitted to the Board. Once they have been endorsed, they are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.css-hgr.be), except as regards confidential advisory reports. Some of them are also communicated to the press and to target groups among healthcare professionals.

The SHC is also an active partner in developing the EuSANH network (European Science Advisory Network for Health), which aims at drawing up advisory reports at the European level.

In order to receive notification about the activities and publications of the SHC, you can send a mail to info.hgr-css@health.belgium.be .

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