Review and Evaluation of the California Environmental Protection Agency’s
"Green Chemistry Hazard Traits"

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1. Accuracy and clarity of the definitions presented

The definitions are presented in two different forms; in the Proposed Regulations as brief statements, and in the Initial Statement of Reasons (ISOR) as extended definitions with explanations and citations. The explanations and definitions are straightforward and clear, and the justifications provided in the ISOR are complete and appropriately cited. I recommend only a few changes or clarifications.

- In the Proposed Regulations definitions, “hazard traits” appear to be defined as toxicologic or environmental effects. However, “toxicological endpoint” is then defined in terms of hazard traits, which appears to be a bit circular. In contrast, “environmental endpoints” are defined by their effects, without reference to traits. It is recommended that “for a specific hazard trait” be deleted from the toxicological definition so that it more closely parallels the environmental definition.

- There is also a lack of parallel between environmental and toxicological in the ISOR definitions and explanations. Under ‘environmental endpoints’, ISOR contains the explanation “Finding environmental endpoints … is evidence that a chemical has one of the environmental hazard traits.” [Subsection 69401.2(d)] In contrast, “The toxicological endpoint is an adverse manifestation of the trait ….” [§ 69401.2(h)] The two definitions should parallel each other. There is a difference between being evidence of a trait (i.e., associated with) and being a manifestation of that trait (i.e., causal).

- It is curious that “radioactive agent” is included in the “chemical substance” [§ 69401.2(c)] definition. This listing, which also includes degradation by-products, would, by its wording, extend to cover radioactivity, itself, separate from its source. However, radioactive agents are not mentioned in the expanded ISOR definitions. If radioactivity by-products (specifically, different types of radiation) are to be specifically identified in the Regulations, they also need to be addressed, and the exact substances defined and delimited (i.e., is radioactivity, apart from its source substances, included in the degradation by-products) in the ISOR.

- “Well-conducted scientific studies.” [§ 69401.2(i)] It should be specified that the studies be peer-reviewed, to distinguish them from studies published in other, less scientifically rigorous media, or presented only at conferences. Studies published as non-peer-reviewed articles may be used if they meet the criteria (i.e., “using methods and analyses which are scientifically valid …”) described in the definition.

- The ISOR makes a good point with regard to the use of well-designed and conducted studies even if they are not GLP. A similar comment can be made regarding studies that are not strictly according to OECD or USEPA Test Guidelines. Many research organizations test chemicals for non-regulatory submissions, and their published studies may be considered by the regulatory authorities when dealing with the particular test substance. Other organizations
may have valid data that predate the formal guidelines. If the study is well performed, it should not be discarded because it was not strictly according to Guidelines, for example, if one or two fewer animals or Ames tester strains were used, or the requisite number of repeat tests were not conducted.

- As a general comment, the term ‘trait(s)’, as it is applied in these regulations, appears to be specific to California regulations and is not, to the best I can determine, used anywhere else to describe adverse biological or ecological properties of substances. “Trait” also implies that the indicated toxic or ecological effect is an inherent property of the substance. This is not only misleading but also potentially alarming. There are many chemicals (that have hazard traits) that are required to sustain life and/or are produced during normal metabolism. The Agency might want to consider adopting a different term.
2. Selection of the toxicological hazard traits

The selection of the toxicological hazard traits appears reasonable and inclusive of health effects of concern, and of many of the mechanisms leading to those health effects. For my evaluation, I concentrated on those traits with which I am most knowledgeable – carcinogenicity, developmental toxicology, reproductive toxicology, endocrine toxicology, genotoxicity, and reactivity in biological systems.

- Carcinogenicity. The ISOR defines carcinogenicity as malignancy [§ 69402.1(a)] based on the IARC definition, whereas the Proposed Regulations also includes benign [§ 69402.1(b)] tumors. Although as noted in the ISOR, benign tumors are considered by agencies in arriving at a carcinogenicity classification, the text does not indicate whether benign tumors, by themselves, are sufficient for the classification. The blending of US (i.e., NTP, EPA) and international (IARC) rules, can be confusing because, in general, the US agencies will consider benign tumors, by themselves, as sufficient for classifying a substance as a carcinogen, whereas the IARC will also require malignancy. The role and contribution of benign tumors, and whether they can stand alone, to the classification needs to be better defined.

- The Proposed Regulations identify a number of findings that would lead to a classification of “suggestive evidence of carcinogenicity.” The first three of these [§§ 69402.2(b)(1)(2)(3)] are based on evidence of cancer in animals, and are valid, whereas the next three [§§ 69402.2(b)(4)(5)(6)] are based solely on the mechanistic considerations of genotoxicity, general mechanistic evidence, and QSAR methods. Although, I agree that positive results in these latter three procedures can constitute suggestive evidence of carcinogenicity, unlike the animal tests, they are not tests for carcinogenicity and not proof of carcinogenicity. As the Proposed Regulations are currently worded, I am concerned that a result from one of these mechanistic studies suggesting a positive response could be given equal weight to an animal study showing a positive response. This is not justifiable given the variety of different studies and systems (especially genotoxicity and QSAR) and their varying levels of predictability for carcinogenicity.

- Although IARC’s use of mechanistic information is cited as a justification for the inclusion of these studies, IARC does not use these studies for classification, but only to support an up- or down-grade in classification when there are existing, positive animal cancer studies. It is recommended that these three mechanistic categories be considered, as is done by IARC, as supporting an upgrade in classification when there is an existing, positive animal cancer study, and not at the same level of evidence as existing animal studies.

- One example of mechanistic evidence presented in the ISOR is the metabolism of a wide variety of benzidine-based dyes to benzidine, which is a known human carcinogen. This ‘mechanism-based’ example is accurate and valid, but in a
completely different category than, for example, ‘changes in physiology.’ In the benzidine example, the mechanistic studies show that regardless of the dye administered, the test substance is actually benzidine. In contrast, the physiological changes, as well as genetic damage, are events leading to the development of cancer, but are far from sufficient, and far less persuasive (than the benzidine example) for induction of cancer.

- The QSAR explanation in ISOR [§ 69402.2(b)(6)] reads as if EPA’s OncoLogic program is being recommended to the exclusion of many other programs that may be no less robust. Although those other programs may be proprietary, they are widely used and relied upon by industry and US Government regulatory bodies. I agree that proprietary systems should not be mentioned by name because it would imply endorsement of the named systems to the exclusion of others that may not be named. However, it should be made clear that other proprietary and non-proprietary systems are equally acceptable; the only limitation should be supportive evidence showing their predictive effectiveness for the effect in question.

- Developmental toxicity. Similar to my comments regarding Carcinogenicity mechanistic studies [§ 69402.4(b)], evidence from mechanistic and QSAR studies [subsections (4)-(7)] should be assigned less weight than findings in animal tests [subsections (1)-(3)].

- Reproductive toxicity. The ISOR [§ 69402.5(a); on pg. 40, last paragraph] correctly notes that developmental toxicology is a component of reproductive toxicology. It should also be noted that endocrine toxicology is a major component of reproductive toxicology (this information should also be included in the Endocrine Toxicology section [§ 69403.2]), and the inter-relationships of endocrine toxicology with reproductive and developmental toxicology should be noted. Many of the endpoints of male and female reproductive toxicity are the same as those used for evaluating male and female endocrine disruption.

- The effects constituting ‘suggestive evidence’ are appropriate, with the exception of the QSAR indication [§ 69402.6(b)(6)]. Evidence from such systems are less well developed than the QSAR systems for carcinogenicity and genotoxicity, and should not be afforded equal status to results of animal studies, or even to results from relevant mechanistic studies absent convincing evidence of their effectiveness.

- Endocrine toxicity. This section [§ 69403.3] of the ISOR should refer back to the overlap between endocrine toxicity and developmental and reproductive toxicities.

- Although I do not provide specific comments on epigenetic toxicology [§ 69403.4], I have concerns with its incorporation among the other toxicity traits. Epigenetic changes are a required, normal component of organism development and not hazardous, per se. Although epigenetic effects are emerging as an important
mechanism of toxicity, to the best of my knowledge there are, as of now, no standard
tests to measure such effects and no clear consensus on what changes or level of
change, and at what life stage of the organism, would constitute an adverse effect. I
am concerned that, for example, a chemical such as the amino acid, methionine,
which is involved with DNA methylation, would be considered hazardous because
changes in dietary levels could lead to changes in DNA methylation state based on
animal or in vitro tests. At the present time, I believe it is premature to list epigenetic
effects as a hazard trait equivalent to the other traits in this section [§ 69403].

• Genotoxicity. The inclusion of sister chromatid exchanges (SCE) on a equal footing
with the other genetic endpoints [§69403.5(b)] is not recommended. Unlike the other
endpoints identified, less is known about the causes or consequences of SCE. They
are also known to often be formed in the absence of direct DNA damage or as a
consequence of test protocol factors and artifacts. This has led to the decision by the
OECD to ‘retire’ its 1987 SCE Test Guideline, and to the absence of SCE tests from
those currently considered or recommended by regulatory authorities in the US and
elsewhere. The current thinking about SCE is that they may serve as a biomarker of
chemical exposure, or as an indicator of non-specific DNA damage. As such, SCE
are more appropriately placed with the commonly used DNA damage endpoints. As
a result, the 2nd sentence in the first paragraph on pg. 64 of the ISOR (“It is common
to perform mammalian evaluation of SCE in vivo or in vitro.”) is incorrect and should
be deleted.

− Bacterial DNA damage is currently listed among the “commonly used” assays of
DNA damage. This endpoint should be deleted because the tests used have been
shown to lack reproducibility across laboratories and some of them are not well
defined genetically, i.e., the repair-proficient and repair-deficient cell lines are not
isogenic. Another factor to consider is that the tests are no longer being performed
routinely except, perhaps, as an initial screen prior to performing standard genetic
toxicity tests.

− Reactivity in Biological Systems. This trait is not well defined or delimited. The
ability to “catalyze electron transfer” [§ 69403.14(b)] can refer to a myriad of natural,
and endogenous chemicals, including some vitamins, and processes vital for
sustaining life. The Proposed Regulations should be carefully written so that
molecules such as vitamins A and C, and vital cellular constituents such as cysteine
and glutathione, and not considered to have this trait. Alternatively, or in addition,
there should be a clear statement that the presence of this trait may just as often be
beneficial as hazardous and that, unlike traits such as genotoxicity and developmental
toxicity, the determination of hazard from electron transfer activity is highly context-
dependent.
3. Selection of the environmental hazard traits

This is an area that I am not qualified to address.
4. Selection of the exposure potential hazard traits

The only areas in this section that I feel qualified to address are bioaccumulation and lactational or transplacental transfer.

Bioaccumulation [§69405.2]. The definition and criteria appear to be adequate.

Lactational or Transplacental Transfer [§69405.5]. The definition and criteria appear to be adequate.
5. Selection of the physical hazard traits

This is an area that I am not qualified to address.
6. Methodology for identifying strong evidence and suggestive evidence for toxicological and environmental hazard traits

My comments on the methodologies for identifying evidence for toxicological hazard traits have been included in my comments on the individual hazard traits. The methodologies presented for identifying strong evidence are valid and appropriate. My concerns, however, are with the presentations of methodologies for identifying suggestive evidence.

In general, the various methods listed are all valid, but are associated with widely different levels of assurance regarding the specific trait. For example, as noted in my above comments, as the Proposed Regulations are currently written, results of an animal test for the apical endpoint, and a QSAR prediction, may be given equal weight as suggestive evidence (for human hazard). While I agree that they can both provide suggestive evidence, and be supportive of strong evidence, they do not merit, and should not be given, equal weight in the absence of strong evidence.

Another issue to be considered is that not all tests that can be used to address the same hazard trait are equal or equivalent in their abilities to identify or predict the trait. As examples, there are many in vitro and in silico tests currently available that are designed to measure what are believed to be events leading to the development of a tumor, but there is limited information from the majority of these tests as to their predictivity or their relevance for the apical endpoint. For example, in the area of genotoxicity, whereas gene mutations are considered highly predictive for cancer, tests for effects such as sister chromatid exchanges in vitro or in vivo, and chromosome aberrations in vitro, are considered to yield high positive rates but be poorly predictive for other genotoxic effects or cancer. Similarly, the QSAR systems available for genotoxic prediction can have widely differing predictive abilities depending on the algorithms they are based on, and the training sets used to develop and maintain the systems.

The ISOR, and possibly the Proposed Regulations, should recognize these differences in effectiveness among test procedures designed to address the various suggestive evidence endpoints, and caution that information on the reliability and relevance of the particular procedure, or test endpoint, needs to considered when evaluating test results or data for suggestive evidence.