

# COSMETIC INGREDIENT REVIEW

## CIR Precedents

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Endocrine Activity

9/2017

This document is a compilation of issues discussed by the CIR Expert Panel along with precedent language used in CIR Reports to articulate the Panel's views. Standard formats used in Panel Reports are also addressed. This is intended to provide background on issues and serve as a reference explaining the reasoning behind previous Panel decisions.

## BACKGROUND

Concerns have been growing over the past several decades about the potential for exposures to some chemicals to cause adverse health effects by altering the normal functioning of the human endocrine system. The Cosmetic Ingredient Review (CIR) continually monitors developments of the research and the regulation of such substances as a matter of long-standing policy. CIR safety assessment reports include data from *in vitro* (e.g., estrogen-receptor binding) and *in vivo* (e.g., uterotrophic) assays that address the potential for ingredients to bind to and interact with endocrine receptors and other components of the endocrine system, as well as reproductive toxicity studies that identify adverse responses for safety assessment.

The CIR Expert Panel considers ingredients that have demonstrated endocrine activity in such tests as potential endocrine disrupting chemicals (EDCs), depending on the relevance, quality and concordance of the available studies, the doses and concentrations tested and the dose- or concentration-response relationships observed in such studies, the affinities of the ingredients for endocrine receptors or other components of the endocrine system, the potency of endocrine-active ingredients compared with endogenous hormones, and other important factors that contribute to an assessment of the overall weight-of-the-evidence (WoE). Such assessments depend, at the outset, on a clear definition of what constitutes an EDC, understanding of the distinction between endocrine activity and endocrine disruption, and differentiation of endocrine-mediated effects from other likely mechanisms of action (MOAs).

These factors are discussed in greater detail below.

### Definitions and Distinctions

In 2002, the World Health Organization (WHO) International Program on Chemical Safety (IPCS) defined an EDC as “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.”<sup>1</sup> By this definition, EDCs cause adverse health effects in living organisms specifically by altering the function of the endocrine system.

This definition has three important elements:<sup>2,3</sup>

- The substance must act through an endocrine MOA that alters the function of the endocrine system
- The substance must cause an adverse health effect
- The adverse effect must be causally related to, and occur as a consequence of, the altered endocrine function

All three of these elements are necessary to identify a chemical as an EDC.

The IPCS (2004) defines an adverse health effect as “change in morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influence.”<sup>2</sup> An “adverse effect” in this context means toxicity, including pathology or functional impairment, in an intact organism, their progeny or (sub)populations through a hormonal or hormone-like MOA.<sup>2,4</sup>

An adverse effect reflects exceedance of the body’s normal ability to modulate endocrine function adaptively.<sup>2,4</sup> An increase or decrease in endocrine activity does not indicate a health risk to a living organism, unless it can be shown to lead to harmful effects.

Endocrine disruption is distinct from endocrine activity, which is simply the ability of a chemical to interact with the endocrine system without necessarily posing a health risk.<sup>5</sup> The endocrine system is designed to respond to environmental fluctuations, and the responses are considered to be adaptive when they are transient and within the normal homeostatic range.<sup>2,6,7</sup> The responsive nature of the endocrine system is essential to health. Thus, the potential for interaction with the endocrine system is distinguished from the disruption of physiological or developmental processes that may result from such interactions.

Furthermore, *in vitro* data alone are not sufficient to classify an ingredient as an EDC. Endocrine activity observed in *in vitro* tests and some *in vivo* assays is not sufficient to classify a substance as an EDC if the tests do not indicate whether the alterations cause actual harm in a living organism or its offspring. Such a substance may be considered endocrine-active but not an EDC.<sup>2,8</sup>

Thus, for example, if the objective is to establish whether or not a test article is a reproductive EDC, it should be tested for its “reproductive” activity (i.e., the ability to alter the development or the function of the reproductive system) *in vivo*, rather than just for its sex-steroid activity *in vitro*.<sup>1</sup>

Many endocrine active substances may lack sufficient potency, compared to endogenous hormones, or exposures may be so low that no effects occur.<sup>5</sup> In other cases the body naturally adjusts, and the exposure causes no health effect.

### **Dose, Dose-Response and Potency**

Doses administered in experimental animal studies are often orders of magnitude greater than possible consumer exposures, to produce an effect on the endpoint(s) of interest.<sup>2,5</sup> Excessive doses of any chemical increase the chances of systemic toxicity and effects on endocrine endpoints that are mediated indirectly by other effects. Results from studies in which there is systemic toxicity cannot be used to identify and characterize the endocrine activity of a test substance. For example, alterations in endocrine function may be the indirect effects of weight loss caused by exposure to the substance.

The issue of dose-response relationships for EDCs at low doses continues to be highly controversial.<sup>1</sup> This is because, for example, EDCs often act by mimicking or antagonizing the actions of endogenous hormones. These hormones are typically substantially more potent than exogenous EDCs and are present in the body at physiologically-functional concentrations. Thus, dose-response relationships of EDCs are often different from those of other chemicals that do not act directly on the endocrine system. Consequently, dose-response relationships vary for different chemicals, endocrine mechanisms, and timing, frequency and duration of exposure. This is true for endocrine-mediated carcinogenesis and developmental, reproductive, immunological, and neurological effects.

The reported low-dose effects of EDCs have come under intense scrutiny concerning the adequacy of traditional toxicology testing paradigms for detecting low-dose effects.<sup>1</sup> Participants of a workshop addressing this issue concluded that low-dose effects often are not replicated consistently, and the toxicological significance of the reported effects is often questionable.<sup>9</sup>

At the receptor level, potency is determined by the affinity of a substance for binding to a receptor or other endocrine-system component and the efficacy with which it activates or inactivates the component.<sup>2,10</sup> Endogenous hormones characteristically exhibit strong binding affinity and high efficacy for activation of their corresponding receptors. Thus, endogenous hormones are potent modulators of endocrine function. In contrast, exogenous chemicals are rarely as potent as hormones because of lower affinity, lower efficacy, or both.<sup>2,11,12</sup> The presence of exogenous chemicals generally will not alter hormone binding to any significant extent at low doses, and biological thresholds for potency can be expected.<sup>2,10</sup>

The ability of a substance to produce a biological effect *in vivo* may be substantially different from the activity measured in *in vitro* assays.<sup>2,8</sup> *In vitro* studies can be relevant for investigating MOA and the potential for endocrine activity. However, *in vitro* tests may not provide useful information on dose-response relationships, do not take into account the toxicokinetics of a substance in the body (i.e., absorption, distribution, metabolism and elimination), and do not account for homeostasis or other pathways and processes that may be responsive to *in vivo* exposures. Thus, hazard identification may employ *in vitro* screening tests, but evidence of these effects must be verified *in vivo*.

It has been hypothesized that the combination of several weakly acting substances may be additive or synergistic and, thus, cause adverse effects.<sup>4</sup> However, such effects are improbable, based on theoretical and practical considerations.<sup>4,7,13</sup>

Assuming dose additivity for specific toxic effects presupposes that the chemicals in a mixture are true congeners, produce the same spectrum of biological effects by the same mode of action, are metabolized by the same biological

processes, and exhibit parallel dose-response curves.<sup>14</sup> There are numerous direct and indirect mechanisms by which substances may affect hormones or exert hormone-like activity, which limits the possibility of additive (“cocktail”) effects. The potential for additivity is reduced further by differences in toxicokinetic pathways, which are rarely, if ever, identical for different substances of this nature.<sup>4</sup>

Furthermore, there are major quantitative and qualitative differences in the affinities or activities of weak ligands for cell receptors, compared to those of strong ligands.<sup>4</sup> In principle, weak ligands may occupy and trigger cell receptors at high concentrations, but low concentrations of weak ligands will not influence the receptor binding or receptor-mediated effects of strong ligands.

For example, one study investigated the estrogenic responses to mixtures of synthetic chemicals combined with phytoestrogens at several concentrations in vitro and doses in vivo. The results showed that low concentrations or doses of the chemical mixtures failed to increase estrogenic responses, in vitro or in vivo, compared with the responses to phytoestrogens alone.<sup>4,15</sup> Significantly increased responses to phytoestrogens occurred only when each synthetic chemical was near or above its individual response threshold. In vitro, high concentrations of the synthetic chemicals in the presence of phytoestrogens yielded greater than additive responses, but mixtures of the chemicals in the absence of phytoestrogens produced less than additive responses. In vivo, the responses to high doses of the synthetic chemicals in the presence of phytoestrogens were consistent with additivity. The authors concluded that mixture effects are likely to be of concern only when the components of the mixture are present at or near their individual response thresholds.<sup>15</sup>

Thus, additivity may be limited to substances with moderate-to-high potencies at doses near their individual response thresholds, and is not likely for substances with low potencies or at low doses.<sup>3,4,14,15</sup> There is no evidence of additivity for such substances at exposures within the range of likely human exposures, despite the presence of thousands of natural, weak hormone-receptor agonists and antagonists in food and the environment.<sup>4,13,16</sup> Furthermore, there is no theoretical justification for extrapolating data from the high exposures tested in the available studies to assess the risks associated with exposures to the low doses that can reasonably be expected among consumers.<sup>4,14</sup> Most mixture studies are not relevant for evaluating human health safety or risk because human exposures are typically orders of magnitude lower than doses that cause detectable responses.

### **Mechanisms or Modes of Action (MOAs)**

As noted above, one of the three key elements of the definition of an EDC is that the chemical must act through an endocrine MOA that alters the function of the endocrine system. Thus, by this definition, it is important to know that the critical adverse effect of a chemical is caused by a primary or direct endocrine MOA, rather than the secondary or indirect manifestation of a non-endocrine MOA or non-endocrine toxicity, before it can be considered as a potential EDC.<sup>4</sup>

The possible primary or direct MOAs of EDCs include inhibition of hormone synthesis, transport, or metabolism and activation of receptors through processes such as receptor phosphorylation or the release of cellular complexes necessary for hormone action, in addition to direct interactions with hormone receptors.<sup>1,3</sup> Furthermore, multiple receptor systems act in concert (“cross talk”) to regulate biological functions. These, and many other factors, should be considered when considering mechanistic information on EDCs to support human health safety or risk assessments. Of particular concern are species, inter-individual, and tissue specificities in endocrine-signaling pathways.

The MOAs are poorly understood for most associations reported between exposure to EDCs and biological outcomes.<sup>1</sup> This makes it difficult to distinguish direct from indirect effects and primary from secondary effects of exposures. Although there is considerable information on the early molecular events involved in the responses to hormones, there is comparatively little known about the relationships between these molecular events and adverse health effects, such as cancer and reproductive toxicity.<sup>1</sup> This knowledge gap limits the ability to causally link an endocrine-specific MOA and an adverse effect. It will continue to be difficult to attribute adverse effects to endocrine-mediated pathways until such data become available. However, this knowledge gap does not preclude the assessment of safety or risk from appropriate studies from which relevant and reliable no-observed-adverse-effect levels (NOAELs) can be derived.

Considerable homology exists in the endocrinology of vertebrates. However, there are differences among some species in endocrine function that warrant consideration in safety and risk assessments.<sup>1</sup> For example, the role of specific hormones in reproductive function and development can vary substantially between human beings and non-mammalian test animals. In addition, species differences in metabolism can cause marked differences in responses to exposure. Thus, safety and risk assessments should address the significance of such differences, to the extent that the differences are known, and characterize the uncertainties associated with using data from animal studies to evaluate the potential for adverse health effects from the use of cosmetic ingredients.

### **Weight-of-Evidence (WoE) Assessment**

A WoE assessment is essential for determining the conditions under which observed effects of exposures can be attributed to an endocrine-mediated MOA.<sup>1,5</sup>

The critical elements of a WoE assessment include an assessment of the relevance, appropriateness, usefulness, quality and reliability of the studies available for informing the safety assessment process.<sup>5</sup> Also important is the evaluation of the consistency of the pattern of responses across studies for or against explicitly defined hypotheses. The hypotheses are typically defined to assess the premise that a substance interacts as an agonist or antagonist with components of the estrogen, androgen, or thyroid pathways or of the aromatase or steroidogenic enzyme systems, for example. One key concern is how dose responses observed in experimental animal studies compare to potential human exposures.<sup>2</sup>

Several additional factors that have been outlined for evaluating the available data against these hypotheses in an overall WoE assessment include temporality, strength of the association, biological gradient (i.e., dose response), biological plausibility, and evidence of recovery.<sup>2,17-19</sup> Furthermore, evaluating the MOA of a substance is critical because the MOA is central to the overall assessment of whether or not a substance can be considered to be an EDC.<sup>1,2</sup>

All of the relevant information should be considered in an organized and structured manner. The goal of this approach is to reconcile different results from different studies. (Cook et al., 1994).<sup>1,20</sup>

### **FRAMEWORK FOR DISCUSSION SECTIONS OF SAFETY ASSESSMENTS**

[INGREDIENT NAME(S) OR GROUP NAME, e.g., Trimethyl Pentanyl Diisobutyrate] ([CONCENTRATIONS OR DOSAGES TESTED; e.g., 0.001, 0.01, 0.1 and 1 mM]) was tested for endocrine receptor agonist and antagonist activity in [NATURE OF THE TESTS, e.g., multiple cell lines]. [Provide a brief statement of the overall results, e.g., the results were positive for hER1 agonist activity and negative for mAhR, hPPAR $\gamma$ , and hTR  $\beta$  agonist activity. All cell lines were negative for endocrine receptor antagonism]. These tests are not sufficient to characterize this/these ingredient(s) as endocrine disrupting chemicals (EDCs), based on scientific definitions and criteria developed to identify EDCs. A detailed summary and discussion of the Panel's approach to evaluating ingredients for the potential to act as endocrine disruptors is available at <http://www.cir-safety.org/cir-findings>.

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