15

STEFANOVA Desislava (ENTR)

From: [redacted]@cosmeticseurope.eu>
Sent: 07 June 2013 16:30
To: VAN DER JAGT Katinka (ENV); GIRAL-ROEBLING Anne (ENTR)
Cc: [redacted]
Subject: Cosmetics Europe input on document on thresholds from 7th Ad Hoc meeting on EDs
Attachments: Thresholds on EDs - 7 Ad Hoc meeting - Cosmetics Europe input.doc

Dear Mrs. Van-Der-Jagt and Mrs. Giral,

We would like to thank the European Commission the opportunity to provide comments to the document presented at the 7th Ad Hoc meeting on EDs (existence or not of thresholds for EDs).
Please find attached the document containing our comments. Please note that Cosmetics Europe comments are in red in the text to be easily identified.
We have deleted most of the original text, except the questions so that it is more reader friendly.
We look forward to continuing the work with the Commission on this topic.

I would also like to wish you a very good and sunny weekend.

Best Regards,

[redacted]
Science Issue Manager

Cosmetics Europe

Save the date:
"Personal Care – An Essential Component Of Living"
Cosmetics Europe General Assembly, 12 - 14 June 2013

Cosmetics Europe-The Personal Care Association
Avenue Herrmann-Debroux 40
1160 Brussels
Tel. +32
Fax +32
Email: [redacted]@cosmeticseurope.eu
www.cosmeticseurope.eu

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
ENVIRONMENT
Directorate D - Water, Marine Environment & Chemicals
ENV.D.3 – Chemicals, Biocides and Nanomaterials

Brussels, 24 May 2013
ED-AD-HOC-7/2013/14

THE COMMUNITY STRATEGY FOR ENDOCRINE DISRUPTORS

7TH AD HOC MEETING OF

COMMISSION SERVICES, EU AGENCIES AND MEMBER STATES

Centre de Conférence A. Borschette, room 4B, rue Froissart 36, Brussels
30 May 2013 (09:30 – 17:30)

Concerns: **Discussion on endocrine disruptors, should they be considered as non-threshold chemicals?**

Agenda Point: **4**

Action Requested: **This document contains guiding questions for brainstorming and discussion on the question if endocrine disruptors should be considered as non-threshold chemicals.**

The participants to the meeting are invited to:

- reflect on the questions taking into account relevant documentation.

- contribute to the discussion during the meeting by sharing the outcome of their reflection

- send comments in writing by 7 June 2013 to the following addresses: katinka.van-der-jagt@ec.europa.eu
anne.giral@ec.europa.eu

Cosmetics Europe would like to welcome the opportunity to provide comments for the discussion concerning the existence of thresholds for Endocrine Disruptors.
Overall, it is the view of the cosmetics industry that there is sufficient scientific evidence that endocrine disrupters should not be treated differently to other chemicals of concern. The current risk assessment and risk management approaches cover all adverse effects from chemicals, also those caused by endocrine disruptors. Thus, EDs should be evaluated on a case by case approach, considering all available data instead of assuming a non-threshold approach for all EDs by default.

1. Discussion on what the science is telling us about the existence of thresholds for endocrine disruptors

Can the presence of thresholds never be confirmed or rejected by experimental data, because all methods for measuring effects have a limit of detection which will obscure thresholds, if they exist?

While it is theoretically not possible to experimentally show the existence of a threshold by standard toxicology studies, it is possible to experimentally demonstrate that thresholds are likely by using sensitive methods to query the mode of action through which endocrine-active chemicals work. Small molecule hormones act through binding a receptor, which then elicits biological effects by initiating the expression of specific genes. This gene expression is a necessary step for the biological response to estrogens, androgens, thyroid hormones. Gene expression can be measured very sensitively using modern biotechnology. Available methods like microarray techniques, real-time RT-PCR, and bead-based oligonucleotide methods have sensitivities in sub-attomole range, which translates to the ability to detect just a few molecules of mRNA per cell. Using these sensitive methods, a threshold was demonstrated for gene expression changes for potent and weak estrogens in developing fetal reproductive system (Naciff et al, 2005, Boobis et al, 2009). Interestingly, the apparent threshold for gene expression is not far below (order of magnitude or less) the NOAEL for traditional tox endpoints.
The endocrine system provides a mechanism in the body called homeostasis through feedback mechanisms involving various organs and organ systems. Homeostasis is the ability to maintain a constant internal environment in response to environmental changes. It is a relevant concept in living organisms, which are exposed to stressors, whether they have an endocrine or any other mode of action (e.g. temperature control, pH balance, water and electrolyte balance, blood pressure, and respiration). Thus, thresholds of adversity exist and are the rule for all endpoints, including those arising from endocrine disruption. This is supported by experimental studies showing no adverse effects in vivo at low doses, i.e. environmentally- and/or human relevant dose levels. Conversely, if no thresholds existed for hormonally active substances, human exposure to food-born and more potent hormonally active substances should pose a potentially greater problem than that caused by chemicals. A simple example: cattle fed clover only may become infertile due to the hormonal activity of coumestrol. Cattle grazing on a normal pasture that contains some clover as well as other plants are not at risk of infertility. Thus coumestrol-induced infertility clearly has a threshold.

Another argument proposed by supporters of the non-threshold approach for EDs is that because steroids are present in the embryo before the entire pituitary-gonadal

axis is set up, that there can be no homeostasis. However, the hypothalamic-pit-gonadal axis is only one of many homeostatic mechanisms available for modulating hormonal activity. Others that are present include the presence of binding proteins that change the level of free hormone available for receptor interaction, the ability of cells to eliminate hormones via metabolism, and the ability of cells to downregulate receptor levels in response to hormonal stimulation. In mammalian embryos, there is also the presence of a maternal organism that tightly regulates steroid hormone levels and does have an intact HPG axis, and a liver.

Finally, there is also the idea that thresholds cannot be determined for EDs since endocrine disrupters are acting on an existing background of endogenous hormones. Thus, a little additional hormone would be enough to elicit an unwanted response. Again, the facts argue differently. First, changes in endogenous hormone levels needed to change physiological state tend to be marked. Good examples of this are the large swings in estradiol, and later progesterone levels needed to achieve a normal menstrual cycle. Small changes are insufficient. The LH surge needed to stimulate ovulation is another. Second, even if the endogenous hormone levels are at the edge of eliciting a response in a single cell such that a single molecule of an endocrine disrupter could change the state of that cell, it's only one cell, and one cell in the uterine wall, or one cell in the developing reproductive system is not sufficient to drive an adverse response.
It's worth noting that this whole "additivity to background" argument comes from the literature on thresholds for genotoxic carcinogens, but the argument also requires the notion that cancer results from the clonal expansion of a single cell into a tumor. This second half of the argument has been absent in the ED debate. However, genotoxic cancer is a special case, and should not be dealt with here.

Should, from a pure scientific perspective, evaluations on whether effects of EDs exhibit a threshold or not be based on a combination of biological plausibility and experimental observations, using expert judgement after a case by case analysis of all data available?

On the basis of the WHO/IPCS definition an endocrine disruptor has to demonstrate adverse effects (pathology or functional impairment) in an intact organism. Transient, inconsistent and minor fluctuations at the biochemical and molecular level may be considered adaptive, i.e. non- adverse. In many cases, the putative adverse findings have not been replicated in a more robust study design and the toxicological significance of many of these mostly transitory effects has not been determined. Here it should be mentioned that a biological response does not equal an adverse effect. Weak hormonal activity can be advantageous, detrimental or neutral for the organism.

Are thresholds of adversity likely to exist but may be very low and subject to different factors such as mode of action and toxicokinetics?

Since hormones act via receptors and the response is dependent on hormone concentration, it follows that a certain level of receptor occupancy is required before a response is produced. Because most receptor-based physiological responses can

be triggered when only a small fraction of available receptors are activated by a strong agonist (a ligand with high affinity and intrinsic activity), receptor ligands with very low affinity and no intrinsic activity (weak antagonists) will fail to interfere with endogenous agonists unless their concentrations reach levels that obstruct access to the receptor by shear mass action.
Toxicity is the result of multiple steps at multiple levels of biological organization. While some of these events may not be thresholded, if any, even one is thresholded then the entire pathway is thresholded. Although we can agree that the binding of one molecule to one receptor is a non-thresholded response, this does not translate into a biological response, adverse or not. A simple example of this: one acetylcholine molecule can bind to one ACh receptor on the membrane of a muscle fiber. That binding causes the receptor to open an ion channel, letting a little sodium into the cell and resulting in a very small change in membrane potential (a nanovolt or two). However, this results in no response from the muscle fiber because a nanovolt change in membrane potential is insufficient to activate the voltage-sensitive ion channels that propogate an action potential along the cell membrane. It takes millions of molecules of ACh acting within a few milliseconds to achieve that change. Even if that occurs, if it is an isolated event it would lead to the twitch of a single muscle fiber, not enough to even move the muscle. That would require thousands of millions of ACh molecules acting on thousands of muscle fibers simultaneously. So, even though the initial molecular event is unthresholded, the ultimate action is thresholded because at least one (in this case two) of the steps required for muscle contraction are thresholded. So it is for endocrine active agents. While binding of a molecule to receptor is not thresholded the resulting action is. It requires more than one occupied receptor to elicit meaningful changes in gene expression in a single cell, and changes in a single cell are unable to change the physiological or developmental state of an organism.

Are endocrine disruptors 'special' with regard thresholds compared to other chemicals?

Existing *in vivo* data do not justify adopting a non-threshold approach by default for endocrine disruptors. As endocrine modes of action are either receptor-mediated or are interfering with steroid biosynthesis involving enzyme interaction, the existence of a threshold is clearly to be expected as for effects with other modes of action. Thresholds of adversity exist because, within a certain range, the body can repair damage and detoxify chemicals to which it is exposed. The threshold may be low in certain development phases, but it may be determined with appropriate methods. In fact, data show that endpoints like reproductive organ weights from standard reproductive toxicity studies (OECD) were equally sensitive compared to additionally added parameters such as hormone levels. Adverse effect may only result in case of overloading the homeostatic and detoxifying mechanisms of the organism. A no-effect level was also observed for ethinyl oestradiol (an active ingredient of the contraceptive pill): when injected in adult men at high doses (60 µg/day) it affected sperm motility and density; in contrast, 20 µg/day, still a huge dose in terms of hormonal potency, had no effect on sperm motility and density (Lübbert et al., 1992). On the basis of weight of evidence the example of the contraceptive pill demonstrates that the mode of action of endocrine disruptors must have a clear threshold of adversity. Taking into account that the estrogenic potency of the contraceptive pill is by magnitudes greater than that of suspected chemical EDs, i.e. pill: 16675 estrogen equivalents (µg/d), organochlorines: 0.0000025 estrogen

equivalents (μg/d), severe adverse effects should be expected after exposure of infants in utero to daily intake of the contraceptive pill. It is assumed that while about 500 million women in US and EU use the pill annually, about 5% (25 million/year get pregnant while using it. However, no adverse effects were reported in male infants exposed in utero to the contraceptive pill (Hemminki et al, 1999; Ramlau-Hansen et al., 2009). In addition, taking the example of phytoestrogens with an estrogenic potency of up to 100x higher than that of chemicals or cosmetic ingredients (e.g. phthalates, parabens). Phytoestrogens (soya beans, cabbage etc) are consumed daily for long periods of time by billions of people without producing adverse effects.
It is also noteworthy that DES, although it is several times more potent than oestradiol, appears to be have a no- effect level in humans: human in utero exposure to maternal doses of a total of 1.4 g DES over 101 days (approximately 0.25 mg/kg/day), a huge dose in terms of oestrogenic potency, did not produce urogenital abnormalities or abnormal sperm parameters in male offspring (Fish,2000).
Finally, if the hypothesis that ED could be "special" regarding thresholds on the basis of their receptor-mediated modes of action were correct, then all substances with cell receptor affinities should lack thresholds. Our knowledge of pharmaceutical molecules (many of which are receptor- or subreceptor-specific) clearly shows that a critical mass of receptor occupancy is needed to generate a pharmacologically relevant effect.

2. Discussion on what are the uncertainties related to identification of endocrine disruptors and to determination of their thresholds (including comparison with the uncertainties faced with other chemicals)

What uncertainties exist when performing hazard assessment of endocrine disruptors and when setting threshold?

There is no scientific evidence that EDs should be treated in a different way compared to other chemicals of concern. As such, the uncertainties that exist when performing hazard assessment for EDs are similar to uncertainties for other toxicants. These uncertainties are addressed in many ways (e.g. applying safety and extrapolation factors) to identify safe regulatory levels at which chemicals, including EDs, can be used.
There is some concern regarding the lack of testing methods to identify EDs, which can cover the entire scope of the endocrine system, other than the typical EATS. However, as stated in the EFSA Opinion published in March 2013, "despite the fact that the existing internationally standardized assays might miss some endocrine-sensitive endpoints, this should not necessary lead to the non-identification of EDs. Given the complexity of the endocrine system with its multiple signaling pathways and cross-talks, an ED is expected to produce a pleiotropic response with a range of effects, some of which are likely to be observed in an appropriate guideline study" (EFSA, 2013). Moreover, the existing OECD framework to test and assess chemicals for endocrine disrupting properties, updated in 2012, provides a tool box in which the various test methods, useful for the identification of EDs are placed. This framework also defines tests to evaluate dose-response relationships and adverse effects.
An additional uncertainty mentioned several times is the alleged "low dose" hypothesis and the fact that EDs might present a non monotonic dose response

curve. Thus, it is claimed that the current testing methods used for other chemicals would not apply for EDs as the threshold identified through these methods would not be correct.
However, there is no consensus in the scientific community regarding the existence of low dose effects and particularly, how they translate into adverse effects. At least there is a general agreement that low dose and non monotonic dose response relations are not equivalent (as stated in the EFSA Opinion), and above all not limited to endocrine disruptors. Moreover, the existence of these phenomena does not imply the absence of thresholds.

Are the uncertainties faced or their combination when performing hazard assessment of EDs higher than with other chemicals?

Again, on the basis of the WHO/IPCS definition an endocrine disruptor has to demonstrate adverse effects (pathology or functional impairment) in an intact organism. Interference with the endocrine system is a mechanism of action and not an adverse effect itself. An adverse effect on the organ or tissue level clearly has a threshold which can be determined using accepted test methods. This is valid for acute, subchronic as well as long-term effects and also applies to effects on reproduction. These testing methods are designed to provide data to establish a NOAEL (No Observable Adverse Effect Level) and thus a threshold. Given the nature of the design of toxicological experiments where only a limited number of dose levels can be used, the true threshold is often significantly higher than the threshold determined in the tests.
In conclusion, there is no reason to assume that levels of uncertainty are generally different for EDs compared to other substances of concern.

Are the current, recognized and available methods sensitive enough to detect EDs and to provide enough information on the mode of action? Is it possible with the current methods to determine thresholds of adversity? Can we use those methods to perform hazard assessment of EDs?

Standard regulatory *in vivo* toxicity studies are considered sensitive enough to detect substances with an endocrine-mediated mode of action including the definition of a threshold of adversity. Under the Long Range Initiative (LRI) a reproduction toxicity study was conducted including standard regulatory and additional endpoints to verify whether traditional dose-response assumptions and risk assessment regimes are still valid. In this study, an effect dose, a NOAEL dose and the ADI dose (acceptable daily intake) were administered. No effects were seen at the ADI dose confirming the safety of the use levels established from regulatory end points for a pesticide with a potential endocrine mode of action. The additionally added parameters (e.g. hormone levels) were not more sensitive compared to standard regulatory endpoints like reproduction organ weights. Clear thresholds and dose-responses were observed (BASF, 2013). The endocrine system is comparable across many species including humans. However, the physiology such as the extent of hormone levels during pregnancy is different across species resulting in species-specific sensitivity. This has been confirmed by studies comparing the oestrogenic effects of phytohormones in

rodents, humans and non-human primates (Cline et al.,2001). In terms of sensitivity, rodents are more sensitive to endocrine disruptors than humans, because rodents and humans differ considerably with regard to hormonal physiology during gestation (extent of involvement and hormonal control of the corpus luteum, organs involved in progestin and estrogen secretion, the specific estrogens produced, and estrogen blood levels attained in the mother and embryo). For example, soy phytoestrogens are known to affect fertility and the foetus in rodents, while they were shown not to induce oestrogenic effects in humans or non-human primates at dietary levels (Cline et al., 2001). On the basis of these species differences (particularly, the markedly higher estrogen levels attained in human pregnancy compared to the mouse), it would appear unlikely that low doses of EDC of low potency produce adverse endocrine disruptive effects during human pregnancy (Witorsch 2002, Food Chem Toxicol 40: 905-912).

3. Discussion on whether a safe threshold can be determined with reasonable certainty for endocrine disruptors (including comparison with other chemicals)

Is it possible, on a case by case analysis, depending on the data set available for the substances, considering all available data and using expert judgement, to determine a threshold of adversity when the substance would seem to exhibit a threshold behaviour for some end points?

Yes it is. The current approach of the authorities is to consider whether a safe threshold can be set on a case by case basis for each of these substances. In some cases depending on the available data, it is not possible to establish a safe threshold, in other cases it is. There is no scientific basis for abandoning this case-by-case assessment of substances, and introducing an 'a priori' assumption that a safe threshold cannot be set in any case. Each substance should be evaluated by competent authorities and Scientific Committees, and all available data should be taken into consideration for a true weight of evidence approach.

is it more difficult to set safe thresholds for EDs with reasonable certainty compared to other substances of concern?

No it is not more difficult to set safe thresholds for EDs. The essence of a safe threshold is that it is safe: that is to say, it is a level of exposure at which a substance does not have any adverse effect. As a general rule, such a level can be determined with reasonable certainty for endocrine disruptors (as for any substance) by applying standard toxicological methodology. There is no reason to compare endocrine disruptors for example with carcinogens and mutagens for which the adversity may be readily demonstrated.

4. Regulatory identification of EDs and discussion on any other arguments for and against considering endocrine disruptors as non-thresholds chemicals (e.g. whether or not are endocrine disruptors of particular concern, etc)

What are the views of participants on a 'a priori' approach for EDs?

We believe that safe levels can be defined on a case by case situation avoiding the use of the "a priori" approach. A substance can only be called an endocrine disruptor if an adverse effect can be observed. Interference with the endocrine system is a mechanism of action and not an adverse effect itself. An adverse effect on the organ or tissue level clearly has a threshold which can be determined using accepted test methods. This is valid for acute, subchronic as well as long-term effects and also applies to effects on reproduction. These testing methods are designed to provide data to establish a NOAEL (No Observable Adverse Effect Level) and thus a threshold. Given the nature of the design of toxicological experiments where only a limited number of dose levels can be used, the true threshold is often significantly higher than the threshold determined in the tests.
Taking into account the state of science pertaining to endocrine disruptors and their effects we are convinced that these substances are sufficiently safe. This includes the identification of adverse effects and the derivation of suitable limit values. Like for any other mode of action, a science based approach should be used to evaluate whether each chemical presents or not a safe threshold. There is no scientific reason to consider substances exhibiting endocrine disrupting effects to be non-threshold substances.

What are the views of participants on allowing a case by case science based approach, using weight of evidence, expert judgment and all available data?

Taking into account the diversity of the endocrine system, potency criteria and because endocrine disruption is a mode of action rather than an endpoint, endocrine disruptors have to be evaluated individually case by case. In some cases due to data gaps, it will not be possible to establish a safe threshold. There is no scientific basis for abandoning this case-by-case assessment of substances, and introducing an 'a priori' assumption that a safe threshold cannot be set in any case.

What are the implications (e.g. Socio Economic) of such views, for all legislations?

The Cosmetics Regulation is based on the principle of risk assessment including both hazard and exposure. Considering EDs as non-threshold substances would result in reduced use or complete loss of ingredients which are virtually safe for the consumer. The loss of ingredients will have a severe impact on industry including increasing costs, loss of products and loss of employment. Consumer safety will be affected by loss of preservatives or loss of innovative ingredients such as UV-filters to protect against the increasing rate of skin cancer in the Western hemisphere.

What are the views of the participants if with the current test methods we can identify a threshold for EDs if it exists and if such a threshold is detected, does it provide enough safety (covering all relevant end points) to regulate on?

Yes, the current test methods cover all relevant endpoints and are able to identify thresholds for EDs providing sufficient protection for both consumers and environment. For the risk assessment generally the NOAEL of the most sensitive species and the most sensitive test system is taken plus safety factors translating the experimental threshold into a rather conservative regulatory threshold. This process uses expert judgement and "worst-case" assumptions and thereby guarantees utmost safety for consumer and environment.

What other arguments exist for and against considering endocrine disruptors as non-thresholds chemicals?