Scientific Peer Review of a) Green Chemistry Hazard Traits Regulation and b) Initial Statement of Reasons:

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Introduction:

The objective as presented in the text of the Proposed Regulations and Initial Statement of Reasons of Chapter 54 on Green Chemistry Hazard Traits to be added to division 4.5 of the California Code of Regulations is "to evaluate and specify the hazard traits, toxicological and environmental endpoints and any other relevant data to be included" in the Toxics Information Clearinghouse. Based on the information provided, it is understood that this Clearinghouse will "provide a decentralized, Web-based system for the collection, maintenance and distribution of specific chemical hazard trait and environmental and toxicological endpoint data" to be available to agencies, the public and industry and government scientists and engineers evaluating chemicals in consumer products.

Comments presented here are based on understanding within this context and experience principally in human health risk assessment.

With few exceptions, the proposed regulations seem thoughtful and inclusive of relevant initiatives, nationally and internationally and reflect considerable prior input (including 4 workshops and public comment). The objective to increase public availability of information on chemical hazards through a Clearinghouse on toxicity in order to provide scientific information as a basis to evaluate chemicals in consumer products is laudable, as are critically important envisaged associated activities of prioritizing chemicals and analysis of alternatives.

And while the objectives are laudable, the bounds of the extent of consideration of hazard in development of the Clearinghouse is (perhaps, understandably) limited to identification of "intrinsic" properties, only, without specification of conditions under which identified hazards are likely to be expressed. While this may not be maximally informative in subsequent establishment of priorities, the need for early designation of undesirable intrinsic hazard traits as an important component in strategies to promote safer alternatives is recognized. However, this necessarily requires collective consideration of more predictive parameters versus toxicological test results, as a basis to more meaningfully address the significant numbers of substances in commerce for which toxicological data are limited. Several of the comments offered here address strategies and tools to meet this longer term objective, as a basis to avoid continuing bias to consideration of data rich substances.

Accuracy and Clarity of the definitions presented

For the most part, the included definitions are clear, being based on thoughtful and inclusive consideration of those used nationally and internationally with transparent and reasoned rationale

(pages 4 – 5 of the Proposed Regs, pages 7 – 19, Initial Statement of Reasons). A few suggestions for clarification and one addition are noted below.

While it is clearly indicated that the ***hazard traits*** included in the framework address intrinsic properties of a chemical substance, excluding dose-response and exposure estimation (pages 14 and 15 of the Initial Statement of Reasons), the specific focus of the content of the Clearinghouse in the context of hazard identification versus hazard characterization is unclear. While the framework seems progressive in recognizing "mechanistic similarity" and the implied importance of understanding how chemicals induce effects as a basis to be more predictive of human health risk, it is silent currently on how hazard and mechanistic data are appropriately combined to characterize hazard in a more predictive sense.

It is also indicated (page 8 of the Initial Statement of Reasons) that any perturbation that would lead to toxicity and disease would be considered an ***"adverse effect"*** in the context of the U.S. EPA definition proposed for adoption in the Regulations versus any measurable effect. In fact, toxicity is expected to result from sufficient perturbation of homeostasis (i.e., a cascade of failures of normal biological control mechanisms leading to disease). It seems unlikely, then, that early perturbations can be considered adverse in their own right but potentially with sustained exposure, could lead to adverse effect.

Supporting documentation in the Initial Statement of Reasons (page 9), relevant to the definition of ***"authoritative organization"*** references "legal and administrative processes" applied to help ensure validity of their products. While some examples are provided concerning the nature of process that ensures scientific integrity (e.g., NAS, the federal government), there is no clear delineation of criteria for acceptability of products of authoritative organizations. These could include, for example, transparency of process, transparency and inclusiveness of identification of relevant data, nature of peer engagement including peer input, consultation and review in addition to public availability of products. Specification of these criteria would increase transparency of prioritization of classifications of different agencies considered in the context of strong and suggestive evidence for various endpoints. (Note also that the "French" government is erroneously distinguished here from European governments on page 9 of the Initial Statement of Reasons).

In relation to ***"well conducted scientific studies"*** (page 5 of the proposed regulations, page 18 of the Initial Statement of Reasons), I applaud particularly, proposal not to require that a study be conducted in accordance with Good Laboratory Practice (GLP) as a basis to evaluate hazard, in view of the desirability of acquiring data on the widest range of compounds and to more meaningfully focus on data relevant to more mode of action based predictive approaches.

In view of the need for more predictive technologies for hazard as a basis to avoid bias to data rich chemicals in the Clearinghouse, it is recommended that consideration be given to also referencing the definition of "adverse outcome pathway" developed by the U.S. EPA, as follows: "The documented, plausible, and testable processes by which a chemical induces molecular perturbations and the associated biological responses which describe how the molecular perturbations cause effects at the

subcellular, cellular, tissue, organ, whole animal and (when required) population levels of observation"[1]. The "adverse outcome pathway" including "molecular initiating events" (MIE) provides an extremely helpful construct in developing more predictive indicators of hazard consistent with the objectives of Green Chemistry approaches (see additional reference in comments below).

Selection of the Toxicological Hazard Traits

The list of toxicological hazard traits is long and relatively inclusive (n=14) framed predominantly on types of hazards identified in traditional toxicity testing studies in animals, with only one ("reactivity in biological systems") being more predictive in nature. Reliance on such a large number of traditional toxicological endpoints as a basis for identifying hazard traits (e.g., carcinogenicity, developmental and reproductive toxicity, cardiovascular toxicity, dermatotoxicity, hematotoxicity, hepatotoxicity etc.) versus simpler descriptors such as "reactivity in biological systems" seems likely to bias content of the Clearinghouse and as a result, priority setting and evaluation, to data rich substances.

In addition, while it is indicated in the Initial Statement of Reasons (page 21), that "Absence of data does not constitute absence of hazard", there is no indication of how chemicals with limited data will be addressed. Identification of the critical, simpler, more predictive undesirable parameters for hazard would seem to be one of the important objectives of Green Chemistry approaches. While it is recognized that establishment of repositories of information such as that envisaged in the Clearinghouse may be helpful in more meaningfully identifying some of these parameters, biasing content at the outset to data rich substances through the nature of delineated hazard traits will necessarily detract from this objective. Given the disparity of data available on hazard traits for different chemicals, it would be helpful to understand how it's envisaged to minimize content bias to data rich chemicals.

This seems an important aspect in addressing higher level objectives and one of the reasons that exposure parameters were weighted heavily in identification of priorities for assessment for human health priorities from amongst all 23, 000 entries on the Domestic Substances List in Canada. On the basis of relatively simple information available for all substances (i.e., simple use profiles and volume and locations of use), it was possible to relatively rank all 23, 000 Existing Substances in relation to their potential for exposure, ensuring their unbiased consideration.

Interestingly, also, in categorization (i.e., priority setting for assessment) of the Domestic Substances List in Canada, hazard classifications of authoritative organizations for carcinogenicity, genotoxicity and reproductive/developmental toxicity, where available, correlated well with reactivity of the compounds.

Specification of such a large number of toxicological hazard traits may also complicate codification of data from encompassing studies in the Clearinghouse. Consideration of patterns within and across systems is critical in interpretation of endpoint specific data and provides important clues about how chemicals may be inducing effects. For example, it's appropriately indicated in the Initial Statement of

[1] Ankley, G.T., et al. (2010). "Adverse Outcome Pathways: A Conceptual Framework to Support Ecotoxicology Research and Risk Assessment." Environmental Toxicology and Chemistry 29(3): 730-741.

Reasons (page 7) that "Thus some hazard traits are indicative of other hazard traits" with relevant examples being provided throughout the text (e.g., perturbation of the thyroid axis leading to neurodevelopmental effects. Pg. 37) but it is unclear how this interdependence will be universally addressed in a hazard characterization/mode of action context across endpoints.

For example, in a more predictive mode of action context, available data on patterns of carcinogenicity and genotoxicity are jointly considered as a basis to delineate potential molecular initiating (MIEs) and key events in a hypothesized adverse outcome pathway between exposure and tumours. The initial chemical – biological interaction can then be modelled, as a basis to be more predictive for a wider range of chemicals. The likelihood of mutation being an early, rate limiting key event (i.e., acting through a mutagenic mode of action) is also addressed in this manner.

Consideration of the interrelationship of endpoints (e.g., cancer and genotoxicity) is also critically important in considering weight of evidence analysis as a basis to interpret the output of existing predictive models, such as quantitative structure activity analysis, for both statistically based models and those with mechanistic underpinning (i.e., those where MIEs have been identified). Output, for example, for both cancer and genotoxicity is considered based on weight of evidence criteria such as consistency and biological plausibility; output of individual models is also weighted based on the nature of the relevant training sets and applicability domains, specificity, sensitivity, etc.

In addition, subdivision of the hazard traits into such a large number of different types of largely traditional toxicological endpoints could be considered somewhat incongruent with transition to more progressive testing strategies, to address earlier often common manifestations of effects relevant to several organ systems.

3. Selection of the Environmental Hazard Traits

While this is not my area of expertise, I note the interdependence of several of the toxicological hazard traits identified here to those related to human health (e.g., domesticated animal toxicity, wildlife growth, survival and reproductive impairment). Generic content of the comments on selection of the Toxicological Hazard Traits above apply here, also – e.g., bias to data rich substances, patterns within and across effects and the need for simple, predictive descriptors. In relation to the latter, it's unclear, for example, why "reactivity in biological systems" wouldn't also be relevant here.

4. Selection of the Exposure Potential Hazard Traits

Inclusion of surrogates of exposure as "hazard traits" for the Green Chemistry initiative is progressive and laudable. Their definition as «properties of chemicals that increase exposure of humans and wildlife once those chemicals are released into the environment" is also clear and appropriate.

The basis for enthusiastic support of their inclusion relates to experience in categorization of the Domestic Substances List in Canada[2], that surrogates of exposure are likely to be much more discriminating in priority setting than those related to hazard, *per se.* (i.e., There is much greater

[2] http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/final_framework-int-cadre-eng.php

variation in potential for exposure across chemicals than there is in potency for critical endpoints). In addition, availability of data on surrogates of exposure is likely to be much more even across chemicals (i.e., there is less likelihood of bias to data rich chemicals).

I wondered, though, whether other relatively simple parameters were considered for inclusion, such as molecular weight (preventing absorption), lipophilicity as measured by log octanol water partition coefficients (related to absorption, lactational transfer and bioavailability), and some measure of potential for release, given likely application in products (i.e., to what extent the substance is likely to be bound). For the latter aspect, it's recognized that "classification of a chemical substance as having a hazard trait does not depend on its potential uses" (Page 117, Initial Statement of Reasons). It's unclear, however, how use pattern analysis and likelihood of release in products will be addressed in priority setting and analysis. (Could this be addressed, for example, in the envisaged product ingredient network?).

5. Selection of the Physical Hazard Traits

Again, this lies outside of my area of expertise, but seemingly reflects important considerations relevant to potential hazards in consumer products. Harmonization with the GHS is helpful.

6. Methodology for Identifying Strong Evidence and Suggestive Evidence for Toxicological and Environmental Hazard Traits

Consideration of the strength of evidence for various hazard traits through distinction between strong and suggestive evidence is helpful. Inclusion of only two categories minimizes complexity, while indicating the need to consider and appropriately weight different types of information. Compatibility with global classification schemes has also seemingly been maximized, with justifiable rationale, where this is not the case.

Structuring on the basis of the framework used by the International Agency for Research on Cancer with endpoints that are manifestations of the trait and other relevant data that provide less direct evidence aligning in large measure with the strong and suggestive categories also seems appropriate. Reliance on assessments of other agencies, organized hierarchically followed by data, for which sources for the latter are also hierarchically considered moving from *in vivo* evidence in animal studies addressing hazard identification to mechanistic data to predictive models, also seems sensible.

Subdivision of the toxicological hazard traits into separate articles addressing carcinogenicity, developmental toxicity and reproductive toxicity versus other toxicological hazard traits, also seems appropriate, given the extent of consideration of classifications of the former by international and national agencies.

However, text in the Proposed Regulations and Initial Statement of Reasons is restricted to the nature of specific types of available data that may be considered in the category of "strong" or "suggestive" evidence. It does not address the critical interplay of all sources of data in robustly considering weight of evidence or the need for integration of mechanistic data at early stage as a basis to be more

predictive of risk. Important in this context are traditional criteria or factors for consideration of weight of evidence in hazard characterization which have been applied widely both nationally and internationally[3]. These include consistency[4], specificity and biological plausibility.

Guidance in considering the appropriate extent of evidence in the "suggestive" category is also limited. There is, for example, repeated reference to consideration of mechanistic data indicating hazard potential from cell-based, tissue-based or whole organism-based assays showing perturbation of known physiological, biochemical or other pathways. And while this is forward looking from the perspective of incorporating evolving methodologies to characterize adverse outcome pathways (i.e., key events in mode of action), criteria or factors to take into consideration in considering what constitutes meaningful perturbation are not specified. For example, there is reference (page 47 of the Initial Statement of Reasons) to endocrine toxicity via mechanisms likely to be involved in reproductive toxicity providing evidence of reproductive toxicity, though it is additionally considered that: "Single measurements of hormonal changes may be insensitive indicators of any damage because of large normal variability in females". There is also repeated reference in the Initial Statement of Reasons to results of gene expression arrays, principally in the context of understanding genes in various systems targeted by specific chemicals

In relation to "suggestive" evidence, there is also repeated reference to predictive (in particular, quantitative structure activity analysis) methods for, for example, carcinogenicity, developmental toxicity and reproductive toxicity. Reference is to "validated" Quantitative Structure Activity Analysis models with no indication of the basis for "validation" (This requires explicit delineation). In general, such models are not "validated" *per se;* rather, their appropriate application is considered in the context of specified purpose (priority setting, screening, etc.) and consideration of principles such as those specified by OECD including a defined endpoint, an unambiguous algorithm, a defined domain of applicability, appropriate measures of goodness-of–fit, robustness and predictivity and a mechanistic interpretation[5], if possible.

Factors to be considered in assessing collective weight of evidence of output of predictive models are also not addressed. These include mechanistic underpinning (i.e., identification of a molecular initiating event), the nature of the relevant training sets and applicability domains, specificity, sensitivity, etc. Interestingly, in this context, for example, there is no reference to QSAR, specifically, in relation to genotoxicity, where its application is most justified, in light of mechanistic underpinning and relatively large training sets, due to availability of results of *in vitro* assays.

It might also be helpful to characterize the difference in weight of evidence for strong vs. suggestive evidence in the context of degree of confidence/uncertainty. Similarly, I wondered if there had been

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[3] See for example, Boobis et al. (2008) Crit. Rev. Toxicol. 38 87-96 and http://epa.gov/cancerguidelines/.

[4] While not addressed explicitly, the need for some consistency in relation to "Other Toxicological Hazard Traits" is implicit in the specified need to have two or more well conducting scientific studies demonstrating the hazard trait.

[5]See, for example, http://www.oecd.org/dataoecd/33/37/37849783.pdf; http://www.oecd.org/dataoecd/45/52/40705314.pdf

any thought given to specification of relevant degrees of uncertainty for various datasets supporting hazard for inclusion in the Clearinghouse.

There is also repeated reference to structural and mechanistic similarity to known toxicants in relation to suggestive evidence for "Other Toxicological Hazard Traits" but no indication of international sources of information on category approaches[6] or how it might be used.

The Big Picture:

In reading the regulation, are there any important scientific issues relevant to identifying hazard traits that have not been addressed in response to the points listed above?

While there is recognition that methods to predict hazard are evolving, suggested content of the Clearinghouse for hazard traits is (likely necessarily) delineated primarily on the basis of methods and endpoints considered in traditional toxicity testing studies in animals. There is less development of considerations related to predictive properties as a basis for characterizing undesirable characteristics of chemicals, as a basis for minimizing bias to consideration of those which are data rich (see also comments in response to other questions above).

Taken as a whole, are the proposed hazard traits, examples of environmental and toxicological endpoints and other relevant data based on sound scientific knowledge, methods and practices?

It seems likely that content here could be additionally developed to address principles for transparent and consistent consideration of weight of evidence for hazard and to incorporate recent developments internationally in the development and application of tools such as quantitative structure activity analysis (see also comments in response to other questions above).

[6] See, for example, http://www.oecd.org/document/23/0,3343,en_2649_34365_33957015_1_1_1_1,00.html