INITIAL STATEMENT OF REASONS

Proposed Division 4.5, Title 22, Cal. Code of Regulations, Chapter 54
Green Chemistry Hazard Traits

Table of Contents

I. Purpose

Health and Safety Code section 25256.1 requires the Office of Environmental Health Hazard Assessment (OEHHA) "to evaluate and specify the hazard traits, toxicological and environmental endpoints, and any other relevant data to be included" in a Toxics Information Clearinghouse ("Clearinghouse"). The primary purpose of the Clearinghouse is to serve as a web-based portal for information on the toxicity of chemicals in commerce. The Clearinghouse will be developed and maintained by the Department of Toxic Substances Control (DTSC).[1]

The law envisions that the 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." As such, it will provide basic scientific information that will be available to agencies, the public, industry and government scientists and engineers evaluating chemicals in consumer products.

This information is applicable to a multimedia context in that it may be used to evaluate chemicals in consumer products that may be released into air, water, soil, or otherwise contaminate the environment. The Clearinghouse will include information developed from studies in humans, animals, tissues, cells, cell components, and ecosystems.

Health and Safety Code section 25252 requires DTSC[2] to evaluate and prioritize chemicals by developing criteria that include, but are not limited to, traits, characteristics, and endpoints, developed by OEHHA, for the Toxics Information Clearinghouse established under Health and Safety Code section 25256.1.

This proposed regulation would implement OEHHA's mandate under Health and Safety Code section 25256.1. The regulation:

- identifies and defines specific hazard traits
- identifies four general categories of hazard traits: toxicological, environmental, exposure potential and physical
- lists non-exclusive general categories of endpoints for each toxicological and environmental hazard trait
- lists non-exclusive general categories of "other relevant data" for each toxicological and environmental hazard trait

[1] See Health and Safety Code section 25251, *et seq*.
[2] Assembly Bill 1879, Feuer, Chaptered September 2008, codified at Health and Safety Code section 25252 *et seq* and Senate Bill 509, Simitian, Chaptered September 2008, codified at Health and Safety Code section 25251 *et seq*.

- shows how endpoint and other relevant data can be used as evidence in evaluating whether or not a chemical substance has a hazard trait
- shows how data can be used to evaluate whether or not a chemical substance has an exposure potential or physical hazard trait

Health and Safety Code section 25256.1 requires OEHHA to evaluate hazard traits after one or more public workshops. OEHHA conducted four public workshops related to this mandate. The first workshop was conducted in Sacramento in January 2009 to receive preliminary ideas about hazard traits, endpoints and any other relevant data to include in the Toxics Information Clearinghouse.

Two workshops, conducted in March and May, 2010, explored the science underlying hazard traits. These two workshops were conducted in collaboration with the following University of California (UC) entities: UC Center for Occupational and Environmental Health, UCLA Law and Environmental Health Sustainable Technology Policy Program, UC Berkeley Center for Green Chemistry, and the UC Toxic Substances Research and Teaching Program. OEHHA invited scientific experts from the federal government, academia, industry and environmental groups to make presentations, provide advice and otherwise participate in these workshops.

The fourth workshop was conducted on August 23, 2010, in conjunction with the release of a pre-regulatory draft proposal. OEHHA developed and released the pre-regulatory draft to provide the workshop material for a substantive discussion of any questions or concerns about the draft, prior to the commencement of the formal regulatory process.

In addition to the workshop, the public was invited to submit written comments on the pre-regulatory draft regulation. The public comment period lasted from August 11 to September 13, 2010. The workshop was well-attended and many written comments were received. OEHHA carefully reviewed the public comments received when developing this proposed regulation.

The information OEHHA received from these workshops helped inform OEHHA's efforts when developing the proposed regulation. Some of the general comments OEHHA received on the pre-regulatory draft are discussed in this statement of reasons in order to explain why OEHHA chose one path over another to develop specific provisions of the regulation. All the written public comments received by OEHHA during the pre-regulatory comment period are available for public inspection.

Further, OEHHA developed the proposed regulation in consultation with DTSC and other state agencies. This proposed regulation complements the regulations currently proposed by DTSC for Chapter 53[3].

II. Effort to Avoid Duplication or Conflicts with Federal Regulations

The proposed regulations do not duplicate or conflict with existing federal regulations. No federal regulation comprehensively establishes hazard traits, endpoints and other relevant data to be included in a web-based database. Further, according to DTSC's Initial Statement of Reasons for Chapter 53, California's Green Chemistry Initiative was developed, to a great extent, to address structural weaknesses in the federal Toxic Substances Control Act of 1976 ("TSCA").

As discussed below, the proposed regulation, to the extent possible, relies on designations, criteria, definitions and practices of federal, California and international regulatory and public health bodies as allowed by Health and Safety Code section 25256.1. These proposed regulations do not duplicate or conflict with any of the federal regulations reviewed. They are complementary to them or expand the concepts embodied in those regulations. Among other things, references to specific federal sources that were used in the development of the proposed regulation are provided in the footnotes.

III. Studies Relied On

Chapter 54 relies upon general principles and concepts of toxicology and risk assessment, standard guidance, criteria and practices used by national and international bodies, including documents produced by the National Toxicology Program, U.S. Environmental Protection Agency, World Health Organization, and the International Agency for Research on Cancer. The peer-reviewed scientific literature and key texts used by academic institutions to teach toxicology and risk assessment were also used. This statement of reasons cites the documents consulted, and OEHHA will include them in the administrative record for the regulation.

IV. Alternatives Considered

In accordance with Government Code subsection 11346.5(a)(13), OEHHA has determined that no reasonable alternative considered by OEHHA, or that has otherwise been identified and brought to the attention of OEHHA, would be more effective in

[3] Department of Toxic Substances Control, Text of Proposed Regulations, Division 4.5, Title 22, California Code of Regulations, Chapter 53. Safer Consumer Product Alternatives, post hearing version, November 2010, available at: http://www.dtsc.ca.gov/LawsRegsPolicies/upload/SCPA_Regs_15Day_Revisions_COURTESYCLEAN.pdf

carrying out the purpose for which this action is proposed, or would be as effective and less burdensome to affected private persons than the proposed action. In addition, the proposed regulation imposes no requirements on any person or business since it only identifies hazard traits, endpoints and other relevant data that DTSC will use in its development of the Toxics Information Clearinghouse.

V. Detailed Discussion of the Proposed Regulations

Article 1. General

The information contained in Article 1 is necessary in order to understand the structure and meaning of the subsequent Articles. It contains explanations of the approach and defines critical terms used in the subsequent articles.

§ 69401 Purpose and Applicability

Section 69401 explains that this regulation was developed in response to the legislative mandate in Health and Safety Code section 25256.1 for OEHHA "to evaluate and specify the hazard traits, toxicological and environmental endpoints, and any other relevant data to be included" in a Toxics Information Clearinghouse.

§ 69401.1 Hazard Trait Framework

Section 69401.1 explains the relationship between hazard traits, endpoints and other relevant data. The framework provides a way for organizing the information to be included in the Toxics Information Clearinghouse. The most general categories are the four major types of hazards: toxicological, environmental, exposure potential and physical. Within each of these there are a number of corresponding hazard traits identified.

The toxicological and environmental hazard traits are directly observed as endpoints in human and non-human studies. For example, the endpoint "decreased fetal weight" in an animal study indicates the developmental toxicity hazard trait. Other relevant data are indirect indicators. For example, an observation that a chemical causes placental insufficiency provides indirect evidence for decreased fetal weight since placental insufficiency can cause decreased fetal weight.

This framework for defining each hazard trait – with endpoints that are manifestations of that trait and other relevant data that provide less direct evidence – is loosely based on the framework used by the International Agency for Research on Cancer for describing

the available evidence on carcinogenicity.[4] Toxicological endpoints correspond to the type of human and animal cancer endpoints that support conclusions regarding sufficiency of evidence for cancer effect in animals and humans. The "other relevant data" correspond directly to the "mechanistic and other relevant data" that IARC finds substantially contribute to overall conclusions regarding carcinogenicity. IARC includes structure activity and other mechanistic information in this category of evidence.[5]

Data on hazard traits – observations of toxicological and environmental endpoints and other relevant data on mechanisms and chemical structure activity – are intertwined and relational. Living organisms are complex and impacts on one organ system are frequently reflected in another. For example, a chemical that causes thyroid insufficiency has the endocrine toxicity hazard trait. Since thyroid hormone is essential for brain development, thyroid insufficiency can cause developmental neurotoxicity. The same chemical that causes thyroid insufficiency and has the endocrine toxicity hazard trait would likely also have the developmental toxicity hazard trait. Thus, some hazard traits are indicative of other hazard traits.

§ 69401.2 Definitions

Subsection 69401.2(a) defines what "adverse effect" means for toxicological and environmental hazard traits. For toxicological endpoints, the proposed regulation adopts verbatim the definition used by the U.S. Environmental Protection Agency[6] [7]:

> "Adverse Effect: A biochemical change, functional impairment, or pathologic lesion that negatively affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge."

This definition supports the identification of outcomes from a broad range of toxicity tests as adverse effects.[8] Comments received on the pre-regulatory draft regulation stated that the definition was not consistent with the viewpoint of toxicity expressed in the 2007 National Research Council (NRC) report Toxicity Testing in the 21st Century: A Vision and Strategy. However, the definition is consistent with the NRC report.

[4] International Agency for Research on Cancer (IARC, 2006). Preamble. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, IARC, Lyon, France, January 23, 2006. Available at: http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf.
[5] IARC 2006 Preamble, pages 15-18.
[6] U.S. Environmental Protection Agency, A Review of Reference Dose and Reference Concentration Processes, U.S. EPA Risk Assessment Forum, EPA/630/P-02/002F, December 2002, page G-1.
[7] U.S. Environmental Protection Agency, Integrated Risk Information System, IRIS Glossary, 2007, available at: http://www.epa.gov/iris/help_gloss.htm
[8] Woodruff et al. 2008 Meeting Report: Moving Upstream—Evaluating Adverse Upstream End Points for Improved Risk Assessment and Decision-Making. Environ Health Perspect 116:1568–1575.

The National Research Council report states that:

> "Biologic responses are viewed as results of an intersection of exposure and biologic function. The intersection results in perturbation of biologic pathways. When perturbations are sufficiently large or when the host is unable to adapt because of underlying nutritional, genetic, disease or life-stage status biologic function is compromised, and this leads to toxicity and disease."

Using the definition in the proposed regulation, any perturbation that would lead to toxicity and disease would be considered "adverse." Perturbations that would not lead to toxicity would not be considered adverse. Toxicity can take many years to manifest, and the reduction of an individual's ability to respond to additional environmental challenges can also lead to toxicity. The definition in the proposed regulation is also consistent with the definition of "adverse" adopted by the American Society for Veterinary Clinical Pathology[9]:

> "Adverse. A biochemical, morphological, or physiological change (in response to a stimuli) that either singly or in combination adversely affects the performance of the whole organism or reduces the organism's ability to respond to an additional environmental challenge."

The definition of adverse does not address dose-response relationships. For example, it gives no indication of how No Observed Adverse Effect Levels, Benchmark Dose Levels or other measures of dose response will be taken into account in the hazard trait framework. As discussed above, dose response is addressed in DTSC's proposed regulations. The proposed definition neither precludes nor endorses the concept of biological thresholds.

For environmental hazard traits, Subsection 69401.2(a) defines as "adverse," changes that affect any of the basic categories of biologic organization specified by the U.S. Environmental Protection Agency in its document Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment[10]: organism (i.e., individual), population, community (i.e., multispecies group in an area), assemblage (e.g., bird community), ecosystem.

[9] L. Boone, D. Meyer, P. Cusick, D. Ennulat, A. Provencher Bolliger, N. Everds, V. Meador, G. Elliott, D. Honor, D. Bounous, H. Jordan, for the Regulatory Affairs Committee of the American Society for Veterinary Clinical Pathology, Position Paper, Selection and Interpretation of clinical pathology indicators of hepatic injury in preclinical studies, Vet Clin Pathol. 2005;34:182–188.

[10] U.S. Environmental Protection Agency. 2003. Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. EPA/630/p-02/004F. U.S. EPA Risk Assessment Forum, Washington DC, October, 2003, pp 8-18.

The environmental hazard traits encompass adverse effects on all living organisms, including microorganisms. Microorganisms are important constituents of the environment. For instance, bacteria are necessary for soil biology and health and are components of the soil food web, sustaining biological diversity, regulating and cycling nutrients, and regulating and detoxifying substances.[11] Soil microorganisms are important to agricultural production.

Subsection 69401.2(b) defines "authoritative organization," in terms of how the scientific findings of authoritative organizations are used. It restricts the identified entities to those organizations that provide the scientific basis for formal public health protections by government entities.

Subsection 69401.2(b) identifies a non-exclusive list of governmental and non-governmental institutions that satisfy the definition of "authoritative organizations," including California agencies. The legal and administrative processes applied by these agencies help ensure the validity of the scientific documents supporting California public health and environmental regulations and guidance. The National Academy of Sciences reports are developed by scientific experts and released after rigorous scientific peer review processes. The federal government has in place a number of procedures, including peer review, data quality and scientific guidance, that are intended to ensure the scientific integrity of products produced for regulatory and public health purposes. Similarly, Canadian, French and European governments, including the European Union and European Commission, have processes in place that help ensure the integrity of their scientific work products. The World Health Organization includes the International Agency for Research on Cancer, an organization renowned and widely respected for its evaluation of the potential carcinogenicity of chemicals using expert review and deliberation. Also within the World Health Organization, the International Programme for Chemical Safety publishes the report series "Environmental Health Criteria Monographs." These monographs present hazard evaluations that are relied upon by other nations and organizations for decision-making. The United Nations houses the World Health Organization, organizations that developed the Globally Harmonized System of Classification and Labelling of Chemicals, the International Labor Organization, and other organizations that provide expert evaluations of chemical hazards.

Subsection 69401.2(c) defines "chemical substance" broadly to encompass chemical materials that could be hazardous and that may be used in consumer products that are sold or used in California. The definition includes chemical elements, compounds and mixtures, particulate matter, metabolites of a chemical, and degradation by-products.

[11] Arlene T, Lewandowski A, Happe-vonArb D, eds. 2000. Soil Biology Primer. Rev. ed. Ankeny, Iowa: Soil and Water Conservation Society, Available at: http://soils.usda.gov/sqi/concepts/soil_biology/biology.html

Chemicals used in consumer products can break down into other chemicals or substances that also are hazardous. Chemicals, including metals, can also undergo transformation into other forms or species, or be incorporated into molecules and structures with different characteristics. This definition ensures that any of the transformation or breakdown products from the original substance are linked to the information on the parent chemical so that they can be considered together. This will reduce the chance that critical hazards related to use of the chemical are missed as it is being evaluated.

A well characterized example of why the definition includes chemical breakdown products is the pesticide DDT (dichlorodiphenyltrichloroethane). In the environment DDT is broken down to DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane) by soil microorganisms. DDT and DDE accumulate in plants and fatty tissues of animals. It is DDE, not DDT, that is found at greatest levels in the environment today, 38 years after DDT was banned in the U.S. DDT, DDE, and DDD are all on the Proposition 65[12] list of chemicals known to the state to cause cancer or reproductive toxicity, and the carcinogenic potential of these chemicals is also recognized by other organizations.[13]

An example of chemicals that are metabolized to well-known toxins are benzidine-based dyes, which are metabolized to the known human carcinogen benzidine.[14]

Chemical mixtures and particulate matter are also chemical substances captured under the section 69401.2 definition. Sometimes toxicity information is available for a mixture and not individual components of the mixture; and sometimes it may be more efficient to consider a chemical mixture in a product as a whole in evaluating its toxicity. Also, since very small particles can have greater toxicity than when present in bulk form, in some circumstances, it is important to consider a particulate matter material and its alternatives explicitly in that form.

Subsection 69401.2(d) defines “environmental endpoints” as adverse manifestations of environmental toxicity. The definition parallels that for “toxicological endpoint” in subsection 69401.2(g) of the proposed regulation. An environmental endpoint is an observation or measurement of an “adverse environmental effect.” “Adverse environmental effect” is similar in meaning to the term “adverse ecological effects”

[12] The Safe Drinking Water and Toxics Enforcement Act of 1986, codified at Health and Safety Code section 25249.5 *et seq.*

[13] Agency for Toxic Substances and Disease Registry, Toxicological Profile for DDT, DDE and DDD, U.S. Department of Health and Human Services, Public Health Service, ATSDR, September 2002, pp, 6-7, available at: http://www.atsdr.cdc.gov/ToxProfiles/tp35.pdf

[14] NTP Report on Carcinogens Background Document for Dyes Metabolized to Benzidine, National Toxicology Program, March 1999, page 1, available at: http://ntp.niehs.nih.gov/files/DyesMetaBenzidine.pdf

defined in the U.S. Environmental Protection Agency's "Guidelines for Ecological Risk Assessment:"

> **"Adverse ecological effects**—Changes that are considered undesirable because they alter valued structural or functional characteristics of ecosystems or their components. An evaluation of adversity may consider the type, intensity, and scale of the effect as well as the potential for recovery."

Finding environmental endpoints following chemical exposure is evidence that a chemical has one of the environmental hazard traits.

"Environmental endpoints" have a related but different meaning than the term "assessment endpoint" used by U.S. Environmental Protection Agency. The terms "environmental endpoint" and "endpoint" are not defined in the U.S. Environmental Protection Agency's "Guidelines for Ecological Risk Assessment." Instead the Ecological Guidelines defines "assessment endpoint":

> **"Assessment endpoint**—An explicit expression of the environmental value that is to be protected, operationally defined by an ecological entity and its attributes. For example, salmon are valued ecological entities; reproduction and age class structure are some of their important attributes. Together 'salmon reproduction and age class structure' form an assessment endpoint."

The U.S. Environmental Protection Agency's "Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment" provides further explanation of the nature of an "assessment endpoint" and how it is used:

> "Ecological risk assessments are preceded by a planning phase in which risk managers, risk assessors, and, as appropriate, interested parties define the management goals. The goals are broad statements of desired conditions such as 'restore the wetlands' or 'sustain the trout population.' The planning phase is followed by the problem formulation phase, in which the assessors define the assessment endpoints based on the management goals. The assessment endpoints are specific entities and their attributes that are at risk and that are expressions of a management goal. The analysis and risk characterization phases of the risk assessment are devoted to estimating the nature and likelihood of effects on those endpoints."

Thus, the term "assessment endpoint" is relative to a particular ecological evaluation. The definition of environmental endpoint in the proposed regulation is more general and better suited to the purposes of the Clearinghouse.

Subsection 69401.2(e) defines the term "hazard traits." This hazard trait definition is based on the organization of the four major areas of hazard addressed by regulatory and public health agencies nationally and worldwide: toxicological, environmental, exposure potential, and physical hazard. Hazard traits are properties of a chemical that fall into these broad categories. The proposed regulation identifies a number of specific hazard traits for these categories. This initial statement of reasons:

- discusses the selection of specific hazard traits,
- explains the hazard trait definitions,
- explains the non-exclusive examples of observable endpoints and other relevant data in the proposed regulation related to these hazard traits, and
- gives some non-exclusive sources of information for the traits, endpoints and other relevant data.

Sources for the definition of these hazard traits are also provided. For reference key sources are tabulated in Appendix Table 1 at the end of this statement of reasons.

The hazard traits identified in the proposed regulation are listed below. For ease of reference, each trait is organized under four general categories. These are shown below in **bold** type. It should be noted that a single chemical substance may fit multiple hazard trait definitions.

Hazard Traits

Toxicological

- Carcinogenicity
- Cardiovascular Toxicity
- Dermatotoxicity
- Developmental Toxicity
- Endocrine Toxicity
- Epigenetic Toxicity
- Genotoxicity
- Hematotoxicity
- Hepatotoxicity and Digestive System Toxicity
- Immunotoxicity
- Musculoskeletal Toxicity
- Nephrotoxicity and other Urinary System Toxicity
- Neurotoxicity
- Ocular Toxicity

Environmental

- Domestic Animal Toxicity
- Eutrophication
- Impairment of Waste Management Organisms
- Loss of Genetic Diversity, including Biodiversity
- Phytotoxicity
- Wildlife Development Impairment
- Wildlife Growth Impairment
- Wildlife Reproductive Impairment
- Wildlife Survival Impairment

- Ototoxicity
- Reactivity in Biological Systems
- Reproductive Toxicity
- Respiratory Toxicity

Physical

- Combustion Facilitation
- Explosivity
- Flammability

Exposure Potential

- Ambient Ozone Formation
- Bioaccumulation
- Environmental Persistence
- Global Warming Potential
- Lactational or Transplacental Transfer
- Mobility in Environmental Media
- Particle Size or Fiber Dimension
- Stratospheric Ozone Depletion Potential

Various Federal and state regulatory agencies have emphasized different hazard traits in their regulatory strategies. This is primarily because each agency has a specific statutory or other mandate it is responsible for implementing. For example, the U.S. Environmental Protection Agency focuses on toxicological, environmental and exposure potential hazards. Its health evaluations for the Integrated Risk Information System, and air, pesticide, and drinking water[15] programs provide information on critical toxicological hazards. U.S. Environmental Protection Agency's water quality criteria, pesticide and superfund programs[16] evaluate environmental hazard traits and exposure potential. A major concern of the U.S. Department of Transportation is physical hazards.[17]

In their comments on OEHHA's pre-regulatory draft some stakeholders suggested that OEHHA should use the United Nations' Globally Harmonized System of Classification and Labeling of Chemicals (hereafter in this section referred to as GHS) as a model for identifying and defining hazard traits. These stakeholders noted that the GHS identifies fewer hazard traits and suggested that OEHHA include fewer traits in its proposed

[15] See for example, U.S. Environmental Protection Agency, National-Scale Air Toxics Assessment of 2002 – Fact Sheet, U.S. EPA Technology Transfer Network. June 2009. Available at: http://www.epa.gov/ttn/atw/nata2002/factsheet.html; U.S. Environmental Protection Agency, Drinking Water Contaminants, National Primary Drinking Water Regulations, available at: http://water.epa.gov/drink/contaminants/index.cfm; U.S. Environmental Protection Agency, Assessing Health Risks from Pesticides, Pesticides: Topical and Chemical Fact Sheets, September 10, 2009, available at: http://www.epa.gov/pesticides/factsheets/riskassess.htm; U.S. Environmental Protection Agency, A Review of Reference Dose and Reference Concentration Processes, U.S. EPA Risk Assessment Forum, EPA/630/P-02/002F, December 2002, e.g., pages 3-4 to 3-6.

[16] See for example, U.S. Environmental Protection Agency, Biological Indicators of Watershed Health, Legal Authority, September 28, 2010, available at: http://www.epa.gov/bioindicators/html/biol2.html; Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments - Interim Final; U.S. Environmental Protection Agency. 1997. Representative Sampling Guidance Document, Volume 3: Ecological, Draft. Edison, NJ: Environmental Response Team, Office of Emergency and Remedial Response, pp 1-18; U.S. EPA Pesticides: Environmental Effects, available at: http://www.epa.gov/pesticides/ecosystem/index.htm

[17] http://www.fmcsa.dot.gov/safety-security/hazmat/complyhmregs.htm

regulation. OEHHA declined to model the proposed regulation after the GHS. GHS is less focused on the toxicological hazards needed for the proposed regulation and more focused on physical hazards as applied to the transportation sector and labeling and safety data sheets for the worker sector.[18] The GHS is not the best model for the type of regulation needed to facilitate the broader analysis of chemical hazards in consumer products envisioned by DTSC's proposed regulations.

On the other hand, this proposed regulation includes many elements of the GHS, but goes beyond it. The broad categories used by the GHS are "Health Hazards," "Environmental Hazards," and "Physical Hazards", which are similar to those in the proposed regulation. Within GHS' environmental hazards are traits that are more relevant to exposure potential (for example, bioaccumulation and rapid degradability). Article 5 of this proposed regulation identifies these as exposure potential hazard traits. GHS covers only two environmental hazards related to aquatic toxicity. GHS has 16 physical hazard traits; these have been generalized to three in Article 6 of this proposed regulation. The GHS health hazards list does not specify certain traits that serve as the basis for weighing human health hazards by many public and environmental health institutions, including but not limited to OEHHA, U.S. Environmental Protection Agency, the National Institute for Occupational Health and Safety, and the Consumer Product Safety Commission (e.g., organ specific toxicity such as nephrotoxicity).

Other stakeholders requested a significant expansion of the number of hazard traits in addition to those included in the pre-regulatory draft. The hazard traits "domestic animal toxicity" and "impairment of waste management organisms" were included. But many of the other suggested hazard traits are already covered by the traits in the proposed regulation or are outside the scope of the regulation since they relate either to the prioritization process which DTSC is conducting and/or are not intrinsic properties of a chemical.

OEHHA received comments suggesting that dose-response characteristics be included in the hazard trait framework. OEHHA's proposed regulation does not include provisions directly addressing dose-response relationships. Dose response information is important in evaluating the toxicity of a chemical substance. Thus, information on these relationships is an important consideration in evaluating safer chemicals to use in products. The hazard trait framework in the proposed regulation addresses hazard evaluation. As currently proposed, DTSC's regulation incorporates dose-response information in prioritizing chemicals of concern and in alternatives analysis. Therefore, dose-response will be included in the Green Chemistry process.

[18] Globally Harmonized System for Classification and Labelling, section 2.5

The hazard trait framework in the proposed regulation also does not directly address detailed exposure assessment, which is another component of risk assessment. However exposure potential hazard traits are included in the proposed regulation. These traits capture the intrinsic properties of a chemical related to the potential for significant exposures subsequent to use or environmental release.

Because the hazard traits included in the framework address intrinsic properties of a chemical substance, but not the specific circumstances of exposure from a particular use, the hazard traits do not provide all the information needed to calculate a risk. The approach used in the proposed regulation is similar to that used by the European Commission under its "CLP" (chemical classification, labeling and packaging) program within "REACH" (Registration, Evaluation, Authorisation and Restriction of Chemical substances):

> "Classification according to CLP is based on intrinsic hazards, i.e. the basic properties of a substance as determined in standard tests or by other means designed to identify hazards. As CLP is hazard based it does not take exposure into consideration in arriving at either a classification or appropriate labeling…"[19]

Subsection 69401.2(f) defines "mechanistic similarity." Mechanistic similarity is a term used throughout the regulation. Mechanistic data are growing in importance with the emerging science enabling us to understand the ways chemical substances act to produce toxicity.[20] These data provide the basis for reducing the need for large scale and resource intensive animal tests, and over time are expected to provide an increasing basis for chemical safety evaluations.[21]

Subsection 69401.2(g) defines what "other relevant data" means for a specific toxicological or environmental hazard trait. Other relevant data indicate the potential for a hazard trait, but are less direct than a measured or observed outcome demonstrating the specific hazard trait. For example, an observation that a chemical causes placental insufficiency provides indirect evidence for decreased fetal weight, since placental insufficiency can cause this effect. As a second example, a chemical that is strongly electrophilic is capable of binding to a number of large molecules in cells and causing a

[19] European Chemicals Agency. Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures. ECHA-09-G-02-EN. August 25, 2008.
[20] U.S. Environmental Protection Agency, Biological (Mechanistic) Research, Human Health Research Program, http://www.epa.gov/hhrp/quick_finder/mechanistic.html; National Research Council (2007). Toxicity Testing in the 21st Century: A Vision and Strategy, National Academy Press, Washington DC, Executive Summary.
[21] National Research Council, Toxicity Testing in the 21st Century: A Vision and Strategy, 2007, Executive Summary. Woodruff et al. 2008 Meeting Report: Moving Upstream—Evaluating Adverse Upstream End Points for Improved Risk Assessment and Decision-Making. Environ Health Perspect 116:1568–1575.

wide variety of adverse impacts. Such a basic physico-chemical property is thus associated with many hazard traits.

Subsection 69401.2(h) defines "Toxicological Endpoint."

U.S. Environmental Protection Agency[22] defines the term "endpoint" as

> "An observable or measurable biological event or chemical concentration (e.g., metabolite concentration in a target tissue) used as an index of an effect of a chemical exposure."

From this broad definition one might consider a "toxicological endpoint" to mean any observation from a toxicological study. However, in numerous reports the U.S. Environmental Protection Agency[23] and other agencies[24] use the term "toxicological endpoint" or "toxic endpoint" more narrowly, to mean an observed or observable *adverse* effect in a study. The toxicological endpoint is an adverse manifestation of the trait that provides the basis for establishing health guidance levels, such as reference doses. Toxicological endpoints in these reports are typically overt observations of the trait in studies of laboratory animals or humans. The proposed regulation includes a more narrow definition of the term consistent with the more narrow use of the term by the U.S.Environmental Protection Agency to mean an adverse effect, not just any measurable biological event.

In some U.S. Environmental Protection Agency reports, the term "toxicological endpoint" is used to mean the major toxicity categories[25], akin to the term "hazard traits" in the proposed regulation. Similarly the United Nations' Globally Harmonized System[26] uses

[22] U.S. EPA 2002. A Review of the Reference Dose and Reference Concentration Processes. U.S. EPA Reference Dose/Reference Concentration (RfD/RfC) Technical Panel, Risk Assessment Forum, EPA/630/P-02/002F. U.S. EPA Washington DC.

[23] See for example U.S. Environmental Protection Agency, General Principles for Performing Aggregate Exposure and Risk Assessments, Office of Pesticide Programs, November 28, 2001; U.S. EPA RED Facts: Diflubenzuron, EPA-738-F-97-008, Office of Pesticide Program, page 2, August 1997; U.S. EPA RED Facts: Alkyl imidazoline, EPA-738-F-95-034, Office of Pesticide Program, page 2, August 1995; U.S. EPA Pesticide Fact Sheet: Fenpropimorph, Office of Prevention, Pesticides and Toxic Substances Program, pages 10-11, March 2006.

[24] C-H Selene, J Chou, M Williams, D Jones, CT DeRosa, Evaluating toxicological end points to derive minimal risk levels for hazardous substances, Int J Hyg Environ Health, 205:71-75; U.S. Food and Drug Administration, Guidance for Industry, Nonclinical Safety Evaluation of Pediatric Drug Products, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Pharmacology and Toxicology February 2006, pp 11-12.

[25] U.S. Environmental Protection Agency, Furniture Flame Retardancy Partnership, Environmental Profiles of Chemical Flame-Retardant Alternatives for Low-Density Polyurethane Foam, U.S. EPA Design for the Environment Program, Volume 1, page 4-1.

[26] United Nations. Globally Harmonized System of Classification and Labeling (GHS), Third Revised Edition, ST/SG/AC.10/30/Rev.3. Compare for example the specific end-points to assess maternal toxicity listed in section 3.7.2.4.4 to the respiratory tract irritation endpoint discussion in section 3.8.2.2.1(c).

the term “endpoint” or “end-point” in the more general sense (i.e., as a hazard trait), but also in the same way that the proposed regulation defines “toxicological endpoint.” In the proposed regulation, the term “hazard trait” is used for the general hazard – such as carcinogenicity - and “toxicological endpoint” to mean specific adverse manifestations of the hazard trait – such as lung cancer.

For many toxicological hazard traits, the adverse effect includes functional or structural impairments. The toxicological endpoints are observations of these impairments, typically in human clinical or epidemiological studies, or laboratory studies conducted in animals. For certain types of toxicity (e.g., genotoxicity, immunotoxicity, dermatotoxicity) more common endpoints are observations of tests conducted with cells or tissues *in vitro* (not in whole animals). The U.S. Environmental Protection Agency currently uses *in vitro* tests, such as genotoxicity and apoptosis, to evaluate mechanisms of toxicity. It has employed a mix of *in vitro* and *in vivo* tests to identify endocrine disruptors in its Endocrine Disruptor Screening Program.[27]

Over time, the use of *in vitro* data to identify toxicological hazards is expected to increase. For example, different institutions in the federal government have entered into a memorandum of understanding to develop *in vitro* test methods that is methods that are not conducted in whole animals:

> “The goals of this MOU are to investigate the use of these new tools to (1) identify mechanisms of chemically induced biological activity, (2) prioritize chemicals for more extensive toxicological evaluation, and (3) develop more predictive models of *in vivo* biological response. Success in achieving these goals is expected to result in test methods for toxicity testing that are more scientifically and economically efficient and models for risk assessment that are more biologically based. As a consequence, a reduction or replacement of animals in regulatory testing is anticipated to occur in parallel with an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation.”[28]

[27] U.S. Environmental Protection Agency. Endocrine Disruptor Screening Program. Tier I Screening Battery. October 21, 2009. Accessible at: http://www.epa.gov/endo/pubs/assayvalidation/tier1battery.htm

[28] July 19, 2010 Memorandum of Understanding on High Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings between the U.S. Department of Health and Human Services (HHS) National Institutes of Health (NIH) National Institutes of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) and the U.S. Department of Health and Human Services (HHS) National Institutes of Health (NIH) National Human Genome Research Institute (NHGRI) NIH Chemical Genomics Center (NCGC) and the U.S. Environmental Protection Agency(EPA) Office of Research and Development and the U.S. Department of Health and Human Services (HHS) U.S. Food and Drug Administration (FDA), pages 1-2

The federal effort is currently focusing on high throughput approaches that evaluate how chemicals act on genes, toxicity pathways, and cell function.[29] Medium throughput assays have been developed already and are currently in use. These assays evaluate "chemicals for their ability to perturb more integrated cellular responses, such as cell proliferation, apoptosis and mutation." [30] The definition in the proposed regulation takes these changing scientific methodologies into account and should be applied broadly to include developing scientific methodologies. For some specific hazard traits named in the regulation, such information is currently accepted methodology and is included in the endpoints listed in the regulation. In other cases, the links to the hazard trait are not as direct, and are included as "other relevant data" in the proposed regulation. Finally, there are some cases where the methods are not sufficiently developed to be utilized either as other relevant data or as endpoints.

Subsection 69401.2(i) of the proposed regulation defines "well-conducted scientific studies." This definition requires *de novo* evidence evaluations to rely on studies that are published in the open literature or are unpublished but submitted to governmental agencies for regulatory purposes. The methods used in these studies must be scientifically valid and the studies must be conducted according to generally accepted principles. This is similar to the requirements for evaluating scientific information in California's Proposition 65.[31]

The proposed regulation does not require a study be conducted in accordance with Good Laboratory Practice in order to be used in evaluating hazard traits because of important limitations. Good Laboratory Practice "is a quality [control] system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported."[32] Various researchers have pointed out the benefits[33] and limitations [34] [35] of restricting health reviews to the use of studies performed using only Good Laboratory Practices. Two of many important limitations is that Good Laboratory Practices are unable to keep pace with the evolving science[36] and that when strictly

[29] National Academy of Sciences, Toxicity Testing in the 21st Century: A Vision and Strategy, page 38.
[30] National Academy of Sciences, Toxicity Testing in the 21st Century: A Vision and Strategy, page 38.
[31] The Safe Drinking Water and Toxics Enforcement Act of 1986. Health and Safety Code, subsection 25249.8(b)
[32] World Health Organization. Good Laboratory Practice (GLP) Handbook. Quality Practices for Regulated Non-Clinical Research and Development. Second Edition. WHO Special Programme for Research and Training in Tropical Diseases.WHO, Switzerland.
[33] Becker R, Janus E, White R, Kruszewski F, Brackett R. Good Laboratory Practices and safety assessments [Letter] Environ Health Perspect. 2009;117:A482–A483.
[34] M Goodwin, Good Laboratory Practices 30 Years on: challenges for industry. Ann Int Super Sanita 44: 239-373, 2008.
[35] Tweedale T. Good laboratory practices and safety assessments: another view. Environ Health Perspect. 118(5):A194, 2010
[36] M Goodwin, Good Laboratory Practices 30 Years on: challenges for industry. Ann Int Super Sanita 44: 239-373, 2008.

applied, they exclude from consideration important health data. While under certain circumstances knowing whether or not a study was conducted in conformance with Good Laboratory Practices provides some assurance that the study was conducted properly, it does not necessarily ensure the study can actually answer the scientific hypothesis being proposed. Therefore, the proposed regulation requires the studies to be scientifically valid and conducted according to generally accepted principles.

Subsection 69401.2(j) defines "wildlife." The proposed definition encompasses all undomesticated animal life, including microorganisms.

In the broadest terms, "wildlife" is defined as "all living things (except people) that are undomesticated."[37] This definition is reflected in California law and guidance. The California Fish and Game Code defines "wildlife" as:

> "all wild animals, birds, plants, fish, amphibians, reptiles, and related ecological communities, including the habitat upon which the wildlife depends for its continued viability."[38]

DTSC defines "wildlife" as "all non-domesticated plants and animals including aquatic plants and animals" in their guidance on ecological risk assessment.[39] In the proposed regulations, plants are treated separately from other organisms and are therefore not included in the wildlife definition. Plants are addressed separately in the proposed regulation in order to capture additional hazards unique to plants, such as the inhibition of photosynthesis.

This approach is common in academia. For example, Allaby's "A Dictionary of Ecology" defines "wildlife" as: "any undomesticated organisms, although the term is sometimes restricted to wild animals, excluding plants."[40]

Microorganisms are critical to terrestrial and aquatic ecosystems. For example, the assessment of chemical impacts on microorganisms involved in nutrient cycling is required by the U.S. Environmental Protection Agency[41, 42, 43] and the Organization for Economic Co-operation and Development[44 45] in the regulation of chemicals

[37] "Wildlife". WordNet. Princeton University. 2010. Available at: http://wordnet.princeton.edu.
[38] California Codes. Fish and Game Codes. Section 711.2. (available at http://www.leginfo.ca.gov/calaw.html).
[39] DTSC. (1996). Guidelines for Ecological Risk Assessment at Hazardous Waste Sites and Permitted Facilities. Part A: Overview. Department of Toxic Substance Control, Sacramento, CA. Page 10.
[40] Michael Allaby. "wildlife." A Dictionary of Ecology. 2004. Encyclopedia.com. 29 Oct. 2010 (http://www.encyclopedia.com).
[41] U.S. EPA. (1996). Ecological Effects Test Guidelines. OPPTS 850.5100. Soil Microbial Community Toxicity Test. Washington, DC: EPA/712/C-96/161.
[42] U.S. EPA. (1996). Ecological Effects Test Guidelines. OPPTS 850.2450. Terrestrial (Soil-Core) Microcosm Test. Washington, DC: EPA/712/C-96/143.

Article 2. Toxicological Hazard Traits – Carcinogenicity, Developmental Toxicity, and Reproductive Toxicity

All the information in the following Articles is necessary in order for OEHHA to meet its statutory mandate to specify the hazard traits, endpoints and other relevant data to be included in the Toxics Information Clearinghouse.[46]

For purposes of clarity and ease of reference, the regulatory provisions in this Article address only the three hazard traits of carcinogenicity, developmental toxicity and reproductive toxicity. These three hazard traits have been well-studied and characterized by a variety of national and international agencies. Therefore, this Article provides more specificity regarding these three hazard traits than others contained in subsequent articles.

§ 69402 General

Section 69402 explains that the hazard traits identified in this article can be demonstrated by "strong evidence" or "suggestive evidence." Sections 69402.2, 69402.4, and 69402.6 define what constitutes strong evidence and suggestive evidence for the three specific hazard trait discussed in this Article. A general discussion of the purpose, use and necessity for specifying the two types of evidence is provided below.

The distinction between strong and suggestive evidence for each of the three endpoints covered by Article 2 of the proposed regulation is provided to assist DTSC, the public and affected industries in understanding the strength of the evidence for hazards associated with chemical substances included in the Clearinghouse. It is intended to promote the inclusion of information from all well-conducted and relevant studies in the Clearinghouse, including information that is insufficient for a finding of strong or suggestive. It also is intended to assist DTSC in its prioritization process, to identify chemicals of concern and to assist DTSC, businesses and other interested parties conducting and interpreting the results of alternative assessments.

The alternative assessments and evaluations that will be conducted under DTSC's proposed regulations will often involve comparisons of different chemicals that exhibit

[43] U.S. EPA. (1996). Ecological Effects Test Guidelines. OPPTS 850.1900. Generic Freshwater Microcosm Test, Laboratory. Washington, DC: EPA/712/C-96/134.
[44] Organization for Economic Co-operation and Development. (OECD 2000) OECD Guideline for the testing of chemicals. Test No. 216: Soil Microorganisms: Nitrogen Transformation Test.
[45] Organization for Economic Co-operation and Development. (OECD 2000) OECD Guideline for the testing of chemicals. Test No. 217: Soil Microorganisms: Carbon Transformation Test.
[46] Health and Safety Code section 25256.1.

various hazard traits. The ability to differentiate between strong and suggestive evidence for a chemical's hazard traits will enable businesses and DTSC to make better-informed decisions. For example, if this regulation were to only describe "strong evidence" for hazard traits, an alternatives assessment might erroneously conclude that a chemical does not have a particular hazard trait even when there is evidence to suggest that it does. Overlooking suggestive evidence for a hazard trait would increase the likelihood of a business or regulatory decision that results in a regrettable substitution of one hazardous chemical for another in a product, thereby defeating one of the key purposes of the DTSC regulatory program. The description of "suggestive evidence" in this regulation will help ensure that such evidence is available in the Clearinghouse and is considered when DTSC, businesses and others weigh the advantages and drawbacks of using various chemicals as alternatives.

A key issue in evaluating the evidence for a hazard trait is the degree to which a chemical has been adequately studied to determine whether or not it has the hazard trait. The proposed regulation adopts a system of evaluating the available evidence for the trait and making a judgment based on that evidence. This is the same approach used by the authoritative bodies named in Subsection 69401.2(b) of the proposed regulation. Absence of data does not constitute absence of hazard, however, and the absence of strong or suggestive evidence does not translate to absence of the hazard trait.

"Strong evidence" and "suggestive evidence" are terms of art used in the scientific community to describe different levels of positive evidence for causality between a possible cause and an outcome in a variety of contexts. Other terms are also used to represent the same level of evidence, but OEHHA chose to use these two well-known terms in the proposed regulation for purposes of clarity and consistency with existing usage.

Some examples of the use of the term "strong evidence" follow:

- Various National Academy of Sciences reports use the term to characterize the evidence supporting causal associations, for example, chemical exposures and health outcomes,[47] factors affecting wetland health,[48] characterizing treatment effectiveness for Gulf War veterans,[49] methods to develop science skills,[50] methods to prepare teachers.[51]

[47] Institute of Medicine, 2010. Secondhand Smoke Exposure and Cardiovascular Effects: Making Sense of the Evidence, Board on Population Health and Public Health Practice, National Academy Press, Washington DC, p 5.
[48] National Research Council, Wetlands: Characteristics and Boundaries, National Academy of Press, 1998, pp 74 and 86
[49] Institute of Medicine, 2001. Gulf War Veterans: Treating Symptoms and Syndromes, National Academy Press, Washington DC, pp 32-34.

- The International Agency for Research on Cancer indicates the term “strong” could be used to describe the “strength of the evidence that any carcinogenic effect observed is due to a particular mechanism.”[52]
- The Agency for Healthcare Research and Quality within the U.S. Department of Health and Human Services conducts evidence-based medicine evaluations and publishes the series of “Evidence Report/Technology Assessment.” In some reports in this series it grades the evidence from a body of literature as “strong,” “moderate,” or “weak” that an activity or factor is related to an outcome.[53]
- The International Programme for Chemical Safety of the World Health Organization uses “strong evidence” to describe the upper end of the continuum of the level of evidence that a chemical poses a hazard.[54]

Some examples of the use of the term “suggestive evidence” follow:

- “Suggestive Evidence” is a label for positive but not definitive evidence of carcinogenicity potential used by the U.S. Environmental Protection Agency.[55]
- “Limited/Suggestive Evidence” of an association is a label given by some Institute of Medicine committees to describe positive but not definitive evidence of causal associations between a chemical exposure or circumstance and a health outcome.[56] [57]
- Suggestive evidence is used as term to describe positive but not definitive evidence of a causal relationship between a chemical exposure and effect in some reports of the International Programme for Chemical Safety.[58]

[50] National Research Council, 2010. Exploring the Intersection of Science Education and 21st Century Skills: A Workshop Summary Board on Education, National Academy Press, Washington DC, p 48

[51] National Research Council, 2010. Preparing Teachers: Building Evidence for Strong Policy. Chapter 2: Seeking Strong Evidence, Center for Education, National Academy Press, Washington DC, p 21.

[52] IARC Preamble, page 21.

[53] Agency for Healthcare Research and Quality, Outcomes of Maternal Weight Gain, Evidence Reports/Technology Assessments, No. 168, AHRQ, DHHS, May 2008 (see e.g., Methods section). Available at: http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=erta168

[54] IPCS Principles for the Assessment of Risks to Human Health from Exposure to Chemicals, Environmental Health Criteria No. 210, World Health Organization, Section 6.3, 1999.

[55] U.S. Environmental Protection Agency 2005. Guidelines for Carcinogen Risk Assessment, U.S. EPA Risk Assessment Forum, Washington DC, page 2-56.

[56] Institute of Medicine, 2003. Gulf War and Health: Volume 2. Insecticides and Solvents, Board on Health Promotion and Disease Prevention, National Academy Press, pp 4-9.

[57] Institute of Medicine, 2006. Amyotrophic Lateral Sclerosis in Veterans: Review of the Scientific Literature, Board on Population Health and Public Health Practice, National Academy Press, Washington DC, pp 11-12.

[58] IPCS, 1999, Environmental Health Criteria 213 Carbon Monoxide (Second Edition), IPCS, World Health Organization, page 7.

§ 69402.1 Carcinogenicity

Subsection 69402.1(a) defines the carcinogenicity hazard trait. The definition is adapted from a definition of a similar term adopted by the World Health Organization's International Agency for Research on Cancer (IARC):[59]

> "In the Monographs, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity."

There are no substantive differences between the definition used in the proposed regulation and the definition used by IARC. IARC is widely recognized as the pre-eminent international agency for the identification of carcinogens.

The National Toxicology Program defines carcinogenicity as "the power, ability, or tendency to produce cancerous tissue from normal tissue." The U.S. Environmental Protection Agency does not define the terms "carcinogen," "carcinogenic," or "carcinogenicity" in its *Guidelines for Carcinogen Risk Assessment.*[60]

OEHHA chose to use the IARC definition in the proposed regulation because it is more specific than that of the National Toxicology Program ("NTP"), but carries the same meaning.

Subsection 69402.1(b) specifies a non-exclusive set of endpoints that indicate the presence of the carcinogenicity hazard trait. The organ systems named are those used by the National Toxicology Program to analyze and report the results of animal studies in its long-term carcinogenicity studies[61] and include all organ systems present in humans. Benign or malignant neoplasia or pre-neoplasia in these organ systems are treated by IARC, the National Toxicology Program, the U.S. Environmental Protection Agency, and other bodies as carcinogenicity endpoints in their evaluations of chemicals for carcinogenicity in humans and animals. Underlying these general carcinogenicity endpoints are more specific ones. For example, there are numerous types of neoplasia of the nervous system, including a variety of brain tumors associated with different cell types. Most evaluations of carcinogenicity aim to evaluate more specific endpoints, and

[59] International Agency for Research on Cancer, Preamble, IARC, World Health Organization, Lyon, 2006, page 2, line 25, et seq.
[60] US Environmental Protection Agency, Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, Risk Assessment Forum, US EPA, Washington DC, March 2005.
[61] *NTP Technical Report Series on Toxicology and Carcinogenesis Studies.* See for example, NTP Technical Report on the Toxicology and Carcinogenesis Studies of Androstenedione (CAS NO. 63-05-8) in F344/N Rats and B6C3F1 Mice, National Toxicology Program, Research Triangle Park, North Carolina, pages 74-78.

guidance exists for classifying human and animal tumors[62] [63]. There are also various experimental and epidemiological designs for evaluating these endpoints. IARC[64] and the U.S. Environmental Protection Agency[65] provide guidance on endpoint and study evaluation.

Subsection 69402.1(c) provides general categories of other relevant data for carcinogenicity. These types of data are currently used in weighing the evidence of carcinogenic potential by IARC, the National Toxicology Program, the U.S. Environmental Protection Agency, OEHHA and other authoritative organizations. The general terminology in the proposed regulation is based on terms used in IARC guidance, particularly in the section describing "mechanistic and other relevant data," specifically in subsection (b) that begins on page 15 of the IARC 2006 Preamble "data on mechanisms of carcinogenesis" and in subsection (d) "other data relevant to mechanisms" that begins on page 17 of that document.

§ 69402.2 Evidence for Carcinogenicity Hazard Trait

Subsection 69402.2(a) describes the types of evidence that constitute strong evidence for the carcinogenicity hazard trait**.** It is based on the evidence and criteria used by a number of well-recognized authoritative organizations and processes. These are explained in more detail below in discussion of Subsections 69402.2(a)(2) - 69402.2(a)(4). The sources of evidence cited in the proposed regulation are not intended to be hierarchical or exclusive. These subsections give examples of sources of strong evidence of carcinogenicity. All the information available for a given chemical should be considered for inclusion in the Clearinghouse.

Subsection 69402.2(a)(1) identifies chemical substances on California's Proposition 65 list as known to the state to cause cancer.[66] Proposition 65 is updated at least annually and is a good source to find chemicals with strong evidence of carcinogenicity. The most current Proposition 65 list should be used when conducting evidence reviews under this provision.

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[62] e.g., IARC Scientific Publications No. 122, International Classification of Rodent Tumors, volumes 1-10; World Health Organization Classifications of Tumors, World Health Organization Press.
[63] A Fritz, C Persey, K Shanmugaratnam, L Sobin, DM Parkin, S Whelan (Eds.) International Classification of Diseases for Oncology, Third Edition, U.S. Interim Version, World Health Organization, Geneva, 2000.
[64] International Agency for Research on Cancer, Preamble, IARC, World Health Organization, Lyon, 2006, pp. 8-10; 12-15.
[65] US Environmental Protection Agency, Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, Risk Assessment Forum, US EPA, Washington DC, March 2005, pp 2-2 to 2-10.
[66] Health and Safety Code section 25249.5 *et seq*, The Proposition 65 list is published at Title 27, Cal. Code of Regulations, section 27001. A current list is maintained for ease of public access: State of California, Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Chemicals Known to the State to Cause Cancer or Reproductive Toxicity, Safe Drinking Water and Toxic Enforcement Act of 1986, available at: http://oehha.ca.gov/prop65/prop65_list/newlist.html

Subsections 69402.2(a)(2) - 69402.2(a)(4) includes those chemical substances meeting the criteria for listing developed by three authoritative organizations: U.S. Environmental Protection Agency, International Agency for Research on Cancer and the National Toxicology Program. Chemical substances found by those organizations to meet their own criteria would have strong evidence of carcinogenicity. If multiple reviews have been undertaken for a given chemical by a given organization, their most current designation should be used.

For chemicals not evaluated by those organizations, evidence for carcinogenicity can be judged against their published criteria. The most current criteria used by these organizations should be used in evaluating the evidence. For example, evaluations should currently be based on the U.S. Environmental Protection Agency's 2005 Guidelines for Carcinogen Risk Assessment, the 2006 International Agency for Research on Cancer Preamble,[67] or the general criteria used by the National Toxicology Program in its 11th Report on Carcinogens. For chemical substances these agencies have evaluated, that agency's findings would take precedence over a third party's analysis using the same criteria, since the agency is authoritative for its own determinations.

Subsection 69402.2(a)(5) includes chemical substances meeting the criteria for "Category 1, Known or Presumed Carcinogen" under the United Nations' Globally Harmonized System for Classification and Labeling of Chemicals, or "GHS."[68] The most current criteria adopted by this body should be used. The United Nations does not maintain a list of chemicals and their classifications:

> "One objective of the GHS is to be simple and transparent with a clear distinction between classes and categories in order to allow for "self classification" as far as possible."[69]

GHS criteria may be applied by various national and international government bodies. For carcinogenicity, the GHS criteria generally rely on *in vivo* evidence. Chemicals meeting the GHS category 1 criteria would likely also meet the criteria of other authoritative organizations such as of the National Toxicology Program and the International Agency for Research on Cancer. Including the GHS in Article 3 of the proposed regulation allows for the use of the GHS classifications when appropriate and is consistent with Subsection 69402.2(a)(7) of the regulation, which is described below.

[67] The International Agency for Research on Cancer publishes the most current preamble in its Monographs. The Preamble was last revised in 2006.
[68] These criteria are currently included in "Chapter 3.6 Carcinogenicity" of the Third Revised Edition of the GHS.
[69] United Nations, 2009. Globally Harmonized System for Classification and Labelling. 3rd Edition, section 1.3.2.1.2.

Subsection 69402.2(a)(6) includes those chemical substances recognized as a known or potential carcinogen in reports of the National Academy of Sciences National Research Council and Institute of Medicine. This includes but is not limited to the reports by the Institute of Medicine[70] that evaluate the impact of chemical exposures of U.S. troops, and by the National Research Council[71] that evaluate environmental and occupational chemicals.

Subsection 69402.2(a)(7) includes recognition as a known or potential carcinogen by California, other states, the United States or other nations. This includes recognition by the European Union as well as its member states. Many governments have in place a number of procedures, such as peer review, data quality and scientific guidance, which is designed to ensure the scientific integrity of work products produced by them for regulatory and public health purposes. Therefore, findings by such governmental entities would constitute strong evidence of carcinogenicity.

Examples of recognition of a chemical substance as causing or potentially causing cancer by a California governmental entity include OEHHA's calculations of risk-specific air concentrations for chemical substances based on a cancer endpoint under the Air Toxics "Hot Spots" or toxic air contaminants programs[72], or establishing health-protective concentrations for drinking water, or its equivalent, for chemical substances based on a cancer endpoint under the Public Health Goals for drinking water program.[73]

Subsection 69402.2(b) describes non-exclusive examples of what constitutes "suggestive" evidence for the carcinogenicity hazard trait. The provisions below are included in the proposed regulation to provide a non-exclusive list of examples of the types of evidence that could be considered "suggestive" of carcinogenicity for a given chemical substance. Including such information in the Toxics Information Clearinghouse is necessary for DTSC and others in their

[70] For example the Institute of Medicine Veterans and Agent Orange series, National Academy Press, available at: http://www.iom.edu/Reports.aspx?series={9748FCC1-A076-4227-BCC1-52266704E5CB}&topic1={2CF2CFE0-3290-4207-BC80-E691658C2074}&page=1

[71] For example, reports produced by the National Research Council's Board on Environmental Studies and Toxicology (see www.dels.nas.edu/best), and published by National Academy Press.

[72] Health and Safety Code sections 44300-44394 or 39650 et seq. The most current findings should be used. For the list available at the time of the proposed regulation is Office of Environmental Health Hazard Assessment, Technical Support Document for Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. Appendix A: Hot Spots Unit Risk and Cancer Potency Values, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Air Toxicology and Epidemiology Branch, May 2009, available at: http://www.oehha.ca.gov/air/hot_spots/2009/AppendixA.pdf

[73] Health and Safety Code section 116365. The most current findings should be used. For an example document, see Office of Environmental Health Hazard Assessment, Public Health Goals for Chemicals in Drinking Water: Benzo(a)pyrene, March 2010. Documents at the time of the proposed regulation are accessible at: http://www.oehha.ca.gov/water/phg/allphgs.html. To determine whether the criteria in the proposed regulation were met the document for the particular chemical would have to be reviewed.

evaluations of chemical substances and is consistent with OEHHA's mandated responsibility to include "other relevant data" to be included in the Toxics Information Clearinghouse pursuant to Health and Safety Code section 25256.1.

Subsections 69402.2(b)(1) and **69402.2(b)(2)** are based on the same citations for the U.S. Environmental Protection Agency and IARC given for Subsection 69402.2(a) above.

Subsections 69402.2(b)(3) includes recognition as a suspected carcinogen or the equivalent by California,[74] other states, the United States or other nations. As noted above, this includes recognition by the European Union as well as its member states. Many governments have in place a number of procedures, such as peer review, data quality and scientific guidance, which is designed to ensure the scientific integrity of work products produced by them for regulatory and public health purposes. Therefore, findings by such governmental entities would constitute suggestive evidence of carcinogenicity.

Subsection 69402.2(b)(4) explains that a chemical substance's possession of the Genotoxicity Hazard Trait defined in Article 3, section 69403.5 of this Chapter constitutes suggestive evidence of carcinogenicity. Genotoxicity as an indicator of potential carcinogenicity is well established and tests for genotoxicity are widely performed to screen chemicals for carcinogenic potential. For example, in explaining the reason for conducting genotoxicity testing, the European Medicines Agency said the following:[75]

> "Genotoxicity tests can be defined as *in vitro* and *in vivo* tests designed to detect compounds which induce genetic damage directly or indirectly by various mechanisms. These tests should enable a hazard identification with respect to damage to DNA and its fixation. Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage, recombination and numerical chromosome changes is generally considered to be essential for heritable effects and in the multi-step process of malignancy, a complex process in which genetic changes may play only a part. Compounds which are positive in tests that detect such

[74] See for example, Office of Environmental Health Hazard Assessment, Public Health Goals for Chemicals in Drinking Water, Cis- and Trans- 1,2-Dichloroethylene, OEHHA, California Environmental Protection Agency, March 2006, pp 24.
[75] European Medicines Agency, ICH Topic S 2 B. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. March 1998. CPMP/ICH/174/95, page 3

kinds of damage have the potential to be human carcinogens and/or mutagens, i.e. may induce cancer and/or heritable defects."

Therefore, in the proposed regulation OEHHA is identifying genotoxicity as suggestive evidence of carcinogenicity.

Subsection 69402.2(b)(5) provides that mechanistic evidence can suggest that a chemical substance has carcinogenic potential. Mechanistic evidence is comprised of data on how an agent may increase the risk of cancer. This can include data indicated in the International Agency for Research on Cancer Preamble to its Monographs on the Evaluation of Carcinogenic Risks to Humans[76], such as "changes in physiology" (e.g., mitosis, cell division, escape from apoptosis, inflammation), "functional changes at the cellular level" (e.g., alterations in DNA repair), "changes at the molecular level" (e.g., genotoxicity, hormonal dysregulation), and other data relevant to mechanisms. The International Agency for Research on Cancer's Preamble is given in the regulation as a basic source.

Mechanistic evidence alone can provide strong evidence of carcinogenicity. For example, chemicals such as dyes that are metabolized to benzidine would fall in this category because they cause specific biological effects that are established as a means of cancer induction. Both the International Agency for Research on Cancer and the National Toxicology Program treat such chemicals as known human carcinogens. In some cases, however, the evidence suggests carcinogenic potential but is not definitive. Such evidence would only be suggestive of carcinogenicity.

Subsection 69402.2(b)(6) describes evidence that is a strong indicator of carcinogenicity from structure activity relationships, including but not limited to those from validated Quantitative Structure Activity Relationship models. Structure activity relationships correlate a chemical's structure with its potential to cause an effect or potency in inducing the effect. Quantitative structure activity relationships quantitatively correlate structure to a biologic activity. For example, the U.S. Environmental Protection Agency has sponsored the development of the computer program OncoLogic™ to evaluate a chemical's carcinogenicity and has made it available to the public via the internet:[77]

[76] International Agency for Research on Cancer, 2006. Preamble, pp 15-17.
[77] U.S. EPA Council for Regulatory Environmental Modeling. OncoLogic™. General Information. Revised July 2, 2010. Available at http://cfpub.epa.gov/crem/knowledge_base/crem_report.cfm?deid=225846

> "OncoLogic™ is a software program that evaluates the likelihood that a chemical may cause cancer. OncoLogic™ has been peer reviewed and is being released by EPA at no cost, to be available to any researcher or organization wishing to evaluate cancer potential of chemicals. This expert system is a computer program that mimics the judgment of experts by following sets of knowledge rules that are based on studies of how chemicals cause cancer in animals and humans."

Strong indications of carcinogenicity from such programs should be considered suggestive evidence that a chemical substance may cause cancer. Therefore, OEHHA is identifying structure activity data potentially providing suggestive evidence of carcinogenicity that should be included in the Toxics Information Clearinghouse.

§ 69402.3 Developmental Toxicity

Subsection 69402.3(a) defines the developmental toxicity hazard trait. This definition is adapted from the U.S. Environmental Protection Agency's Guidelines for Developmental Toxicity Risk Assessment, published in1991, which defines developmental toxicology to be:

> "The study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation."[78]

The U.S. Environmental Protection Agency is a leading authority in the area of developmental toxicity risk assessment, and has published comprehensive guidelines for conducting such assessments.[79] The definition used in the proposed regulation is re-worded for context and slightly expanded for clarity. It is consistent with the more recent definition for developmental toxicity used by the International Programme for Chemical Safety of the World Health Organization adopted in 2001:[80]

> "Developmental toxicity, defined in its widest sense to include any adverse effect on normal development either before or after birth, has become of increasing concern in recent years. Developmental toxicity can result from exposure of either parent prior to conception, from exposure of the embryo or fetus *in utero* or

[78] U.S. Environmental Protection Agency's Guidelines for Developmental Toxicity Risk Assessment, 1991, page 3.
[79] For purposes of this and all the other proposed regulations that are adapted from an existing definition, the most recent decision from that authority should be consulted in interpreting this regulation.
[80] International Programme for Chemical Safety. 2001. Environmental Health Criteria No. 225. Principles for Evaluating Health Risks to Reproduction Associated with Exposures to Chemicals, Chapter 1: Summary and Recommendations.

> from exposure of the progeny after birth. Adverse developmental effects may be detected at any point in the life span of the organism. In addition to structural abnormalities, examples of manifestations of developmental toxicity include fetal loss, altered growth, functional defects, latent onset of adult disease, early reproductive senescence and shortened life span."

Thus, the definition in the proposed regulation reflects current basic understandings of developmental toxicity. Developmental toxicity can result from exposures of the parents, particularly the pregnant female, to a toxic chemical by any route of exposure that results in a sufficiently high internal dose. Developmental effects may be the result of direct exposure of the conceptus to the chemical substance when the developmental toxicant crosses the placenta and reaches the conceptus. Developmental toxicity can also occur even if the toxic chemical does not cross the placenta by, for example, impairment of placental function by the chemical. It may be difficult to distinguish between developmental toxicity that occurs because of direct action of a chemical on the conceptus, or because of the action of the chemical on the female reproductive system, or both. Irrespective of the possible mechanism of action, induction of the adverse effects described above constitutes developmental toxicity.

Prenatal developmental toxicity may occur at a level of exposure lower than that which causes toxicity in the mother. In many cases, however, developmental effects co-occur with manifestations of toxicity in the mother. When adverse developmental effects co-occur with minimal maternal toxicity, they are still considered to be indicative of developmental toxicity, and should not be dismissed out of hand. A widely accepted definition of minimal maternal toxicity is a level that at the least produces marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality[1]. At doses that cause excessive maternal toxicity, information on developmental effects may be difficult to interpret and be of limited value.

The definition in Subsection 69402.3(a) includes a separate explanatory sentence addressing postnatal developmental toxicity. During the postnatal developmental period, effects that are not specific to the developmental period may still constitute developmental toxicity. Effects such as toxicity to specific organs (e.g., liver or kidney) or physiological systems (e.g., the endocrine system) from exposure to a chemical substance may occur in both the developing and adult organism. These effects identify the developmental toxicity hazard trait when the developing organism shows greater quantitative or qualitative susceptibility to the chemical substance than does the adult organism. Greater quantitative susceptibility includes, but is not limited to, a higher proportion of developing organisms than adult organisms showing adverse effects at the

same level of exposure. Greater qualitative susceptibility includes, but is not limited to, developing organisms showing a more severe manifestation of toxicity (e.g., a greater degree of functional impairment of a physiological system, or a greater proportional reduction in specific organ weight) than do adult organisms at the same level of exposure. Assessment of increased quantitative or qualitative susceptibility of the developing organism to chemical substances is now standard regulatory practice in the U.S. Environmental Protection Agency's implementation[81] of the federal Food Quality Protection Act of 1996.[82] The issue of potentially greater quantitative or qualitative susceptibility of the young compared to adults was described by the National Academy of Sciences in 1993[83] and was one of the motivations for certain changes introduced in the Act related to pesticidal exposures of the young.

The definition in the proposed regulation is intended to capture information that may be available for all the issues identified above for inclusion in the Toxics Information Clearinghouse.

Subsection 69402.3(b) lists examples of general endpoints for the developmental toxicity hazard trait. As with the definition of the hazard trait itself, this information is adapted from the U.S. Environmental Protection Agency Guidelines for Developmental Toxicity Risk Assessment, 1991 (page 3):

> "Adverse developmental effects may be detected at any point in the lifespan of the organism. The major manifestations of developmental toxicity include: (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency."

The 2001 World Health Organization guidance[84] similarly states:

> "Adverse developmental effects may be detected at any point in the life span of the organism. In addition to structural abnormalities, examples of manifestations of developmental toxicity include fetal loss, altered growth, functional defects, latent onset of adult disease, early reproductive senescence and shortened life span."

[81] U.S. Environmental Protection Agency, 2002. Children are at Greater Risks from Pesticide Exposures. Available at: http://www.epa.gov/pesticides/factsheets/kidpesticide.htm; Implementation of Requirements under the Food Quality Protection Act (FQPA). Available at: http://www.epa.gov/pesticides/regulating/laws/fqpa/fqpa_implementation.htm
[82] Food Quality Protection Act of 1996 was enacted by Pub.L. 104-170, Title IV, 110 Stat. 1513, see section 401(a) of Pub.L. 104-170, set out as a note under section 301 of Title 21.
[83] National Academy of Sciences. 1993. Pesticides in the Diets of Infants and Children, National Academy Press, page 3.
[84] International Programme for Chemical Safety, Environmental Health Criteria 225 Principles For Evaluating Health Risks To Reproduction Associated With Exposure To Chemicals, IPCS, World Health Organization, Section 1.1 Summary. Available at: http://www.inchem.org/documents/ehc/ehc/ehc225.htm

Normal development of offspring is fundamental to the viability of all animal species, including humans. Exposure to agents affecting development can result in effects ranging from death through structural and growth alterations to functional deficits. These effects can be manifested in a variety of ways including spontaneous abortions, stillbirths, malformations, early postnatal mortality, reduced birth weight, mental retardation, sensory loss, and other adverse functional or physical changes that are manifested postnatally. These are important and commonly measured toxicological endpoints. The ways in which these can be measured are numerous and varied.[85]

Example of general types of developmental toxicological endpoints and data that are gathered to evaluate this trait are listed below. Because science and scientific methods are continuously evolving, and for the sake of brevity, this description cannot be considered comprehensive, but rather should be considered as a sampling of the types of information that can be collected on the toxicity of chemicals to the developing organism. These examples are all discussed in the U.S. Environmental Protection Agency Guidelines.[86]

- Death of the developing organism: This endpoint can occur at any stage of development from conception to sexual maturity. Measurements of the viability of the conceptus can include numbers of implantations (compared to number of corpora lutea), resorptions and numbers of live and dead pups.

- Structural abnormalities: Structural alterations in development include both malformations and variations, assessed at the external, skeletal and visceral levels. A malformation is usually defined as a permanent structural change that may adversely affect survival, development, or function. A variation is a divergence beyond the usual range of structural constitution that may not adversely affect survival or health. Distinguishing between variations and malformations is difficult since there exists a continuum of responses from the normal to the extremely deviant. There is no generally accepted classification of malformations and variations. Other terms that are often used, but no better defined, include anomalies, deformations, and aberrations.

- Altered growth: Altered growth is any alteration in offspring organ or body weight or size. It is generally assessed by changes in body weight, but other metrics such as crown-rump length, cranial circumference or skeletal ossification may

[85] IPCS, EHC 225, Principles For Evaluating Health Risks To Reproduction Associated With Exposure To Chemicals, Chapter 5: Evaluation of Developmental Toxicity.
[86] U.S. Environmental Protection Agency, 1991. Guidelines for Developmental Toxicity Risk Assessment, U.S. EPA Risk Assessment Forum, EPA/600/FR-91/001, pp. 5-31.

also be used. Changes in one endpoint may or may not be accompanied by other signs of altered growth (e.g., changes in body weight may or may not be accompanied by changes in crown-rump length and/or skeletal ossification). Altered growth can be induced at any stage of development, may be reversible, or may result in a permanent change.

- Functional developmental effects: Alterations or delays in the physiological and/or biochemical competence of an organism or organ system following exposure to an agent during critical periods of development pre- and/or postnatally. Functional deficits can be assessed in terms of neurobehavioral functions, and also of other organ system functions such as postnatal renal functional development.

The proposed regulation therefore is intended to provide a non-exclusive list of endpoints for which information and data should be included in the Toxics Information Clearinghouse.

Subsection 69402.3(c) describes other relevant data that may contribute to the evidence for developmental toxicity. U.S. Environmental Protection Agency Guidelines[87] state that:

> "Comparisons of the chemical or physical properties of an agent with those known to cause developmental toxicity may indicate a potential for developmental toxicity. Such information may be helpful in setting priorities for testing of agents or for evaluation of potential toxicity when only minimal data are available."

Proposed Subsection 69402.3(c) reflects this concept and provides more specific examples of properties of a chemical that may contribute to identification of the developmental hazard trait.

§ 69402.4 Evidence for Developmental Toxicity Hazard Trait

Subsection 69402.4(a) gives examples of what constitutes strong evidence for the developmental toxicity hazard trait, based on evidence and criteria used by a number of well recognized authoritative organizations and processes. These are discussed in more detail below.

[87] U.S. Environmental Protection Agency, 1991. Guidelines for Developmental Toxicity Risk Assessment, U.S. EPA Risk Assessment Forum, EPA/600/FR-91/001, page 33. Also available as Federal Register 56(234):63798-63826, section 3.1.3.2.

Subsection 69402.4(a)(1) provides that inclusion of a chemical on California's Proposition 65 list is strong evidence of developmental toxicity[88] for purposes of the Toxics Information Clearinghouse. California's Proposition 65 requires the creation of a list of chemicals known to cause cancer or reproductive toxicity. Developmental toxicity is a type of reproductive toxicity and as an aid for interpretation and communication of listed chemicals, categories of reproductive toxicity including male or female reproductive toxicity and developmental toxicity are provided on the Proposition 65 list.

Therefore the proposed regulation includes designation on the Proposition 65 list as a chemical known to cause reproductive toxicity on the basis of a developmental toxicity endpoint as strong evidence that the chemical substance has that hazard trait. Chemical substances that exhibit male or female reproductive effects are treated later in Subsection 69402.6(a)(1).

Subsection 69402.4(a)(2) provides that the criteria for certain evidence categories under classification systems of the National Toxicology Program is strong evidence that the chemical substance has the developmental toxicity hazard trait. The National Toxicology Program, the nation's preeminent toxicology organization, is a source for guidance and scientific reports. This federal government organization was developed "to coordinate toxicological testing programs within the Department of Health and Human Services, develop and validate improved testing methods, develop approaches and generate data to strengthen scientific knowledge about potentially hazardous substances and communicate with stakeholders."[89] The Program formally reviews the evidence for developmental toxicity of chemicals[90] and publishes the results of the reviews. The Program's findings regarding developmental toxicity provide approaches for how data are evaluated to ascertain the level of evidence of developmental toxicity.

Subsection 69402.4(a)(3) provides that meeting the criteria for classification under the United Nations' Globally Harmonized System for Classification and Labeling of Chemicals (GHS) as a "Category 1: Known or presumed human reproductive toxicant" based on developmental toxicity data is strong evidence that a chemical substance has this hazard trait. The current basis for the classification is laid out in "Chapter 3.7 Reproductive Toxicity" of the 2009 Third Revised Edition of the GHS. As with other documents cited in this proposed regulation, the current version of the document should be used. The GHS classification system subdivides reproductive toxicity into two categories: adverse effect on sexual function and fertility and adverse effects on

[88] Health and Safety Code, section 25249.5 et seq.
[89] Federal Register: December 3, 2003, Vol. 68, No. 232, Page 67691.
[90] e.g., MD Shelby (2005). National Toxicology Program Center for the Evaluation of Risks to Human Reproduction: Guidelines for CERHR Expert Panel Members. Birth Defects Research (Part B) 74:9-16.

development of offspring. The GHS adopted the definition of the 2001 World Health Organization International Programme for Chemical Safety described above, but

> "… considered that the classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women and men and women of reproductive capacity. Therefore for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure."[91]

Thus chemicals that might otherwise be considered developmental toxicants under the GHS might not be required to be classified as a reproductive toxicant. As discussed above, the GHS is designed to be a "self classification" system. Some nations may use the GHS criteria to classify a substance as a reproductive toxicant based on developmental toxicity data. Such classification is strong evidence for reproductive toxicity pursuant to either Subsection 69402.4(a)(3) or Subsection 69402.4(a)(7) of the proposed regulation. The GHS treats "genetically based inheritable effects in the offspring" under a separate category – Germ cell mutagenicity. However, these types of effects would be included under the developmental toxicity hazard trait under the general definition in Subsection 69402.4(a).

Subsection 69402.4(a)(4) provides that the National Institute for Occupational Safety and Health designations relevant to developmental toxicity in the pocket guide[92] is strong evidence of developmental toxicity. The designations can either be made as "symptoms" (e.g., "teratogenic effects") or as "target organs" (e.g., "reproductive system") or both. When the reproductive system is listed as the target organ, the underlying documentation would need to be consulted to determine if the basis for the designation relates to developmental toxicity.

Subsection 69402.4(a)(5) provides that strong evidence of developmental toxicity can be found in reports of the National Academy of Sciences National Research Council and Institute of Medicine. These include but are not limited to the reports issued by the Institute of Medicine[93] that evaluate the impact of chemical exposures on U.S. troops, and by the National Research Council[94] that evaluate environmental and occupational chemical hazards.

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[91] United Nations, GHS 3rd Edition, section 3.7.1.3 Adverse Effects on the Development of Offspring, United Nations,
[92] NIOSH Pocket Guide to Chemical Hazards. See for example, the notation of "teratogenic effects" as a symptom of diphenylamine exposure in the Pocket Guide. Also available at http://www.cdc.gov/niosh/npg/npgd0240.html
[93] For example the Institute of Medicine Veterans and Agent Orange series, National Academy Press, available at: http://www.iom.edu/Reports.aspx?series={9748FCC1-A076-4227-BCC1-52266704E5CB}&topic1={2CF2CFE0-3290-4207-BC80-E691658C2074}&page=1
[94] For example, reports produced by the National Research Council's Board on Environmental Studies and Toxicology (see www.dels.nas.edu/best), and published by National Academy Press.

Subsection 69402.4(a)(6) indicates that strong evidence of developmental toxicity can be obtained through identification of a chemical as having sufficient evidence of carcinogenicity by the International Agency for Research on Cancer, with a clear statement that the chemical substance induces transplacental carcinogenesis. For example, diethylstilbestrol is identified as a known cause of cancer subsequent to *in utero* exposure.[95]

Subsection 69402.4(a)(7) recognizes findings by California, other states, the United States or other nations that a chemical substance poses a developmental toxicity hazard. For example, calculation of a reference concentration for the chemical substance based on a developmental toxicity endpoint, or otherwise indicating the substance has the hazard trait, under the Air Toxics "Hot Spots" or toxic air contaminants programs,[96] or calculation of a health-protective concentration for drinking water, or its equivalent, for the chemical substance based on a developmental toxicity endpoint, or otherwise indicating the substance has the hazard trait, under the Public Health Goals for drinking water program.[97]

Subsection 69402.4(b) gives examples of what constitutes suggestive evidence for the developmental toxicity hazard trait.

Subsection 69402.4(b)(1) provides that the criteria for certain evidence categories under classification systems of the National Toxicology Program is suggestive evidence that the chemical substance has the developmental toxicity hazard trait. For example, "some" or "limited" evidence of adverse effects for the developmental toxicity endpoint would be suggestive evidence that a chemical substance has that hazard trait. The Program formally reviews the evidence for developmental toxicity of chemicals[98] and publishes the results of the reviews. The Program's findings regarding developmental toxicity provide parameters and guidance for others to evaluate the evidence for developmental toxicity.

[95] International Agency for Research on Cancer, Summary of Evaluations, Volume 100A . Available online at: http://monographs.iarc.fr/ENG/Meetings/EvaluationTable100A.pdf

[96] Health and Safety Code sections 44300-44394 or 39650 et seq. See for example Office of Environmental Health Hazard Assessment, Determination of Noncancer Chronic Reference Exposure Levels, Chronic Toxicity Summary for Benzene (Benzol; Benzole; Cyclohexatriene) CAS Registry Number: 71-43-2, March 2000. Also available at: http://oehha.ca.gov/air/hot_spots/2008/AppendixD3_final.pdf#page=24

[97] Health and Safety Code section 116365. See for example Public Health Goals for Nitrate and Nitrite in Drinking Water, Pesticide and Environmental Toxicology Section,Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, 1997, page 1.

[98] e.g., MD Shelby (2005). National Toxicology Program Center for the Evaluation of Risks to Human Reproduction: Guidelines for CERHR Expert Panel Members. Birth Defects Research (Part B) 74:9-16.

Subsection 69402.4(b)(2) provides that the approach by the International Agency for Research on Cancer to identify chemicals with limited evidence of carcinogenicity in animals for transplacental carcinogenesis is suggestive evidence that the chemical substance has that hazard trait. The most current criteria used by that organization should be used in evaluating the evidence. For example, most recent criteria at the time of this proposed regulation are given in 2006 International Agency for Research on Cancer Preamble. The Preamble is republished in the first part of all Monographs.[99]

Subsection 69402.4(b)(3) provides that recognition by California, other states, the United States or other nations that a chemical substance has suggestive evidence or positive but not strong findings of developmental toxicity or the equivalent should be considered suggestive evidence for purposes of the Toxics Information Clearinghouse.

Subsection 69402.4(b)(4) provides that chemicals possessing the Genotoxicity Hazard Trait or Endocrine Toxicity Hazard Trait, when mechanisms of genotoxicity or endocrine disruption are likely to be involved, is suggestive evidence that the chemical substance has the developmental toxicity hazard trait.

The National Academy of Sciences 2007 report "Toxicity Testing in the 21st Century: A Vision and Strategy" gives an example of how one type of endocrine disruption – thyroid disruption – might be considered in the case of a chemical that has not be subjected to long term apical endpoint testing:

> "A recent example of how the high-throughput assays could play out in the near term is the risk assessment of perchlorate. The data on perchlorate include standard subchronic- and chronic-toxicity tests and developmental-neurotoxicity tests, but risk assessments and regulatory decisions have been based on perturbation of iodide-uptake inhibition—the known toxicity pathway through which perchlorate has its effects (EPA 2006; NRC 2006). If a new chemical were found to inhibit iodide uptake, standard toxicity tests would not be necessary to demonstrate the predictable effects on thyroid hormone and neurodevelopment. Regulatory decisions could be based on the dose-response relationship for iodide-uptake inhibition."[100]

Using this process, a chemical that can cause prolonged disruption of iodide uptake would have the endocrine disruption hazard trait. This would also provide suggestive

[99] The International Agency for Research on Cancer publishes the most current preamble in its Monographs. The Preamble was last revised in 2006.
[100] National Academy of Sciences, 2007. Toxicity Testing in the 21st Century: A Vision and Strategy, National Academy Press, Washington DC, page 150.

evidence of developmental toxicity because of the relationship of iodide uptake to thyroid hormone production, and consequences of iodide deficiency for development.

Iodide uptake by the thyroid gland is an essential step in the synthesis of the thyroid hormones T3 and T4.[101] "[E]ven mild [thyroid hormone] insufficiency in humans can produce measurable deficits in very specific neuropsychological functions … the specific consequences of [thyroid hormone] deficiency depends on the precise developmental timing of the deficiency."[102]

Genotoxicity, and mutagenicity in particular, is one mechanism for the induction of developmental toxicity. For example, in explaining the reason for conducting genotoxicity testing the European Medicines Agency explains:[103]

> "Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage, recombination and numerical chromosome changes is generally considered to be essential for heritable effects ... Compounds which are positive in tests that detect such kinds of damage have the potential to be human carcinogens and/or mutagens, i.e. may induce cancer and/or heritable defects."

Subsection 69402.4(b)(5) provides that suggestive evidence also includes strong indications from "supportive studies," as described by the National Toxicology Program, indicating possible developmental toxicity. These studies include those described under subsections 69402.4(b)(6) and 69402.4(b)(7) of these proposed regulations.

Examples of information available in studies conducted by the National Toxicology Program are "toxicokinetics, ADME [absorption, distribution, metabolism and excretion], computational models, structure-activity relationships."[104]

Subsection 69402.4(b)(6) provides that suggestive evidence of developmental toxicity can be found when there are strong indications from mechanistic studies that a chemical behaves similarly to other known developmental toxins. Examples of mechanisms that may result in developmental toxicity include induction of reduced placental blood flow which may result in fetal death, growth retardation or malformation, or interaction with receptors such as the embryonic retinoic acid receptor which may

[101] National Academy of Sciences, 2005. Health Implications of Perchlorate Ingestion. Board on Environmental Studies and Toxicology, National Research Council, National Academy Press, Washington DC, page 5.
[102] RT Zoeller and J Rovet, 2004. Timing of Thyroid Hormone Action in the Developing Brain: Clinical Observations and Experimental Findings. Journal of Neuroendocrinology, 16:809.
[103] European Medicines Agency, ICH Topic S 2 B. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. March 1998. CPMP/ICH/174/95.
[104] National Toxicology Program (2009). Explanation of Levels for Developmental Toxicity.

lead to craniofacial malformations and neurobehavioral deficits.[105] Strong evidence of such effects from assays can be used as suggestive evidence of developmental toxicity for the chemical substance associated with it. A number of drugs are contraindicated in pregnancy mainly due to mechanistic concerns. For example, certain lipid lowering drugs are contraindicated because they could reduce fetal biosynthesis of cholesterol, and subsequent production of steroid hormones, and consequently cause fetal harm:

> "Cholesterol and other products of the cholesterol biosynthesis pathway are essential components for fetal development, including biosynthesis of steroids and cell membranes. Because of the ability of inhibitors of HMG-CoA reductase, such as lovastatin, to decrease the synthesis of cholesterol and possibly other products of the cholesterol biosynthesis pathway, [the drug] is contraindicated in women who are pregnant and in lactating mothers. [the drug] may cause fetal harm when administered to pregnant women. **[The drug] should be administered to women of childbearing age only when such patients are highly unlikely to conceive.** (emphasis in original) If the patient becomes pregnant while taking this drug, [the drug] should be discontinued immediately and the patient should be apprised of the potential hazard to the fetus."[106]

Subsection 69402.4(b)(7) provides that strong indications of developmental toxicity from structure activity relationships, including but not limited to those from validated Quantitative Structure Activity Relationship models, constitutes suggestive evidence. For example, currently evidence is not available for each aminoglycoside antibiotic regarding its potential to damage the inner ear and cause hearing loss or deafness in offspring of exposed mothers. Because of their similar structure and mechanism they all are presumed to pose a potential risk of developmental toxicity.[107] Various quantitative structure activity relationships have been or are under development for the development toxicity hazard trait for use in Green Chemistry applications.[108] For some QSAR models, false negative rates can be quite high[109], and in such cases models provide little guidance regarding whether or not a chemical may be safer than the one currently in the product. When there is clear understanding of the part of the molecule or its metabolite that is involved in toxicity, the results can be significantly more

[105] IPCS, EHC 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals, Chapter 5: Evaluation of Developmental Toxicity.
[106] Physicians Desk Reference, 2006. 60th Edition, Thomson PDR, Montvale, NJ, page 1696.
[107] Schaefer C, P Peters, RK Miller (Eds.) 2007. Drugs During Pregnancy and Lactation: Treatment Options and Risk Assessment. Academic Press; Physicians Desk Reference, 2010.
[108] For example, Cassano, A.; Manganaro, A; Martin, T.; Young, D.; Piclin, N.; Pintore, M.; Bigoni, D.; Benfenati, E. (2010). The CAESAR models for developmental toxicity. Chemistry Central Journal, 4(Suppl 1):S4.
[109] Maslankiewicz L, EM Hulzebos, TG Vermeire, JJA Muller, AH Piersma, Can chemical structure predict reproductive toxicity? RIVM report 6012000005/2005, IRVM Expert Center for Chemical Substances, RIVM, Netherlands.

predictive. Some classes of chemicals such as phthalates are well understood in terms of structure activity relationships and active moieties.[110]

§ 69402.5 Reproductive Toxicity

Subsection 69402.5(a) defines the reproductive toxicity hazard trait. The definition is adapted from that used by U.S. Environmental Protection Agency in its Guidelines for Reproductive Toxicity Risk Assessment. The Agency recognized that developmental toxicity is a component of reproductive toxicity, but for scoping its guidelines defined reproductive toxicity more narrowly:

> "For the purposes of these Guidelines, the following definitions will be used:
>
> "*Reproductive toxicity* - The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."[111]

The U.S. Environmental Protection Agency is a leading authority in the area of reproductive toxicity risk assessment, and has published comprehensive guidelines for conducting such assessments. The definition used in Subsection 69402.5(a) has been re-worded for context. It is also consistent with the guidance and discussion for reproductive toxicity developed by the World Health Organization International Programme for Chemical Safety.[112]

Successful reproduction is fundamental to the viability of all animal species, including humans. Exposure to agents affecting reproduction can result in infertility, reduced fertility or fecundity, or adverse effects on the development of offspring that are produced. Developmental toxicity is generally regarded as a component of reproductive

[110] E Lo Piparo and A Worth, 2010. Review of QSAR Models and Software Tools for predicting Developmental and Reproductive Toxicity, European Union, Joint Research Center, European Commission. Page 10.
[111] U.S. Environmental Protection Agency, Guidelines for Reproductive Toxicity Risk Assessment EPA/630/R-96/009, U.S. EPA Risk Assessment Forum, October 1996, page 5.
[112] International Programme for Chemical Safety. 2001. Environmental Health Criteria No. 225. Principles for Evaluating Health Risks to Reproduction Associated with Exposures to Chemicals, Chapter 5.

toxicity,[113] and male and female toxicity can be treated as two other distinct aspects of reproductive toxicity. Similar to the U.S. Environmental Protection Agency in the development of its guidance,[114] this proposed regulation keeps developmental toxicity as a separate hazard trait and covers both male and female reproductive toxicity under the hazard trait for reproductive toxicity.

Subsection 69402.5(b) lists examples of general endpoints for the reproductive toxicity hazard trait. These endpoints are adapted from the U.S. Environmental Protection Agency Guidelines for Reproductive Toxicity Risk Assessment and are similar to those described in recent guidance developed by the World Health Organization's International Programme for Chemical Safety.

Endpoints of male reproductive toxicity that can be specifically evaluated in male animals and, in some cases, epidemiologically or in human clinical studies include:

- Reproductive organ weights (including testes, epididymides, seminal vesicles, prostate, pituitary)
- Reproductive organ visual appearance and histopathology (including testes, epididymides, seminal vesicles, prostate, pituitary)
- Sperm parameters (including count of sperm numbers and evaluation of sperm morphology and motility)
- Sexual behavior (including mounts, intromissions and ejaculations)
- Hormone levels (including luteinizing hormone, follicle stimulating hormone, testosterone. estrogen, prolactin)
- Development of the male reproductive system (including testis descent, preputial separation, sperm production, ano-genital distance, structure of external genitalia)

Endpoints of female reproductive toxicity that can be specifically evaluated in female animals and, in some cases, epidemiologically or in human clinical studies include[115]:

- Reproductive organ weights (including ovary, uterus, vagina, pituitary)
- Reproductive organ visual appearance and histopathology (ovary, uterus, vagina, pituitary, oviduct, mammary gland)
- Estrous or menstrual cycle normality (vaginal smear cytology)

[113] U.S. Environmental Protection Agency, 1991. Guidelines for Developmental Toxicity Risk Assessment, U.S. EPA Risk Assessment Forum, EPA/600/FR-91/001, page 33. Also available as Federal Register 56(234):63798-63826, page 4.
[114] U.S. Environmental Protection Agency, 1991. Guidelines for Developmental Toxicity Risk Assessment; U.S. Environmental Protection Agency, Guidelines for Reproductive Toxicity Risk Assessment.
[115] U.S. Environmental Protection Agency, 1996. Guidelines for Reproductive Toxicity Risk Assessment, Chapter 3.

- Sexual behavior (including lordosis, time to mating, vaginal plugs or sperm)
- Hormone levels (including luteinizing hormone, follicle stimulating hormone, estrogen, progesterone, prolactin)
- Lactation (including offspring growth, milk quantity and quality)
- Development of the female reproductive system (including normality of external genitalia, vaginal opening, vaginal smear cytology, onset of estrous behavior or menstruation)
- Reproductive senescence (including vaginal smear cytology, ovarian histology, menopause)

Subsection 69402.5(c) describes other relevant data that may contribute to the evaluation of evidence of reproductive toxicity. The U.S. Environmental Protection Agency Guidelines state that:[116]

> "Numerous *in vitro* tests are available and under development to measure or detect chemically induced changes in various aspects of both male and female reproductive systems. These include *in vitro* fertilization using isolated gametes, whole organ (e.g. testis, ovary) perfusion, culture of isolated cells from the reproductive organs (e.g., Leydig cells, Sertoli cells, granulosa cells, oviductal or epididymal epithelium), co-culture of several populations of isolated cells, ovaries, quarter testes, seminiferous tubule segments, various receptor binding assays on reproductive cells and transfected cell lines, and others…"
>
> "The diagnostic information obtained from such tests may help to identify potential effects on the reproductive systems. However, each test bypasses essential components of the intact animal system and therefore, by itself, is not capable of predicting exposure levels that would result in toxicity in intact animals. While it is desirable to replace whole animal testing to the extent possible with *in vitro* tests, the use of such tests currently is to screen for toxicity potential and to study mechanisms of action and metabolism."

This is consistent with the sentiment of the World Health Organization International Programme for Chemical Safety in its 2001 guidance:[117]

> "A variety of *in vitro* test systems, including isolated perfused testis/ovary, primary cultures of gonadal cells, investigation of subcellular fractions of different organs and cell types and *in vitro* fertilization techniques, are available that can

[116] U.S. Environmental Protection Agency, 1996. Guidelines for Reproductive Toxicity Risk Assessment. U.S. EPA Risk Assessment Forum, Washington DC, page 53.
[117] International Programme for Chemical Safety. 2001. Environmental Health Criteria No. 225. Principles for Evaluating Health Risks to Reproduction Associated with Exposures to Chemicals, section 1.1.

> be used in supplementary investigational studies of different aspects of the reproductive system. *In vitro* testing systems are especially useful for screening for toxicity potential and for identifying potential mechanisms of action of potential toxicants. However, these tests are limited in their ability to assess complex, integrative reproductive functions."

In addition, the U.S. Environmental Protection Agency notes other information related to molecular structure that can indicate reproductive toxicity:[118]

> "Comparisons of the chemical or physical properties of an agent with those of agents known to cause reproductive toxicity may provide some indication of a potential for reproductive toxicity."

Thus overall, there is a wide range of "other relevant data" that are generally recognized to provide suggestive or supporting evidence rather than primary evidence for identification of reproductive toxicity. The language in the proposed regulation for "other relevant data" for reproductive toxicity intends to cover this broad range.

§ 69402.6 Evidence for Reproductive Toxicity Hazard Trait

Subsection 69402.6(a) defines what constitutes strong evidence for the reproductive toxicity hazard trait, based on criteria used by a number of well recognized authoritative organizations and processes.

Subsection 69402.6(a)(1) identifies chemical substances with endpoints of male or female reproductive toxicity on California's Proposition 65 list[119] as having strong evidence of reproductive toxicity. The mechanisms and procedures for creation and maintenance of the Proposition 65 list ensure that only chemicals with strong evidence of reproductive toxicity are included.[120]

Subsection 69402.6(a)(2) provides for clear evidence of reproductive toxicity when a chemical substance meets the criteria for listing in certain categories of the classification systems used by the National Toxicology Program. For example, the finding "clear" evidence of adverse effects for the reproductive toxicity endpoint constitutes strong

[118] U.S. Environmental Protection Agency, 1996. Guidelines for Reproductive Toxicity Risk Assessment, section 3.5 page 67.
[119] Health and Safety Code, section 25249.5 et seq.
[120] Office of Environmental Health Hazard Assessment Process for prioritizing chemicals for consideration under Proposition 65 by the "state's qualified experts", California Environmental Protection Agency, OEHHA, Sacramento, California, December 2004; Health and Safety Code section 25249.8; OEHHA, Mechanisms for listing and delisting chemicals under Proposition 65, April 2007.

evidence. The Program formally reviews the evidence for reproductive toxicity of chemicals and publishes the results of the reviews. It systematically publishes reviews.[121] The Program's findings regarding reproductive toxicity for various chemical substances provide examples of how data are evaluated to determine the level of evidence for the male and female reproductive toxicity they establish.

Subsection 69402.6(a)(3) provides that meeting the criteria for classification under the current United Nations' Globally Harmonized System for Classification and Labeling of Chemicals (GHS) as a "Category 1: Known or presumed human reproductive toxicant" based on reproductive toxicity data is strong evidence that a chemical substance has that hazard trait. The basis for these classifications is described in "Chapter 3.7 Reproductive Toxicity" of the 2009 Third Revised Edition of the GHS.[122] The proposed regulation encompasses GHS' term "adverse effects on sexual function and fertility" that in turn is covered by GHS' reproductive toxicity categories:

> "Any effect of chemicals that would interfere with sexual function and fertility. This may include, but not be limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."[123]

GHS goes on to note that:

> "Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately… [t]his is because it is desirable to be able to classify chemicals specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided to lactating mothers."[124]

For the purposes of this proposed regulation, adverse effects on or via lactation would fit into either the developmental or reproductive toxicity hazard traits, depending on the specific nature of the effect.

[121] MD Shelby (2005). National Toxicology Program Center for the Evaluation of Risks to Human Reproduction: Guidelines for CERHR Expert Panel Members. Birth Defects Research (Part B) 74:9-16.
[122] The current version of the cited document should be referenced when applying the proposed regulation.
[123] Globally Harmonized System for Classification and Labelling of Chemicals, Third Revised Edition, United Nations, 2009, section 3.7.1.2
[124] Globally Harmonized System for Classification and Labelling of Chemicals, Third Revised Edition, United Nations, 2009, section 3.7.1.2

Some nations may use the GHS criteria to classify a substance as a reproductive toxicant based on male or female reproductive toxicity data, and such classification would be strong evidence for reproductive toxicity under this subsection or Subsection 69402.6(a)(6) of this proposed regulation.

Subsection 69402.6(a)(4) provides that strong evidence of reproductive toxicity exists where a chemical substance is recognized in reports of the National Academy of Sciences National Research Council and Institute of Medicine. These include but are not limited to the reports by the Institute of Medicine[125] that evaluated the impact of chemical exposures of U.S. troops, and by the National Research Council[126] that evaluate environmental and occupational chemicals.

Subsection 69402.6(a)(5) provides that strong evidence of reproductive toxicity exists where the National Institute for Occupational Safety and Health identifies either "symptoms" (e.g., "reproductive effects," "sterility") or as "target organs" (e.g., "reproductive system," "prostate") or both for a given chemical substance in its pocket guide.[127] When the "reproductive system" is listed as the target organ or "reproductive effects" is listed as the symptom in the pocket guide, the underlying scientific documentation should be evaluated to determine if the basis for the designation relates to developmental or reproductive toxicity.

Subsection 69402.6(a)(6) recognizes that findings by California, other states, the United States or other nations of the chemical substance posing a reproductive toxicity hazard provide strong evidence that a chemical substance has that hazard trait . Examples of this are findings in California agency documents including calculation of a reference concentration for the chemical substance based on a male or female reproductive toxicity endpoint, or otherwise indicating the substance has the hazard trait, under the Air Toxics "Hot Spots" or toxic air contaminants programs[128]; or calculation of a health-protective concentration for drinking water, or its equivalent, for the chemical substance based on a reproductive toxicity endpoint, or otherwise

[125] For example the Institute of Medicine Veterans and Agent Orange series, National Academy Press, available at: http://www.iom.edu/Reports.aspx?series={9748FCC1-A076-4227-BCC1-52266704E5CB}&topic1={2CF2CFE0-3290-4207-BC80-E691658C2074}&page=1

[126] For example, reports produced by the National Research Council's Board on Environmental Studies and Toxicology (see www.dels.nas.edu/best), and published by National Academy Press,

[127] NIOSH Pocket Guide to Chemical Hazards, current copy is available at: http://www.cdc.gov/niosh/npg/ For example, the Pocket Guide entry for acrylamide currently available online at: http://www.cdc.gov/niosh/npg/npgd0012.html

[128] Health and Safety Code sections 44300-44394 or 39650 et seq. For example, see Office of Environmental Health Hazard Assessment, Hot Spots Risk Assessment Guidelines, Determination of Noncancer Chronic Reference Exposure Levels, Chronic Toxicity Summary, 1,3-Butadiene (butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene) CAS Registry Number: 106-99-0, December 2000.

indicating the substance has the hazard trait, under the Public Health Goals for drinking water program.[129]

Subsection 69402.6(b) describes what constitutes suggestive evidence for the reproductive toxicity hazard trait.

Subsection 69402.6(b)(1) is based on the same sets of considerations for the National Toxicology Program indicated in Subsection 69402.6(a). It includes meeting the criteria for listing by certain categories under classification systems of the National Toxicology Program, such as "some" or "limited" evidence of adverse effects for the reproductive toxicity endpoint. The Program publishes guidance for the evidence reviews.[130] The Program's findings regarding developmental toxicity provide parameters and guidance for the evaluations.

Subsections 69402.6(b)(2) provides that recognition of a chemical substance as a possible reproductive toxicant or the equivalent by California, other states, the United States or other nations constitutes suggestive evidence that the chemical substance has that hazard trait.

Subsection 69402.6(b)(3) explains that possessing the Genotoxicity Hazard Trait or Endocrine Toxicity Hazard Trait, when mechanisms of genotoxicity or endocrine disruption are likely to be involved in reproductive toxicity, is suggestive evidence that a chemicals substance has that hazard trait.

As noted by World Health Organization, International Programme for Chemical Safety:

> "Normal human reproduction is regulated by a finely tuned system of coordinated signals that direct the activity of multiple interdependent target cells, leading to the formation of gametes, their transport, release, fertilization, implantation and gestation, and, ultimately, the development of offspring that is eventually capable of successfully repeating the entire process under similar or different environmental conditions."[131]
>
> "Throughout the entire life cycle, all aspects of reproductive function are dependent on various endocrine communicating systems that employ a wide

[129] Health and Safety Code section 116365. See for example Office of Environmental Health Hazard Assessment, Public Health Goal for 1,2-Dibromo-3-chloropropane (DBCP) In Drinking Water, OEHHA, California Environmental Protection Agency, Sacramento, CA, pp 13-16, 23-25.
[130] MD Shelby (2005). National Toxicology Program Center for the Evaluation of Risks to Human Reproduction: Guidelines for CERHR Expert Panel Members. Birth Defects Research (Part B) 74:9-16.
[131] International Programme for Chemical Safety. 2001. Environmental Health Criteria No. 225. Principles for Evaluating Health Risks to Reproduction Associated with Exposures to Chemicals, section 1.1. Summary.

> variety of protein/peptide and steroid hormones, growth factors and other signaling molecules that affect target cell gene expression and/or protein synthesis."

Endocrine toxicity via mechanisms likely to be involved in reproductive toxicity provides evidence of the hazard trait. The World Health Organization International Programme for Chemical Safety provides further illustration:

> "Chemicals with estrogenic or antiandrogenic activity have been identified that are capable of causing reproductive effects in males. While sensitivity may differ, it is likely that mechanisms of action for these endocrine disrupting agents will be consistent or similar across mammalian species. For females, all functions of the reproductive system are under endocrine control and can be susceptible to disruption by effects on the reproductive endocrine system. However, single measurements of hormonal changes may be insensitive indicators of any damage because of large normal variability in females."

Genotoxicity is one important mechanism for the induction of reproductive toxicity,[132] [133] and a number of genotoxins are recognized male reproductive toxicants. Where given mechanisms of genotoxcity are understood to be involved in reproductive toxicity, findings that a chemical substance that induces them would be suggestive evidence of reproductive toxicity.

Subsection 69402.6(b)(4) provides that suggestive evidence of reproductive toxicity also includes strong indications from "supportive studies," as described by the National Toxicology Program, indicating possible reproductive toxicity. These studies would include those included under Subsections 69402.6(b)(5) and 69402.4(b)(6).

Examples of information from such studies provided by the National Toxicology Program are "toxicokinetics, ADME [absorption, distribution, metabolism and excretion], computational models, structure-activity relationships."[134]

Subsection 69402.6(b)(5) provides that suggestive evidence of reproductive toxicity results when there are strong indications that a chemical acts by a mechanism known to be involved in reproductive toxicity. For example, interference with the action of testosterone or blockage of the androgen receptor resulting in significant androgen insufficiency in the developing fetus would be expected to result in many of the male

[132] U.S. Environmental Protection Agency, 1996. Guidelines for Reproductive Toxicity Risk Assessment, section 3.5 pages 11-12.
[133] PP Trivedi, S Kushwaha, DN Tripathi, GB Jena, Evaluation of male germ cell toxicity in rats: Correlation between sperm head morphology and sperm comet assay, Mutation Research 703: 115–121, 2010.
[134] National Toxicology Program (2009). Explanation of Levels for Reproductive Toxicity.

reproductive system malformations caused by the class of chemicals known as phthalates.[135] Typically chemical substances to which this provision would apply would have either endocrine toxicity or genotoxicity hazard traits and would also fit the criteria in Subsection 69402.6(b)(3) of this proposed regulation.

Subsection 69402.4(b)(6) provides that strong indications of reproductive toxicity from structure activity relationships, including but not limited to those from validated Quantitative Structure Activity Relationship models, constitutes suggestive evidence. Structure activity relationships correlate a chemical's structure with its potential to cause an effect or potency in inducing the effect. When this is coupled with some molecular understanding of the mechanism by which a compound may produce an effect, confidence in predictions may increase. For example, there is now a very good understanding of structure activity relationships and a reasonable understanding of mechanism for the class of chemicals known as phthalates and male reproductive toxicity related to their antiandrogenic activity.[136] Whether or not an untested phthalate can produce antiandrogen syndrome can be predicted based with reasonable confidence by considering its particular chemical structure.

Quantitative Structure Activity Relationship models can be based purely upon statistical correlations. Aside from endpoints related to endocrine toxicity, there is limited ability to use such models for predicting reproductive toxicity:[137]

> "At present, the availability of [Quantitative Structure Activity Relationships] for reprotoxicity endpoints (excluding models related to endocrine activity) is limited as a result of the diversity and biological complexity of the endpoints, and the paucity of data suitable for modelling. Available models are potentially useful as a means of supporting hazard identification and priority setting, but not yet for the establishment of toxic potencies for use in risk assessment. Given the nature of the reprotoxicity endpoints, it is unlikely that an entirely structure-based approach will be capable of fully describing and predicting the *in vivo* effects. Thus, available models should not be used in isolation but to contribute to WoE [weight of the evidence] assessments, and to guide experimental testing, where necessary. Batteries of models and *in vitro* tests will need to be developed, and this has been the aim of an EU-funded Reprotect project."[138]

[135] National Academy of Sciences, Phthalates and Cumulative Risk Assessment. The Task Ahead. Board on Environmental Studies and Toxicology, National Academy Press, Washington DC, pages 51-52.
[136] National Research Council (2008). Phthalates and Cumulative Risk Assessment: The Task Ahead. National Academy Press, pp. 48-50; European Chemicals Agency Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals, May 2008, page 109.
[137] E Lo Piparo and A Worth, 2010. Review of QSAR Models and Software Tools for predicting Developmental and Reproductive Toxicity, European Union, Joint Research Center, European Commission, page 19.
[138] http://www.reprotect.eu

In the near term, quantitative structure activity relationship models may provide some support for identifying reproductive toxicity in the absence of other supporting information. However, in the case of certain endocrine-related reproductive toxicities they may potentially provide more compelling data.

Article 3. Other Toxicological Hazard Traits

All the information in the following Articles is necessary in order for OEHHA to meet its statutory mandate[139] to specify the hazard traits, endpoints and other relevant data to be included in the Toxics Information Clearinghouse. An explanation of each of the hazard traits, related endpoints and other relevant data are included in the discussion of each proposed subsection. Subsection 69403.16 of this Article provides a broad description of what constitutes "strong" versus "suggestive" evidence that a given chemical substance has that hazard trait. As noted for the previous Article, it is necessary for DTSC, industry and the public to be able to differentiate between the various types of information and data that may be available on the toxicity of a given chemical. OEHHA is proposing this regulation in order to provide guidance on this question.

§ 69403 General

Section 69403 identifies toxicological hazard traits in addition to those described in Article 2 of the proposed regulation. In total there are 18 toxicological hazard traits identified in the proposed regulation, 15 of which are described in this Article. These are:

Toxicological Hazard Traits

- Carcinogenicity (see Article 2)
- Cardiovascular Toxicity
- Dermatotoxicity
- Developmental Toxicity (see Article 2)
- Endocrine Toxicity
- Epigenetic Toxicity
- Genotoxicity
- Hematotoxicity
- Hepatotoxicity and Digestive System Toxicity
- Immunotoxicity
- Musculoskeletal Toxicity
- Nephrotoxicity and other Urinary System Toxicity
- Neurotoxicity

[139] Health and Safety Code section 25256.1.

- Ocular Toxicity
- Ototoxicity
- Reactivity in Biological Systems
- Reproductive Toxicity (see Article 2)
- Respiratory Toxicity

The toxicological hazard traits identified in this proposed regulation are intended to cover the full range of known toxicological hazards induced by chemical substances. As scientific knowledge advances, others may be identified that can be added to the regulation.

Fourteen of the traits identified in the proposed regulation have a chapter dedicated to them in the standard toxicology reference "Casarett and Doull's Toxicology: The Basic Science of Poisons"[140], and 16 of the 18 toxicological hazard traits are called out as general endpoints for evaluation in the U.S. Environmental Protection Agency's review of processes for establishing reference concentrations.[141] The fact that these traits are included by that Agency and also in a standard toxicology textbook indicates the importance of these traits in toxicological evaluations. Three of these (carcinogenicity, genotoxicity and developmental toxicity) correspond to non-organ directed toxicity chapters in Casarett and Doull's Toxicology.[142] Two additional non-organ directed toxicities have been added to the proposed regulation as hazard traits: epigenetic toxicity and reactivity in biological systems. Like genotoxicity, these toxicities affect fundamental toxicological processes and are associated with other toxicological hazard traits. Ototoxicity or musculotoxicity are additional target organ toxicities beyond those provided in Casarett and Doull's Toxicology. These appear in other standard toxicology reference texts, such as General and Applied Toxicology (Ballantyne B, Marrs TC, Syversen T. eds.) and Encyclopedia of Toxicology (Wexler P, ed.). These toxicities have been induced through human exposure to chemical substances, and the U.S. Environmental Protection Agency included musculoskeletal toxicity as a major system/endpoint for evaluation in toxicity testing.

This Article gives definitions for 15 toxicological hazard traits and provides examples of general types of endpoints that are studied to evaluate the hazard trait. Because science and scientific methods are continuously evolving the listing of endpoints in the proposed regulation is not exhaustive and should not be considered inclusive of all possible endpoints for a given hazard trait. A wide variety of endpoints are used by

[140] Klaassen CD (Editor), Casarett and Doull's Toxicology: The Basic Science of Poisons, 7th Edition, McGraw Hill Medical, 2008.
[141] A Review of the Reference Dose And Reference Concentration Processes. U.S. Environmental Protection Agency 2002 EPA/630/P-02/002F page 3-5.
[142] Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, Chapter 8 p.329-380, chapter 9 p. 381-414, chapter 10 p. 414-452.

researchers in academia, industry, and government, and by risk assessors in government to evaluate the potential damage induced by chemical exposures. The ways in which toxicity can be measured are numerous and varied. Measurements include gross and microscopic observations of tissues and organs, measures of function of the tissue or organ, and measures of cellular integrity or biochemical changes. Evaluation of toxicity can be conducted following exposure of whole animals or of isolated cells or tissues to chemicals. Evaluation of toxicity may also involve clinical studies of human volunteers or involve various other types of epidemiological evaluations.

General examples of "other relevant data" are also provided for the15 hazard traits identified in this article. The examples are meant to be illustrative of data that points in the direction of toxicity potential but, taken alone, may not be enough to establish with confidence that the chemical exhibits the toxicity hazard trait. For instance, elevated gene expression for inflammatory cytokines in an *in vitro* assay is an upstream event that points to the capability of a chemical to cause inflammation in cells. However, the degree to which that occurs in an animal exposed to the chemical may depend on factors not available in an *in vitro* assay, and may need to be studied directly.

Generally, toxicologists divide types of damage to an organ into acute and chronic.[143] Both types of damage are covered by the hazard traits definitions in the proposed regulation. Acute toxicity refers to adverse effects from short-term exposure to a chemical substance, and is usually measured within days following short-term exposure. Exposures in acute toxicity studies in animals can last minutes to 2 weeks. However, the toxicity from an acute exposure may manifest weeks, months or even years following exposure. Chronic toxicity refers to damage induced following long-term exposure to a chemical.

Other relevant data also includes structural and mechanistic similarity to known toxicants. For example, an inadequately tested chemical can have a similar chemical structure to well studied toxicants, or it can be known to act in similar ways on cells and tissues. Chemical similarity information is especially important when there are few toxicity data available to assess a chemical substance, which is true for the majority of chemicals used in commerce in the US.[144]

[143] U.S. EPA 2002. A Review of the Reference Dose and Reference Concentration Processes. U.S. EPA Reference Dose/Reference Concentration (RfD/RfC) Technical Panel, Risk Assessment Forum, EPA/630/P-02/002F. U.S. EPA Washington DC.
[144] National Research Council, Toxicity Testing for Assessment of Environmental Agents, Interim Report, National Academy Press, 2006, pages 99-100.

§ 69403.1 Cardiovascular Toxicity

Subsection 69403.1(a) defines the cardiovascular toxicity hazard trait. This definition is derived from the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms,[145] and covers the full range of adverse effects on the heart itself, arteries, capillaries and veins that may result from chemical stresses.[146] This definition includes damage to the cardiovascular system that affect system functions including the delivery of oxygen, nutrients, metabolites, and hormones to all tissues; the removal of waste products including carbon dioxide; the maintenance of internal homeostasis, cellular and tissue pH, and regulation of body temperature.[147]

For example, cardiovascular toxicity is the main toxicity that serves as a basis for regulating both carbon monoxide[148] and particulate matter by both the U.S. Environmental Protection Agency[149] and the California Environmental Protection Agency.[150] Cardiovascular toxicity has been considered in the establishment of environmental guidance levels for a number of chemical substances, such as the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment's Reference Exposure Levels[151] for inorganic arsenic, methylene chloride, carbon monoxide, and the U.S. Environmental Protection Agency's Reference Dose for sodium fluoroacetate.[152] Cardiovascular endpoints are critical endpoints to the whole organism.

Subsection 69403.1(b) provides a non-exclusive list of examples of general toxicological endpoints for the cardiovascular toxicity hazard trait, including structural and functional impairments of the heart, vasculature, or associated nervous system structures. These general endpoints cover a variety of types of cardiovascular toxicity that may be caused by chemical substances, and measured by researchers or clinics using a variety of methods.

[145] Eaton DC and Gilbert SC Chapter 2 and Gregus Z. Mechanisms of Toxicity. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. P. 45.
[146] Kang JY, Toxic Responses of the Heart and Vascular System. Chapter 18 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008.
[147] Ramos KS, RB Melchert, E Chacon, D Costa. Toxic Responses of the Heart and Vascular Systems. Chapter 18 in Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, ed. 6th edition, 2001, p.598.
[148] U.S. EPA National Ambient Air Quality Standard for carbon monoxide available at : http://www.epa.gov/airquality/urbanair/co/
[149] U.S. EPA National Ambient Air Quality Standard for Particulate Matter, Criteria Document available at: http://www.epa.gov/ttnnaaqs/standards/pm/s_pm_cr.html
[150] California Ambient Air Quality Standard for Particulate Matter, available at: http://www.arb.ca.gov/research/aaqs/std-rs/pm-final/pm-final.htm
[151] California Reference Exposure Levels available at: http://www.oehha.ca.gov/air/allrels.html
[152] U.S. Environmental Protection Agency. Sodium fluoroacetate (CASRN 62-74-8), Integrated Risk Information System.

The endpoints include damage to the tissues of the cardiovascular system from both acute and chronic chemical exposure, which can be measured by pathologic evaluation of the tissues of the heart and blood vessels by microscopy or other imaging techniques, or by monitoring electrophysiology of the heart through electrocardiography. Cell degeneration and death are frequently followed by cell hypertrophy, and fibrotic changes.[153] One manifestation of heart damage and repair from chemical toxicants is cardiac hypertrophy, which can lead to heart failure. A number of metal toxicants including cadmium, arsenic, and cobalt have been shown to cause degenerative changes in the heart muscle, hypertrophy, and cardiac arrhythmia.[154] Some toxic chemicals can initiate or promote atherosclerosis ("hardening of the arteries") and thus contribute to cardiovascular disease and the associated adverse outcomes including heart attack and stroke.[155]

Hallmark toxicological endpoints for adverse functional changes include: decreased cardiac output, abnormal heart rhythm, altered heart rate or heart rate variability, altered conductivity within the heart (contributing to dysrythmias and palpitation), and altered repolarization of the heart (QT prolongation; increasing the risk of sudden cardiac death).[156] These are commonly evaluated by electrocardiography and echocardiography. Cardiac output is a measure of the blood flow through the heart per minute, and sufficient cardiac output is necessary to meet the oxygen and metabolic demands of all the body's tissues. Cardiac output may be altered by toxicants whose effects are on the heart directly, the vasculature, or the nervous system. An example of a more specific measure of cardiac output used to indicate cardiotoxicity is change in the left ventricular ejection fraction. Cardiac dysrhythmias (abnormal heart rhythm) may result from a compound's ability to disrupt the tightly regulated control of ions inside and outside the heart cell necessary for proper electrical activity (and therefore pumping action) of the heart muscle. A number of drugs and environmental chemicals can interfere with ion homeostasis resulting in abnormal heart rhythm.[157] Cardiac arrhythmias can also be caused by a chemical's ability to sensitize the heart to endogenous catecholamines, such as occurs with some halogenated hydrocarbons.[158]

[153] Ramos KS, RB Melchert, E Chacon, D Costa. Toxic Responses of the Heart and Vascular Systems. Chapter 18 in Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, ed. 6th edition, 2001, p. 599-600.
[154] Brautbar N, Wiliams JA III, and Wu MP. Cardiotoxicity of industrial chemicals and environmental pollution. Chapter 12 in: Cardiovascular Toxicology, Acosta D, ed., Fourth Edition, 2008, Informa Healthcare, New York, p. 453-473.
[155] Ramos KS, RB Melchert, E Chacon, D Costa. Toxic Responses of the Heart and Vascular Systems. Chapter 18 in Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, ed. 6th edition, 2001, p.645.
[156] Kang JY, Toxic Responses of the Heart and Vascular System. Chapter 18 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.706-717.
[157] Ramos KS, RB Melchert, E Chacon, D Costa. Toxic Responses of the Heart and Vascular Systems. Chapter 18 in Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, ed. 6th edition, 2001,, p. 612-616.
[158] Ramos KS, RB Melchert, E Chacon, D Costa. Toxic Responses of the Heart and Vascular Systems. Chapter 18 in Casarett and Doull's Toxicology. The Basic Science of Poisons, CD Klaassen, ed. 6th edition, 2001, p.629-630.

Hypertension and vascular damage have also been associated with both lead and arsenic.[159]

A characteristic of a healthy cardiovascular system is a high level of heart rate variability, which can be evaluated by electrocardiography. Heart rate variability may be used to evaluate the effects of toxicant exposure on the vascular endothelium. Studies of toxicant exposure (e.g., environmental tobacco smoke, airborne particulate matter) have shown significant decreases in heart rate variability in exposed individuals.[160]

Other toxicological endpoints relate to the function of the blood vessels including the ability to dilate or constrict appropriately and maintain blood flow. Decreases in flow mediated dilatation reflect decrements in vascular endothelial function and reactivity. Decrements in flow mediated dilatation have been associated with exposure to toxicants that damage the vascular endothelium.[161] [162]

Subsection 69403.1(c) provides examples of other relevant data that may indicate cardiovascular toxicity potential. Perfused organ and isolated heart muscle preparations, aortic rings, isolated cardiomyocytes, tissue slices and tissue culture all provide models which are used to characterize electrophysiological and biochemical cardiovascular responses to chemical exposure. Measurements of biomarkers in the blood indicative of myocardial injury are also considered other relevant data. These include but are not limited to: creatinine kinase, B-Type Natriuretic peptide, C-Reactive protein, and cardiac troponins.[163]

The levels of expression of various genes or protein production can be evaluated in isolated cells of the heart or vasculature following chemical exposures to assess toxicological mechanisms and injury. Gene expression arrays have been developed from heart tissue and used following chemical exposure to understand genes targeted by specific chemicals.[164]

[159] Kang JY, Toxic Responses of the Heart and Vascular System. Chapter 18 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.733-734.
[160] Pope, C. A., 3rd, D. J. Eatough, D. R. Gold, Y. Pang, K. R. Nielsen, P. Nath, R. L. Verrier and R. E. Kanner (2001). Acute exposure to environmental tobacco smoke and heart rate variability. Environ Health Perspect 109(7): 711-6.
[161] Raitakari, O. T., M. R. Adams, R. J. McCredie, K. A. Griffiths and D. S. Celermajer (1999). Arterial endothelial dysfunction related to passive smoking is potentially reversible in healthy young adults. Ann Intern Med 130(7): 578-81.
[162] Woo, K. S., P. Chook, H. C. Leong, X. S. Huang and D. S. Celermajer (2000). The impact of heavy passive smoking on arterial endothelial function in modernized Chinese. J Am Coll Cardiol 36(4): 1228-32.
[163] Kang JY, Toxic Responses of the Heart and Vascular System. Chapter 18 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p.717-718.
[164] Handley-Goldstone HM, Grow MW, and Stegeman JJ. (2005) Cardiovascular gene expression profiles of dioxin exposure in zebrafish embryos. Toxicological Sciences 85:683-693.

Other relevant data would include measures of increased concentration of inflammatory markers such as circulating C-reactive protein, a biomarker of systemic inflammation and an independent predictor of cardiovascular disease.[165] [166] Another example of other relevant data is chemical exposure-associated pro-thrombotic changes in blood such as platelet activation and aggregation or other perturbation of clotting, an important factor in exacerbating ischemic heart disease.[167]

§ 69403.2 Dermatotoxicity

Subsection 69403.2(a) defines the dermatotoxicity hazard trait. The definition is derived from the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms,[168] and its barrier function "as the body's first line of defense against external insult."[169]

Skin protects the body against external insults in order to maintain internal homeostasis. The skin participates directly in thermal, electrolyte, hormonal, metabolic, and immune system regulation. Its protective function is critical as the body's largest interface with the environment. Skin comprises several layers and structures to which chemical toxicants can cause damage, including the dermis, basement membrane, epidermis, and epidermal appendages (hair follicles, sebaceous glands, and ecrine glands).[170] The skin may be a portal of entry and/or a site of biotransformation for many chemical substances, either via enzymatic or photochemical reactions. The dermatotoxicity hazard trait also includes toxicities to the nails and associated structures, including the nail plate, matrix, bed, root, and related structures necessary for nail growth and health.

The definition of the dermatotoxicity hazard trait in the proposed regulation is intended to be sufficiently broad to cover the range of skin toxicities that are currently considered adverse to human health. These skin toxicities are addressed by public health or regulatory agencies such as the U.S. Environmental Protection Agency, OEHHA and the medical community in assessing the potential adverse effects of chemical exposure.

[165] Nogueira JB Air pollution and cardiovascular disease (2009) Rev Port Cardiol 28:715-733.
[166] Araujo JA, Barafas B, Kleinman M et al. (2008) Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circulation Research 102:589-596.
[167] Simkhovich, B. Z., M. T. Kleinman and R. A. Kloner (2008). Air pollution and cardiovascular injury epidemiology, toxicology, and mechanisms. J Am Coll Cardiol 52(9): 719-26.
[168] Eaton DC and Gilbert SC Chapter 2 and Gregus Z. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 45.
[169] Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 74.
[170] Cohen DE and Rice RH 2001. Toxic responses of the skin. Casarett and Doull's Toxicology; The Basic Science of Poisons. Klaassen CD. New York, McGraw-Hill: 653-671.

For example, U.S.Environmental Protection Agency's oral Reference Dose for inorganic arsenic is based on the skin disorders hyperpigmentation and keratosis.[171]

Subsection 69403.2(b) provides examples of toxicological endpoints for the dermatotoxicity hazard trait. These are general endpoints that are intended to include the broad range of toxicities that fall under dermatotoxicity, and are commonly measured by researchers.[172] [173] [174]

Allergic contact dermatitis involves immune-mediated inflammation in response to repeated skin exposure to irritant compounds. Unlike irritant contact dermatitis, allergic contact dermatitis can result from the most limited exposure, following sensitization to a specific chemical.[175]

Irritant contact dermatitis is the most common form of contact-induced disorder. An irritant reaction is a localized inflammation that produces direct cellular injury upon dermal penetration by the irritant agent.[176] Strongly reactive substances may produce an acute irritant response or chemical burn following a single exposure.[177] Because it is not a sensitization response, the intensity of the inflammatory response is proportional to the exposure. Cumulative irritant contact dermatitis is the most common type of irritant contact dermatitis, developing after an extended period of up to years following exposure to chemical substances that may be weak irritants. Multiple simultaneous exposures or subsequent exposure may lead to additive effects and increase the skin's response.[178]

Damage to the skin from chemical exposure can be assessed visually and semi-quantified according to standardized scales. Common endpoints include: corrosion (necrosis), edema, erythema (redness), altered pigmentation, scaling, vesiculation (blistering), and induration (hardness). In test animals, these endpoints are typically

[171] U.S. Environmental Protection Agency Integrated Risk Information System, chronic oral Reference Dose for arsenic available at: http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0278#reforal
[172] Cohen DE and Rice RH 2001. Toxic responses of the skin. Casarett and Doull's Toxicology; The Basic Science of Poisons. Klaassen CD. New York, McGraw-Hill: 653-671.
[173] O'Malley M 2001. Regulatory evaluation of the skin effects of pesticides. Handbook of Pesticide Toxicology, Academic Press. 1: 299-333.
[174] Marzulli and Maibach's Dermatotoxicology, Zhai H, Wilhelm K-P, and Maibach HI, eds., Seventh Edition 2008, CRC Press.Chapters 40, 42, 44, 45, 51, 52, 58, 61, 62, 66, 75, 86, 88, 92, 102.
[175] Rice RH and Mauro TM. Toxic Responses of the Skin, Chapter 19 in: Casarett and Doull's Toxicology; The Basic Science of Poisons. Klaassen CD. New York, McGraw-Hill 2008, p. 746-750.
[176] Weltfriend S and Maibach HI. Irritant Dermititis: Clinical Heterogeneity and Contributing Factors. Chapter 13 in: Marzulli and Maibach's Dermatotoxicology, Zhai H, Wilhelm K-P, and Maibach HI, eds., Seventh Edition 2008, CRC Press. P.125-128.
[177] Rice RH and Mauro TM. Toxic Responses of the Skin, Chapter 19 in: Casarett and Doull's Toxicology; The Basic Science of Poisons. Klaassen CD. New York, McGraw-Hill 2008, p. 746.
[178] Ngo MA and Maibach HI 2010. Dermatotoxicology: historical perspective and advances. Toxicol Appl Pharmacol 243(2): 225-38.

assessed visually and include measurements of the size of the area affected, degree of redness, and relative severity. Morphological changes associated with pathology are often easier to detect in cell cultures such as of keratinocytes or other epithelial cells. Tests for determining the irritancy potential of specific chemicals involve either single or repeated application of the material to the skin,[179] and methods involving *in vitro* skin systems.[180] Photoirritation, or phototoxicity, is defined as a nonimmunologic sunlight-induced response to a photoactive agent. Light can stimulate certain photoactive chemicals that are on or in the skin, resulting in phototoxic or photoallergic skin reactions.[181] With exposure to ultraviolet light, some chemicals produce an allergic reaction. Unlike with phototoxicity, photoallergic reactions require prior sensitization, with reactions resulting from subsequent contact with the chemical.

Other examples of dermatotoxicity endpoints include acne and contact urticaria. Various forms of acne have been associated with chemical exposures via diverse routes. In particular, chloracne, an acne-like eruption of blackheads, cysts, and pustules, is associated with over-exposure to certain halogenated aromatic compounds, such as chlorinated dioxins and dibenzofurans.[182] While chloracne may derive from dermal exposure, this is an example of a toxic manifestation in the skin that may be the result of exposure by inhalation or ingestion.

Contact urticaria, or hives, is a response developing 30–60 minutes after skin exposure to a chemical substance. The reaction can remain localized or extend beyond the site of contact. Systemic symptoms may be seen in cases of strong hypersensitivity or in widespread exposure and extensive percutaneous absorption of the allergen.[183]

The potential dermatotoxicity of chemicals is often tested *in vitro*; thus, some *in vitro* measurements are considered toxicological endpoints as we have defined them. Skin corrosion by chemical substances can be tested *in vitro* in a three-dimensional reconstructed human epidermis (RhE) model comprising normal, human-derived epidermal keratinocytes, which have been cultured to form a multilayered, highly

[179] Ngo MA and Maibach HI 2010. Dermatotoxicology: historical perspective and advances. Toxicol Appl Pharmacol 243(2): 225-38.
[180] OECD 2009a. OECD Guideline for the testing of chemicals; Draft proposal for a new guideline: *In vitro* skin irritation: reconstructed human epidermis (RhE) test method. Paris, Organisation for Economic Co-operation and Development.
[181] Moulton-Levy NM and Maibach HI. Photoirritation (Phototoxicity, Phototoxic Dermatitis), Chapter 21 in Marzulli and Maibach's Dermatotoxicology, Zhai H, Wilhelm K-P, and Maibach HI, eds., Seventh Edition 2008, CRC Press., p. 209-213.
[182] Cohen DE and Rice RH 2001. Toxic responses of the skin. Casarett and Doull's Toxicology; The Basic Science of Poisons. Klaassen CD. New York, McGraw-Hill: 653-671.
[183] Ngo MA and Maibach HI 2010. Dermatotoxicology: historical perspective and advances. Toxicol Appl Pharmacol 243(2): 225-38.

differentiated model of the human epidermis.[184] Other effects on skin may also be tested *in vitro* with a RhE-based model that measures initiating events in the cascade of skin irritation or damage.[185]

Subsection 69403.2(c) provides examples of other relevant data for the dermatotoxicity hazard trait. This refers generally to *in vitro* measures of skin toxicity in cell-based models, and analysis of structural and mechanistic similarity to other dermatotoxicants.

Gene expression arrays developed to investigate gene expression during the elicitation of dermatitis may be useful to delineate genes affected in the skin following chemical exposure.

Changes in protein and cytokine production can also be measured in cultured skin cells to give insight into toxicological mechanisms and injury.[186] For example, the main pathological mechanisms of irritancy include skin barrier disruption, induction of a cytokine cascade and involvement of the oxidative stress network, which result in a visible or subclinical inflammatory reaction.

High reactivity and high lipid solubility are examples of chemical properties that suggest potential for dermatotoxicity due to enhanced tissue destruction or absorption.

§ 69403.3 Endocrine Toxicity

Subsection 69403.3(a) defines the endocrine toxicity hazard trait. The definition includes the full range of adverse effects on endocrine health that may result from chemical exposures, including endocrine disruption. The hazard trait is based on descriptions of endocrine toxicity and disruption used by the U.S. Environmental Protection Agency, the European Union[187], and in standard toxicology texts.[188]

[184] OECD 2004. OECD Guideline for the testing of chemicals; Draft proposal for an update of test guideline 431: *In vitro* skin corrosion: reconstructed human epidermis (RhE) test method. Paris, Organisation for Economic Co-operation and Development.
[185] OECD 2009a. OECD Guideline for the testing of chemicals; Draft proposal for a new guideline: *In vitro* skin irritation: reconstructed human epidermis (RhE) test method. Paris, Organisation for Economic Co-operation and Development.
[186] Ale IS and Maibach HI. Mechanisms in irritant and allergic contact dermatitis., Chapter 16 in: Marzulli and Maibach's Dermatotoxicology, Zhai H, Wilhelm K-P, and Maibach HI, eds., Seventh Edition 2008, CRC Press. P. 159-161.
[187] Commission of the European Communities (1999). Community Strategy for Endocrine Disrupters - a range of substances suspected of interfering with the hormone systems of humans and wildlife. Brussels, 17.12.1999. Available at: http://ec.europa.eu/environment/endocrine/documents/comm1999_en.htm.
[188] Capen CC (2008). Chapter 21: Toxic Responses of the Endocrine System. In: Klaassen, Curtis D. (2008). Casarett and Doull's Toxicology - The Basic Science of Poisons (7th Edition). McGraw-Hill.

Subsection 69403.3(b) provides general toxicological endpoints for the endocrine toxicity hazard trait. Endocrine toxicity endpoints include observations of adverse effects on endocrine organs. The general endpoints named include those named in standard texts, which describes a variety of endpoints that would fit into this general category, for the pituitary, adrenal cortex, adrenal medulla, thyroid, parathyroid, ovary and testis. It also includes endpoints based on the definition of endocrine disruptor in the scientific literature[189] and used by U.S. Environmental Protection Agency.[190]:

> "An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and or behavior."

Subsection 69403.3(c) identifies other relevant data for the endocrine toxicity hazard trait. These data in and of themselves would typically not provide strong evidence of the endocrine disruption hazard trait but would support such findings and could provide suggestive evidence. (See discussion of Evidence for Toxicological Hazard Traits below at page 91). Other relevant data include results of studies on receptor binding, computational approaches and *in vitro* studies that are used by the U.S. Environmental Protection Agency, in academia and by various authoritative organizations to explore the potential of a chemical to cause endocrine disruption.

§ 69403.4 Epigenetic Toxicity

Subsection 69403.4(a) defines the epigenetic toxicity hazard trait. The definition is adapted from the definitions for epigenetics used by a number of research groups and institutes throughout the world. For example, the Epigenome Network of Excellence (NoE), a European consortium consisting of 81 research groups, defines epigenetics as:

> “The studies of heritable changes in gene function that occur without a change in the sequence of nuclear DNA and the processes involved in the unfolding development of an organism.” [191]

Similarly, the Epigenomics Program at the National Institutes of Health describes epigenetics as:

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[189] TM Crisp, ED Clegg, Ralph L. Cooper, WP Wood, DG Anderson,KP Baetcke, JL Hoffmann, M Morrow, DJ Rodier, JE Schaeffer, LW Touart, MG Zeeman, YM Patel, Environmental Endocrine Disruption: An Effects Assessment and Analysis, Environmental Health Perspectives, 106(SupplementI): 11-56, 1998.

[190] Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, et al. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the U.S. EPA-sponsored workshop. Environmental Health Perspectives 104(S-4):1-26.

[191] The Epigenome Network of Excellence (NoE). http://www.epigenome-noe.net/WWW/index.php

> "An emerging frontier of science that involves the study of changes in the regulation of gene activity and expression that are not dependent on gene sequence."

For the purposes of its program, National Institutes of Health further states that:

> "Epigenetics refers to both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable."[192]

The broad definition of epigenetic toxicity in the proposed regulation identifies epigenetic changes resulting from exposure to chemicals and permits flexibility in recognizing new endpoints that are emerging from ongoing dynamic research in epigenetics or epigenetic toxicology.

Subsection 69403.4(b) provides examples of toxicological endpoints that may be used to indicate the presence of the epigenetic toxicity in an individual or its offspring, resulting from exposure to a chemical substance.

DNA methylation, histone modification, nucleosome remodeling, or non-coding RNA, are the major epigenetic mechanisms that are currently used to identify epigenetic changes at the cellular, individual, or population level.[193] These endpoints are included within those provided in this subsection.

DNA methylation is an important endpoint. It is the covalent addition of a methyl group to the fifth carbon of the cytosine ring to form 5-methyl cytosine (5meC). It is actively involved in regulating cell differentiation and function. When too much or too little methylation occurs, it can often negate a gene's function and thus causes unwanted alterations in the cell and may result in disease. For example, too little DNA methylation (hypomethlation) is believed to initiate chromosome instability and activate oncogenes. Conversely, too much DNA methylation (hypermethylation) may initiate the silencing of tumor suppressor genes. In the aging process, DNA methylation in the genome decreases as cells age.[194] [195]

There are a number of laboratory methods that are currently used to evaluate the status and patterns of DNA methylation in cells or tissues. In general, these methods include

[192] The National Institutes of Health. http://nihroadmap.nih.gov/epigenomics/
[193] Portela A and Esteller M. 2010 Epigenetic modifications and human disease. Nature Biotechnology 10:1057-1068.
[194] Beckerman M (2009). Epigenectics. In: Cellular Signalling in Health and Disease. M. Beckerman eds. Springer. pp 249-70.
[195] Szyf M (2007). The dynamic epigenome and its implications in toxicology. Tox Sci 100:7-23.

a combination of methylation detection strategies and identification of genes that are subject to DNA methylation[196]

Another endpoint that indicates the presence of epigenetic toxicity is histone modification. Histones are globular proteins that make up the nucleosome, the basic structural unit of chromatin. These proteins are subject to modifications including but not limited to, lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, and lysine ubiquitination and sumoylation. Histone code, or the pattern of histone modifications within a cell or DNA sequence can be analyzed by several laboratory methods. Chemical-induced histone modifications indicate abnormal changes in the epigenome.[197] [198]

Subsection 69403.4(c) provides general, non-exclusive categories of other relevant data that may be used to indicate the potential of epigenetic toxicity in an exposed individual or its offspring.

Numerous studies have utilized a large variety of methods to characterize the epigenetic status in normal or abnormal cells and to evaluate the potential of environmental factors to cause epigenetic changes either in mammalian cells or in other models such as zebrafish, Drosophila, and honeybees.[199]

Including epigenetic toxicity as a hazard trait will ensure that information on chemical effects on genes produced by this emerging field in toxicology will be available to the Toxics Information Clearinghouse.

§ 69403.5 Genotoxicity

Subsection 69403.5(a) defines the genotoxicity hazard trait. The definition is derived from the general definition of genotoxicity as the occurrence of a chemical substance-induced change to the hereditary material (cellular genome, including DNA sequences or chromosomes) that have the potential to be heritable at the cellular level, and genetic processes in living cells.[200]

[196] Schumacher A and Petronis A. Epigenetics of Complex Diseases: from General Theory to Laboratory ExperimentsCTMI (2006) 310:81–115.
[197] Szyf M (2007). The dynamic epigenome and its implications in toxicology. Tox Sci 100:7-23.
[198] Schumacher A and Petronis A. Epigenetics of Complex Diseases: from General Theory to Laboratory ExperimentsCTMI (2006) 310:81–115.
[199] Lockett GA, Wilkes F, Maleszka R (2010). Brain plasticity, memory and neurological disorders: An epigenetic perspective. Neuro Report 21:909-13.
[200] Preston JR and Hoffman GR in: Cassarett and Doull, 7th Edition, 2008, Chapter 9, Genetic Toxicology, p. 381.

Genotoxicants can potentially cause damage to all of the cells in an organism, including both germ cells (the cells that give rise to the sperm or ova of organisms that reproduce sexually) and somatic cells (all the other cells in an organism). Germ cell genotoxicity can prevent reproduction or result in deleterious heritable changes in offspring. Somatic cell genotoxicity can result in gene mutations or chromosomal damage, which are associated with increased cancer risk.[201]

A gene is a DNA sequence in a living organism that codes for a protein or a ribonucleic acid (RNA) sequence that has a function in the organism. All proteins and functional RNA chains are specified by genes. Genes code for the information needed to build and maintain cells. Genes are considered to be units of heredity in organisms, and pass genetic traits to offspring.

Damage to the genome (genes, noncoding DNA organized into chromosomes), or genotoxicity, can result in the disruption of cellular functions, which depend on protein and functional RNA chain synthesis. Genotoxicity can result in the production of partly functional or non-functional proteins and RNA chains, which in turn causes disruption of cellular function.

The definition of the genotoxicity hazard trait in the proposed regulation is meant to be sufficiently broad to cover the range of genotoxic effects that are considered adverse to human health and covered by agencies such as U.S. Environmental Protection Agency and OEHHA, and the medical community in addressing potential adverse effects. In addition, the definition is intended to encompass genotoxicity to terrestrial wildlife.

Subsection 69403.5(b) provides examples of genotoxicity endpoints. Genotoxicity endpoints include but are not limited to those indicating: DNA damage (such as DNA adduct formation and unscheduled DNA synthesis) mutations in genes, chromosomal aberrations, sister chromatid exchange, aneuploidy or polyploidy in humans, animals, or cell lines.

The list in this subsection is nearly identical to that given as examples in the IARC Preamble:[202]

> "The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy."

[201] Preston JR and Hoffman GR in: Cassarett and Doull, 7th Edition, 2008, Chapter 9, Genetic Toxicology , p. 383-384.
[202] IARC Monograph on the Evaluation of Cancer Risk to Humans. 2006, International Agency for Research on Cancer, World Health Organization. Preamble, pp 16-17.

The endpoints identified in this subsection can be measured in cells, animals and humans. The non-exclusive list of endpoints in the proposed regulation is intended to cover the range of genotoxicity assays, including systems named by IARC in its most recent Preamble:

> "Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals."

Some examples of more specific endpoints are provided here for the general genotoxicity endpoints described in the proposed regulation.

DNA damage endpoints include: Alkylation; apurinic site induction; apyrimidinic site induction; base damage; bulky adduct formation; double-strand breaks; single-strand breaks; DNA-protein crosslink formation; intercalation; interstrand crosslinks; intrastrand crosslinks; phosphotriester formation; pyrimidine dimer formation; radical formation.

Some commonly used assays of DNA damage include: Alkaline elution DNA strand breakage; COMET single cell gel electrophoresis DNA strand breakage; chemical covalent DNA binding (bulky adducts); bacterial DNA damage; mammalian cell DNA repair (unscheduled DNA synthesis).
.
Unrepaired DNA damage can result in gene mutations or chromosomal damage.

Gene mutation endpoints include: base pair mutations, frame shift mutations or small deletions. Some common tests of gene mutation include: reverse mutation in bacteria or fungi; forward mutation in bacteria, fungi, mammalian cells *in vitro*, mammalian *in vivo*; plant gene mutation; insect sex-linked recessive lethal mutations; and fish gene mutation.

Chromosomal damage classifications include structural chromosome aberrations, changes in chromosome number, and sister chromatid exchanges.

Types of chromosome aberrations include intra-chromosomal exchanges (inversions, interstitial deletions) and inter-chromosomal exchanges (dicentric chromosomes and reciprocal translocations). Common assays measure chromosome aberrations in plants, insects, fish,or mammalian (*in vitro* and *in vivo*).

Sister chromatid exchanges (SCE) may be due to errors in the chromosomal replication process during the S phase of mitosis. It is common to perform mammalian evaluation of SCE *in vivo* or *in vitro*.

Aneuploidy, that is a cell has extra copies or missing chromosomes, can be induced by interference with chromosomal movement (disruption of tubulin polymerization or spindle microtubule stability) during cell division. Common assays detect aneuploidy in fungi, plant cells, and mammalian cells in *in vivo* and *in vitro* experiments.

Other common assays of chromosome damage include micronucleus evaluation *in vitro* or *in vivo* in peripheral blood or bone marrow, fungal induced recombination and fish chromosomal damage.

Subsection 69403.5(c) provides examples of other relevant data that can provide evidence for the genotoxicity hazard trait described in standard sources.[203]

§ 69403.6 Hematotoxicity

Section § 69403.6(a) defines the hematotoxicity hazard trait, following the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms.[204] This definition covers the full range of adverse effects on the blood and blood forming tissues. [205] [206]

Hematotoxicity is the basis of a number of health protective levels developed by the U.S. Environmental Protection Agency and the California Environmental Protection Agency. One notable example is benzene, for which levels set by both agencies relate to both carcinogenicity and the non-cancer changes induced in the hematopoietic (blood-forming) system. Other aromatic compounds such as styrene[207] are regulated on the basis of hematological effects. The hematotoxicity of arsine is the basis of US Environmental Protection Agency's chronic RfC ("reference concentration") for this chemical.[208]

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[203] e.g., Preston RJ and Hoffman GR. Genetic Toxicology. Chapter 9 in: Toxicology. The Basic Science of Poisons. Klaassen CD, ed. Seventh Edition, 2008 p.385-386. and Gregus Z. Mechanisms of Toxicity. Chapter 3 in:Toxicology. The Basic Science of Ppoisons, Klaassen C. ed. Seventh Edition 2008 p. 55-58.

[204] Eaton DL and Gilbert SC Chapter 2 and Gregus Z. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 11, 45.

[205] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 455.

[206] Marrs TC, Warren S. Haemotology and Toxicology. Chapter 31 in: General and Applied Toxicology, Ballantyne B, Marrs, TC, Syversen T, eds., Third Edition, 2009 Wiley and Sons., p 742-762.

[207] US EPA IRIS database. http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0104#reforal

[208] US EPA IRIS database. http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0672

Subsection 69403.6(b) provides examples of general endpoints for the hematotoxicity hazard trait. These are generally classified by the site of impact, which also determines the nature of the resulting functional deficits depending on the role of the affected component. The most widely recognized role of the hematologic system is the delivery of oxygen from the lungs to tissues throughout the body by means of the red blood cells, or erythrocytes. Integral to the hematologic system is the blood-forming tissues that produce erythrocytes, primarily the bone marrow. The erythrocytes are also involved in the transport of carbon dioxide from tissues to the lung, in the maintenance of a constant pH in blood, and help modulate the inflammatory, and also have a role as a carrier and/or reservoir for drugs and toxins.

Toxicant effects on the hematologic system are frequently manifested as a decrease in the circulating red blood cell mass, (anemia), and occasionally an increase (erythrocytosis). Some toxicants affect the oxygen affinity of hemoglobin. This may lead to an increase in the red blood cell mass (erythrocytosis). In general, either decreased production or increased destruction of erythrocytes may lead to anemia.[209]

Circulating blood cells also include leukocytes (white blood cells), which can be further subdivided into neutrophils, eosinophils, basophils, monocytes and lymphocytes. These cells play a central role in the inflammatory response and host defense. Leukocytes are susceptible to chemical agents which impact their formation, resulting in a decline in the numbers of one or more cell types.[210] Various leukocyte classes are also targets for chemical carcinogenic effects. Adverse changes in leukocyte functions are specifically addressed in the discussion of the immunotoxicity hazard trait.

The hematologic system also encompasses hemostasis, the multicomponent system responsible for prevention of blood loss from sites of vascular injury and maintaining circulating blood in a fluid state. The platelets (thrombocytes) are essential for formation of a stable hemostatic plug in response to vascular injury and the maintenance of vascular integrity. Toxic chemicals may affect the rates of either formation or destruction of thrombocytes, most often resulting in thrombocytopenia (reduced numbers of circulating thrombocytes). A number of chemicals are known to affect particular components of the hemostatic process, including platelet function and the cascade of control factors and proteolytic enzymes which implement the conversion of fibrinogen to fibrin in clot formation (e.g., aspirin, warfarin, coumarins).[211]

[209] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.456-465.
[210] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.465-469.
[211] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 471-476.

The primary blood forming tissue of the body is bone marrow. However, in the fetus hematopoiesis (i.e., production of blood cells) also occurs in the spleen, liver, thymus and lymph nodes. The spleen and lymphoid tissues are also considered part of the hematologic system. The spleen has little function in blood cell production in the healthy human adult, but plays a critical role in the clearance of defective or senescent cells, as well as host defense.

Measurements of serum composition are also frequently used as measures of toxicity affecting the blood itself, as well as indicators for toxicity at other organ sites. Hematological parameters of relevance to hematotoxicity include the serum concentrations of haptoglobin, lactic dehydrogenase, free hemoglobin, vitamin B12, folate, iron, ferritin, and the direct or indirect red cell antiglobulin test.[212]

Common functional toxicological endpoints involve decreased oxygen transporting capacity of hemoglobin.[213] A number of compounds (e.g., lead) can interfere with one or more steps in erythroblast heme synthesis and result in sideroblastic anemia. A number of chemicals can cause other forms of anemia including hemolytic anemia (quinidine), megaloblastic anemia (colcicine), and aplastic anemia (benzene).

Methemoglobinemia and carboxyhemoglobinemia are disorders associated with a reduction in the capacity of RBCs to transport oxygen. Whereas methemoglobinemia results from chemicals (e.g., nitrites and nitrates) oxidizing iron in hemoglobin from the ferrous state to the ferric state, carboxyhemoglobinemia results from the complexation of carbon monoxide with hemoglobin.

Chemicals can also cause an increase or decrease in blood clotting activity. Toxicants may interfere with the platelet response by causing thrombocytopenia (reduced platelet number) or interfering with platelet function, or both.[214] Alterations in the components or systemic activation of this system can lead to the clinical manifestations of deranged hemostasis, including excessive bleeding and thrombosis. The most common toxic effects of xenobiotics on clot formation are related to a decreased level of one or more of the critical proteins necessary for this process, or interference with cofactors (e.g., Vitamin K) resulting in poor clotting and excessive bleeding.

Subsection 69403.6(c) provides examples of other relevant hematotoxicity data.

A variety of measures *in vitro* of function in isolated blood cells have been used as indicators of toxicity. Structural measures such as resistance to hemolysis have also been determined *in vitro.* Such measurements contribute to the understanding of the

[212] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 458.
[213] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.460-462.
[214] Bloom JC and Brandt JT. Toxic Responses of the Blood, Chapter 11 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 471-476.

mechanism of action of toxicants as well as contributing to the identification of the hematotoxicity hazard trait. Structural or mechanistic similarity to other chemical substances with the hematotoxicity hazard trait is also an important indicator of potential hazard.

§ 69403.7 Hepatotoxicity and Digestive System Toxicity

Subsection 69403.7(a) defines the hepatotoxicity and digestive system toxicity hazard trait as the occurrence of adverse effects on the structure or function of the liver, gall bladder, or gastrointestinal tract following exposure to a chemical substance. The definition of the hepatotoxicity hazard trait is derived from the general definition of toxicity as adverse effects of an agent on the structure or function of living organisms,[215] and is meant to be sufficiently broad to cover the range of liver and digestive system toxicities[216] that are considered adverse to human health by regulatory agencies such as U.S. Environmental Protection Agency and OEHHA and the medical community in addressing potential adverse effects.

The liver is a critical organ for maintenance of the body's metabolic homeostasis. Venous blood from the stomach and intestines flows though the liver before entering the systemic circulation; the liver is therefore the first organ to process ingested nutrients, vitamins, drugs, or environmental toxicants for use, storage, or excretion into bile. The major functions of the liver include nutrient homeostasis, protein synthesis, biotransformation and detoxification, formation of bile and biliary excretion. Hepatocytes are the primary functional cells of the liver and they make up 80% of the mass of the liver. Bile is a yellow fluid that contains bile acids, bilirubin, and other proteins, ions, metals, and biliary excretion is an important process for removing toxicants from the body. Bile plays a key role in the absorption of dietary fat and disruption of normal bile production via hepatotoxicity can have adverse effects such as steatorrhea or excess fat in the feces.

The U.S. Environmental Protection Agency based its reference concentration for chronic inhalation of carbon tetrachloride on fatty changes in the liver, and the oral reference dose for carbon tetrachloride on elevated serum sorbitol dehydrogenase activity.[217] The U.S. Environmental Protection Agency also based the oral reference

[215] Eaton DC and Gilbert SC Chapter 2 and Gregus Z. Mechanisms of Toxicity. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 45.
[216] Jaeschke H. Toxic Responses of the Liver, Chapter 13 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p. 557-582.; Spainhour, C. Gastrointestinal function and toxicology in canines, Chapter 6 in Toxicology of the Gastrointestinal Tract, Gad, SC, ed. CRC Press2007, p.181-197.
[217] U.S.EPA Integrated Risk Information System; available at http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0020

dose for chloroform on fatty cyst formation in liver and elevated serum glutamic-pyruvic transaminase.[218]

Subsection 69403.8(b) provides examples of toxicological endpoints for the hepatotoxicity and digestive system toxicity hazard trait. These are important and commonly measured toxicological endpoints that include a variety of types of liver and digestive system toxicity. Measurements involve gross and microscopic observations of liver tissue, which can be conducted following exposure in whole animals or in cell-based assays. Clinical chemistry also can be used to measure toxicity to the liver.

Damage to the liver from both acute and chronic chemical exposure can be measured by pathologic evaluation of the liver tissue both at the gross and microscopic levels. Histopathological findings of chemically-induced liver toxicity include, but are not limited to: liver enlargement; fatty liver or steatosis (an appreciable increase in the fat content of the liver); liver cell damage or death; damaged or blocked sinusoids, cannaliculi, or bile ducts; or fibrotic changes.[219] Chemically induced sinusoidal damage is considered an early effect in chronic veno-occulsive or vascular liver disease. Cirrhosis is characterized by the accumulation of extensive amount of fibrous tissue, specifically collagen fibers in response to direct liver injury or inflammation. With progressive collagen deposition the liver structure is altered limiting its functional capacity irreversibly. [220]

An important signal of liver toxicity is the increase of serum levels of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and increased bilirubin levels that indicate that the function of the liver is suboptimal. These are used in the clinical setting to evaluate liver damage. The enzymes are released into the blood as the liver cells die. Jaundice, dark urine color and pale stool color are specific toxicological endpoints for liver toxicity and can be used to indicate frank functional impairment.[221]

Bile formation is an important function of the liver. Chemical-induced damage to hepatocytes and bile duct cells can lead to cholestasis, interruption of bile formation, which in turn causes the accumulation of bile acids in the liver, and ultimately to elevated levels of bile salts and bilirubin in the serum. The accumulation of bile acids in

[218] U.S.EPA Integrated Risk Information System; available at: http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0025
[219] Jaeschke H. Toxic Responses of the Liver, Chapter 13 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p. 562-567.
[220] Jaeschke H. Toxic Responses of the Liver, Chapter 13 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p. 564-567.
[221] Jaeschke H. Toxic Responses of the Liver, Chapter 13 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p. 564.

hepatocytes can ultimately lead to premature liver cell death.[222] Damage to the hepatic bile ducts (cholangiodestructive cholestasis) is also indicated by a sharp increase in serum levels of alkaline phosphatase. Significant reduction of bile flow leading to impaired fat absorption and increased lipid in the feces may also result in deficiencies in fat-soluble vitamins (e.g., D, K, and E) particularly in cholestatic children. Steatorrhea, which results in foul-smelling, bulky stools, occurs when fecal fat exceeds six percent of dietary fat.

Subsection 69403.7(c) provides examples of other relevant data for hepatotoxicity and digestive system toxicity. For example, non-parenchymal cells such as Kupffer cells, stellate cells and neutrophils which play a role in chemical-induced inflammation in the liver, may secrete inflammatory chemical messengers in response to a chemical insult which can be measured. Immune response such as the migration of neutrophils and other inflammatory cells into the liver can be measured and is considered to be a factor in the hepatotoxicity induced by some chemicals.[223]

Mitochondrial injury is involved in chemical-induced microvesicular steatosis, nonalcoholic steatohepatitis (NASH), and cytolytic hepatitis.[224] Mitochondrial functional parameters such as respiration, membrane potential, reactive oxygen species (ROS) production, and mitochondrial complex activity can be studied in isolated mitochondria. These types of data are thus relevant to evaluation of the hepatotoxicity potential of a chemical.

Isolated hepatocytes can be examined for a variety of responses including cell death, generation of reactive oxygen species, degeneration of cellular organelles, changes in gene expression indicative of hepatotoxicity. Thus, such cell assays can contribute relevant data to the evaluation of the hepatotoxicity of chemicals.

Information that the chemical is similar in structure to other liver toxicants or acts on the cell in similar ways to chemicals that induce liver toxicity is also relevant to the evaluation of chemical-induced hepatotoxicity. Such information is especially important when there is little toxicity data to assess a chemical, which is the case for the majority of chemicals in commerce in California.

[222] Rust C. et al. (2009). Bile acid-induced apoptosis in hepatocytes is caspase-6-dependent. J. Biol. Chem. 284(5):2908-2916.
[223] Jaeschke H. Toxic Responses of the Liver, Chapter 13 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008,p. 573-576.
[224] Fromenty B. and D. Passayre. (1995). Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol. Ther. 67(1):101-154.

§ 69403.8 Immunotoxicity

Subsection 69403.8(a) defines the immunotoxicity hazard trait. The definition derives from the basic definition of toxicity as adverse effects on the structure and/or function of a living organism[225] and is intended to include the full range of adverse effects on the immune system.[226] It is similar to the U.S. Environmental Protection Agency definition provided in their Biochemicals Test Guidelines for testing of pesticides and toxic substances:

> "Immunotoxicity refers to the ability of a test substance to induce dysfunction or inappropriate suppressive or stimulatory responses in components of the immune system." [227]

A similar definition is given in U.S. Environmental Protection Agency's Health Effects Test Guidelines:[228]

> "Immunotoxicity refers to the ability of a test substance to suppress immune responses that could enhance the risk of infectious or neoplastic disease, or to induce inappropriate stimulation of the immune system, thus contributing to allergic or autoimmune disease."

The immune system functions to protect against infection and cancer. The immune system is a complex network of various primary lymphoid organs (e.g., bone marrow, thymus, fetal liver), secondary lymphoid organs (e.g., spleen, lymph nodes, mucosa-associated lymphoid tissues), tertiary lymphoid tissues (sites where immune system cells exert their effects), cell types and molecules.[229] They work individually and in cooperation with one another to maintain the homeostasis of the body to defend against invading pathogens. Chemical toxicants can cause damage to immune system cells, or lymphoid tissues, or can interfere with the immune response itself, including changing the production or function of molecules involved in the immune response (e.g., soluble mediators, antibodies, proteins in the complement cascade) or the interactions of those molecules with cells or pathogens.

Toxic chemicals can adversely affect the immune system resulting in immunosuppression, hypersensitivity diseases, or autoimmunity. The result can be

[225] Eaton DC and Gilbert SC Principles of Toxicology, Chapter 2, p. 11 and Gregus Z. Mechanisms of Toxicity. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 45.
[226] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p. 486.
[227] Biochemicals Test Guidelines OPPTS 880.3550. Immunotoxicity EPA 712-C-96-280, February, 1996, U.S. Environmental Protection Agency, Prevention, Pesticides and Toxic Substances, Washington D.C.
[228] Health Effects Test Guidelines OPPTS 870.7800 Immunotoxicity, EPA 712-C-98-351, U.S. Environmental Protection Agency, Prevention, Pesticides, and Toxic Substances April 1998.
[229] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p. 487-488.

increased incidence of infectious disease, more prolonged or severe infections, elevated cancer risk,[230] [231] and allergic diseases. This is further supported from *in vivo* and *in vitro* studies of experimental animals that environmental chemicals can inhibit the immune system and alters host resistance to infectious agents or tumor cells.[232]

Autoimmune diseases, where an individual's own immune system attacks tissue or organs, resulting in functional impairment, inflammation and permanent tissue damage has been associated with industrial chemical exposure (e.g.,crystalline silica, solvents) and drugs (e.g penicillamine and procainamide-induced lupus).[233] There are a number of well known industrial chemicals that induce contact hypersensitivity, for example, toluene diisocyanates, and ethylenediamine. Immunotoxicity is the basis for the risk assessment and regulation of chemicals by the U.S. Environmental Protection Agency, National Institute for Occupational and Safety and Health recommendations for occupational standards, and California Environmental Protection Agency including nickel[234], isocyanates[235], and chromates.[236]

Subsection 69403.8(b) provides examples of important and commonly measured toxicological endpoints for the immunotoxicity hazard trait.[237] [238] Damage to the immune system from both acute and chronic chemical exposure can be measured by pathologic and functional evaluation of the lymphoid organs and their cells. Structural toxicological endpoints can be evaluated by gross and microscopic observations of lymphoid organs and tissues. For example, a change in size, weight or architecture of the thymus is an important indicator of chemical induced effect. Other endpoints include increased lymphocyte apoptosis, cellularity of the cortex and medulla, and increase or decrease in the epithelial component of the thymus. The structural integrity of mucosal tertiary lymphoid tissue and lymph nodes can also be measured as an endpoint to evaluate immunotoxic effects.

[230] Ehrke M, Mihich E 1985. Effects of anticancer agent on immune response. Trend pharmacol Sci 6:412-417.
[231] Penn 2000. Post transplant malignancy: The role of immunosuppession. *Drug Saf* 23:101-13.
[232] Luster MI, Rosenthal 1993. Chemical agents and the immune response. *Environ Health Prospec*. 100:219-236.
[233] Cooper GS, and Miller FW. Environmental influences on autoimmunity and autoimmune diseases. Chapter 25; Uetrecht JP. Drug-induced autoimmune disease, Chapter 26 in: Immunotoxicology and Immunopharmacology. Luebke R, House R, and Kimber I, eds. 2008 CRC Press, p. 437-453; 455-468.
[234] Cal/EPA OEHHA Reference Exposure Level for nickel available at: http://www.oehha.ca.gov/air/allrels.html.
[235] http://www.cdc.gov/niosh/topics/isocyanates/ Many isocyanates occupational standards are based on respiratory or skin sensitization and asthma including methylene diphenyl diisocyanate, toluene-2,4-diisocyanate, hexamethyl diisocyanate, methyl isocyanate.
[236] Occupational standard partly based on sensitization dermatitis; http://www.cdc.gov/niosh/npg/npgd0138.html
[237] Biochemicals Test Guidelines OPPTS 880.3550. Immunotoxicity EPA 712-C-96-280, February, 1996, and Biochemicals Test Guidelines OPPTS 880.3800 Immune Response, EPA 712-C-96-281 February, 1996. U.S. Environmental Protection Agency, Prevention, Pesticides and Toxic Substances, Washington D.C.
[238] Health Effects Test Guidelines OPPTS 870.7800 Immunotoxicity, EPA 712-C-98-351, U.S. Environmental Protection Agency, Prevention, Pesticides, and Toxic Substances April 1998.

Functional endpoints measure the integrity of the immune system including immunocompetence,[239] and can include evaluation of immune cell functions, and measures of levels of molecules involved in either innate or acquired immunity. These end points can be measured *in vivo*, *in vitro* and/or with a combination of *in vivo* chemical exposure followed by *in vitro* measurements of isolated immune system cells.

Many toxicological endpoints include measurements that can be easily assessed in peripheral blood, such as complete blood count with differential, immunoglobulin concentration, specific antibody levels, lymphocyte subset characterization of T and B cells, delayed type hypersensitivity, NK cell function, lymphocyte and cytokine measurements, and autoantibody titres.[240] Numbers and ratios of macrophages, neutrophils, eosinophils, basophils, mast cells, and natural killer cells in peripheral blood or other tissues can be used to assess impacts of chemicals on immune function. The impact of chemical exposure on the ability of macrophages to engulf and kill pathogens, or ability of natural killer cells to destroy infected and malignant cells can be assessed *in vitro*. The effects of chemicals on the numbers and ratios of the various lymphocyte subsets, and maturation of lymphocytes can be assessed with flow cytometry which measures the fluorescence of antibody-cell-surface marker complexes. These types of data provide information on the integrity of immune function, and are often used in conjunction with assays on immune function.

Functional endpoints to assess the effects of chemicals on acquired immunity include the plaque or antibody—forming cell assay, which tests the ability of an animal to mount a defense in response to a specific antigen.[241] Such tests can measure a chemical's effects on the activity of multiple cell types involved in the acquired immune response including T cells, B cells and other antigen presenting cells, as well as a number of processes involved in the response including cytokine production, antibody production, and proliferation and differentiation of lymphocytes.

The impacts of chemical exposure on cell-mediated immunity can also be measured with a number of assays including the cytotoxic t-lymphocyte assay (CTL), the delayed hypersensitivity response (DHR) assay and the T-cell proliferative response assays.[242] The CTL assay measures the ability of T-cells to proliferate and kill tumor cells in culture. The DHR assay evaluates the ability of T cells to recognize foreign antigen and secrete soluble mediators to draw in other immune cells.

[239] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p.503-522.
[240] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p.503-522.
[241] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p.504-505.
[242] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p.505-506.

Another toxicological endpoint used to evaluate the impact of chemicals is host resistance *in vivo* to pathogenic microorganisms.[243] In these studies experimental animals are exposed to a chemical and the response to infections with bacteria are measured and compared to unexposed animals.

As discussed in subsection 69403.15 immunotoxic effects of inhaled toxicant can be assessed in bronchoalveolar lavage fluid (BALF)[244]. An example of measurement of the BALF indicative of immunotoxic effects in the lung is increased numbers of cells from the immune system indicating an immune response (e.g., elevated polymorphonuclear leukocytes, lymphocytes, macrophages and monocytes, and eosinophils).

Subsection 69403.8(c) provides examples of other relevant data for the immunotoxicity hazard trait. For example, high antigenicity of a chemical indicates ability to provoke a hypersensitivity response. Structural similarity or data from mechanistic studies can also provide insight into the potential for a chemical to be immunotoxic.

§ 69403.9 Musculoskeletal Toxicity

Subsection 69403.9(a) defines the musculoskeletal toxicity hazard trait. Chemical toxicants can cause damage to various parts of the musculoskeletal system including bones, teeth, muscles, cartilage, tendons, ligaments, joints, and connective tissue. The definition of the musculoskeletal toxicity hazard trait is based on the general definition of toxicity as an adverse effect of chemicals or agents on the structure or function of living organisms.[245] The definition is meant to be sufficiently broad to cover the range of musculoskeletal toxicities[246] [247] that are considered adverse to human health by regulatory agencies such as U.S. Environmental Protection Agency, OEHHA,[248] and the medical community in addressing potential adverse effects. For example, the U.S. Environmental Protection Agency reference dose for chronic oral exposure to strontium was based on rachitic bone.[249] The basis for the public health goal for fluoride in

[243] Kaminski NE, Faubert Kaplan BL, Holsapple MP. Toxic Responses of the Immune System, Chapter 12 in Toxicology: The Basic Science of Poisons, Klaassen CD, ed. 2008, p.507.
[244] Henderson R Bronchoalveolar lavage: A tool for assessing the hlth status of the lung. Chapter 15 in Concepts in Inhalation Toxicology McClellan and Henderson, eds. Hemisphere Publishing, 1989.
[245] Eaton DC and Gilbert SC Principles of Toxicology, Chapter 2, p. 11 and Gregus Z. Mechanisms of Toxicity. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 45.
[246] Kenaston MA, Abramson EA, Pfeiffer ME, Mills EM. Skeletal Muscle Toxicology. Chapter 61 in: General and Applied Toxicology., Ballantyne B, Marrs TC, Syversen T. eds. 2009, John Wiley and Sons.
[247] Lansdown ABG. Cartilage and bone as target tissues for toxic materials. Chapter 62 in: General and Applied Toxicology., Ballantyne B, Marrs TC, Syversen T. eds. 2009, John Wiley and Sons.
[248] OEHHA (2008). Technical Support Document for the Development of Noncancer Reference Exposure Levels. Available at: http://www.oehha.ca.gov/air/hot_spots/pdf/NoncancerTSD071808.pdf
[249] U.S.EPA Integrated Risk Information System, available at http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0550

California is tooth mottling, a musculoskeletal toxicity.[250] Substances such as heavy metals (e.g., lead) and polycyclic aromatic hydrocarbons interact with cells of the skeletal system and adversely affect musculoskeletal development.[251]

Subsection 69403.9(b) provides examples of toxicological endpoints for the musculoskeletal hazard trait.

Measurements involve gross and microscopic observations of tissues including biopsy specimens, electrophysiological tests, manual measures of muscle function, measures of bone density, measures of cellular integrity or biochemical changes, and can be conducted following exposure in whole animals or in cell-based assays.
The musculoskeletal system's main function is support and movement of the body. Clinical instruments or tests available to study muscle function include manual muscle testing, muscle endurance tests, flexion tests with an inflatable pressure biofeedback unit, dynamometry and functional lifting tests.

In humans, studies have evaluated symptoms such as pain, stiffness, and inflammation of the joints, pain in the muscles, difficulty walking due to bone spurs, and fractures of bones pressing against the skin.

Phossy jaw (phosphorus necrosis of the jaw) is an example of musculoskeletal toxicity formerly noted in humans who worked with fumes of white phosphorus in the match industry. Workers suffered with toothaches and swollen gums, and some had serious brain damage. Osteonecrosis of the jaw has also been reported among patients receiving bisphosphonates for osteoporosis and other ailments.[252]

Structural damage to the musculoskeletal system from both acute and chronic chemical exposure can be measured by pathologic evaluation of the tissues of the system including bones, muscles, cartilage, tendons, ligaments, joints and connective tissue. This is done typically by gross examination, by light and electron microscopy, and by bone densitometry testing.[253] Common endpoints include: degeneration of cells including ultrastructural changes, or hypertrophy; cell necrosis (death) including occurrence of dead cells in the musculoskeletal tissues; cell proliferation as measured by hyperplasia, and metaplasia; altered ratio of cells (generally a result of damage and

[250] Public Health Goal for Fluoride in Drinking Water, Office of Environmental Health Hazard Assesssment, Cal/EPA; available at http://www.oehha.ca.gov/water/phg/pdf/fluor_c.pdf
[251] Holz JD, Sheu TJ, Drissi H, Matsuzawa M, Zuscik MJ, Puzas JE (2007). Environmental agents affect skeletal growth and development. Birth Defects Res C Embryo Today. 81(1):41-50.
[252] Hess LM, Jeter JM, Benham-Hutchins M, Alberts DS (2008). Factors associated with osteonecrosis of the jaw among bisphosphonate users. Am J Med. 121(6):475-483.
[253] Bhattacharyya MH (2009). Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. Toxicol Appl Pharmacol. 238(3):258-65.

subsequent repair); increased or decreased thickness of bone; mottling of teeth; and other altered structural features including other morphometric changes.[254] [255] Severe injury to the system can result in structural and functional changes leading to altered mobility, immobility, and even paralysis.

The presence of myoglobin in the urine is an indication of muscle damage.[256] Imaging studies can also measure certain types of musculoskeletal damage.[257] [258] These are used in animal studies and in studies of humans with occupational musculoskeletal disease from chemical exposure. Radiographic changes that can be assessed using X-rays can be diagnostic of breaks, bone spurs, and other malformations. Tumors of the muscle and bone can also be seen with imaging techniques.

Subsection 69403.9(c) provides examples of other relevant data for the musculoskeletal hazard trait. Consideration of structural or mechanistic similarity identifies chemical structures or actions on the cell similar to those of other chemicals that induce musculoskeletal toxicity. Such information is especially useful when, as is often the case, there are few musculoskeletal toxicity data to assess a chemical. Changes in gene expression and protein production can also be measured in cells of the musculoskeletal system and give insight into toxicological mechanisms and injury. This can be done following *in vivo* exposure using immunohistochemical techniques and *in vitro* in cell cultures. Results of gene expression arrays following *in vitro* exposure (e.g., exposure of rat bone cells to cadmium) can be useful to understand genes targeted in the musculoskeletal system by specific chemicals.[259]

§ 69403.10 Nephrotoxicity and Other Toxicity to the Urinary System

Subsection 69403.10(a) defines the nephrotoxicity hazard trait. The definition is derived from the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms,[260] and covers the full range of adverse

[254] Kazantzis G (2004). Cadmium, osteoporosis and calcium metabolism. Biometals. 17(5):493-8.
[255] Chachra D, Vieira AP, Grynpas MD (2008). Fluoride and mineralized tissues. Crit Rev Biomed Eng. 36(2-3):183-223.
[256] Bagley WH, Yang H, Shah KH (2009). Rhabdomyolysis. Intern Emerg Med. 2(3):210-8.
[257] McGonagle D, Tan AL (2008). What magnetic resonance imaging has told us about the pathogenesis of rheumatoid arthritis--the first 50 years. Arthritis Res Ther. 10(5):222.

[258] Scirè CA, Meenagh G, Filippucci E, Riente L, Delle Sedie A, Salaffi F, Iagnocco A, Bombardieri S, Grassi W, Valesini G, Montecucco C (2009). Ultrasound imaging for the rheumatologist. XXI. Role of ultrasound imaging in early arthritis. Clin Exp Rheumatol. 27(3):391-4.
[259] Ohba K, Okawa Y, Matsumoto Y, Nakamura Y, Ohta H (2007). A study of investigation of cadmium genotoxicity in rat bone cells using DNA microarray. J Toxicol Sci. 32(1):107-9, 2007.
[260] Eaton DC and Gilbert SC Principles of Toxicology, Chapter 2, p. 11, and Gregus Z. Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008 p. 45.

effects on the kidney itself, and on components of the urinary system such as the ureters and urinary bladder, that may result from chemical stresses:

> "The functional integrity of the mammalian kidney is vital to total body homeostasis because the kidney plays a principal role in the excretion of metabolic wastes and in the regulation of extracellular fluid volume, electrolyte composition and acid-base balance… A toxic insult to the kidney therefore could disrupt any or all of these functions and could have profound effects on total body metabolism."[261]

The American Society of Nephrology in their 2005 Research Report[262] describes a range of kidney disease types along these lines that would be considered adverse effects in the proposed nephrotoxicity hazard trait definition.

Nephrotoxicity is the basis for a number of health protective levels developed by the U.S. Environmental Protection Agency and the California Environmental Protection Agency. Most notably, for cadmium and its salts nephrotoxicity is the basis of California's Chronic Reference Exposure Level (for the Air Toxics Hot Spots program) and Public Health Goal (for the drinking water program)[263] and of the U.S. Environmental Protection Agency's Maximum Contaminant Level for drinking water[264] and their RfD.[265] Nephrotoxicity is extensively cited in the corresponding standards for mercury. Effects on the kidney are also cited in the derivation of health protective levels for a number of organic compounds, including ethylbenzene, ethylene glycol, methyl *t*-butyl ether and phenol.[266]

Subsection 69403.10(b) provides examples of general endpoints for the nephrotoxicity hazard trait, including structural and functional impairments of the kidney and components of the urinary system. These endpoints cover a variety of types of nephrotoxicity that may be caused by chemical substances, and that may be measured using a variety of methods.[267] [268]

[261] Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008 p. 583.
[262] American Society of Nephrology Renal Research Report: J Am Soc Nephrol 16: 1886–1903, 2005
[263] http://www.oehha.ca.gov/water/phg/pdf/122206cadmiumphg.pdf
[264] U.S. EPA (1986). Drinking Water Health Criteria Document on Cadmium. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, DC.
[265] U.S. EPA (2005). Cadmium. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, DC. Available at: www.epa.gov/iris/subst/0141.htm.
[266] http://www.oehha.ca.gov/air/allrels.html
[267] Scnellman RG. Toxic Responses of the Kidney, Chapter 14 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 586-599.
[268] Toxicology of the Kidney, Tarloff JB and Lash LH, eds., CRC Press, 2005., Chapter 3, p.-148, Chapter 4, p. 149-190, Chapter 5, p. 191-214.

Nephrotoxicity endpoints are typically classified by site of impact, and this is the case for several of the general endpoints named in this subsection. Impacts at any of these sites may affect the primary functions of filtration, reabsorption of water and valuable components of the glomerular filtrate, and the associated vascular homoeostasis and responses to physiological controls.[269] Key sites within the kidney itself include the glomerulus, proximal tubule, Loop of Henle, distal tubule, and the papilla. Other key sites within the urinary system include the epithelium and musculature of the ureters and urinary bladder. Pathological changes in structure at any of these sites may be observed directly by histopathological examination. Such changes include alteration of cell structure and cell loss. Direct damage may also be inferred from the appearance of dead cells and proteinaceous casts in the urine, and the release of characteristic marker enzymes into the bloodstream, which are identified by clinical chemistry.

Accumulation of proteins in inappropriate locations, e.g. immune complexes in the glomerulus, or chemically mediated accumulation of small proteins in the intercellular matrix can also be seen with histopathological evaluation.

The endpoints named in this subsection also include principal functional endpoints that relate to the production of urine as a means of eliminating soluble waste products and toxins from the body and providing homeostasis for electrolytes.[270] The simplest measure is the volume of urine formed. Marked reductions in this volume typically indicate a decline or failure of the glomerular filtration process, while marked increases indicate abnormal tubular reabsorption of water and/or solutes. Urine composition provides additional important functional endpoints, where changes in osmolarity, pH, or concentrations of glucose, protein or electrolytes from usual values are indicators of impaired kidney function.[271] The glomerular filtration rate may be measured directly by determining the clearance of non-reabsorbed solutes such as creatinine or inulin. Clearance values for reabsorbed solutes such as glucose may be used as indicators of tubular function. Plamsa levels of urea, creatinine and low molecular weight proteins are also endpoints used to indicate kidney damage.[272]

Various experiments *in vitro* have also been designed to investigate the occurrence and mechanisms of nephrotoxicity. Examples of *in vitro* methods that generate data used as toxicological endpoints for nephrotoxicity include the use of kidney slices or isolated

[269] Schnellmann RG. Toxic Response of the Kidney, Chapter 14 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p. 593-594.
[270] Scnellman RG. Toxic Responses of the Kidney, Chapter 14 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008, p.594-595.
[271] Hart SE and Lewis LB, Assessing Renal Effects of Toxicants *In vivo*, Chapter 3 in: Toxicology of the Kidney, Tarloff JB and Lash LH, eds., CRC Press, 2005, p. 95-121.
[272] Hart SE and Lewis LB, Assessing Renal Effects of Toxicants *In vivo*, Chapter 3 in: Toxicology of the Kidney, Tarloff JB and Lash LH, eds., CRC Press, 2005, p. 85-95.

nephrons to measure the uptake of nephrotoxic chemicals by kidney tissue, identify susceptible cell types within the kidney, and examine cellular endpoints of toxicity including metabolic and functional changes in kidney cells.[273] Since the kidney has significant capacity for xenobiotic metabolism (including many of the cytochrome P-450 enzymes also found in the liver), a number of organic chemicals are toxic as a result of their activation to reactive intermediates in this tissue. A smaller number of examples are known where damage to the epithelium of the ureters or bladder occurs from similar activation of xenobiotic chemicals, or by hydrolytic re-activation of conjugates formed elsewhere and excreted in the urine. Demonstration of such metabolic processes in kidney or other urinary system tissues *in vivo* or *in vitro* provides supporting evidence for the nephrotoxicity hazard trait.

Subsection 69403.10(c) gives examples of other relevant data that may indicate the presence of nephrotoxicity. Data on these mechanisms from isolated primary cell cultures or cell lines[274] typically provide supportive evidence for determining whether or not a chemical has the nephrotoxicity hazard trait. These are included as examples of other relevant data in this subsection.

Given the very substantial proportion of the resting blood flow which passes through the kidney, and its use and regulation of blood pressure to drive glomerular filtration, it is understandable that nephrotoxicity often leads to adverse impacts on the cardiovascular system. Conversely, adverse impacts on the cardiovascular system which affect blood pressure or blood flow may have impacts on the kidney. Marked changes in these cardiovascular measures may therefore provide indirect evidence of nephrotoxicity.

§ 69403.11 Neurotoxicity

Subsection 69403.11(a) defines the neurotoxicity hazard trait. The definition is consistent with and expands upon the U.S. Environmental Protection Agency's definition in its Guidelines for Neurotoxicity Risk Assessment:[275]

> "This section defines the key terms and concepts that EPA will use in the identification and evaluation of neurotoxicity…Neurotoxicity is an adverse change

[273] Kirkpatrick DS, Gandolfi AJ, *In Vitro* techniques in Screening and Mechanistic Studies: Organ Perfusion, Slices, and Nephron Components., Chapter 4 in: Toxicology of the Kidney, Tarloff JB and Lash LH, eds., CRC Press, 2005, p. 149-189.
[274] Ford, SM, In Vitro Techniques in Screening and Mechanistic Studies: Cell Culture, Cell-Free Systems, and Molecular and Cell Biology, Chapter 5 in: Toxicology of the Kidney, Tarloff JB and Lash LH, eds., CRC Press, 2005, p.191-213.
[275] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment. Washington, D.C., U.S. Environmental Protection Agency, page 8. Available at: http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF.

> in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent (Tilson, 1990)."

The nervous system functions to regulate the body's responses to internal and external stimuli. As such it serves a critical function, an impact of a chemical substance on the nervous system is typically important for another organ or organ system. Thus, a chemical substance that possesses the neurotoxicity hazard trait typically possesses other related hazard traits.

The U.S. Environmental Protection Agency explained the breadth of effects covered by the definition as follows:[276]

> "Structural neurotoxic effects are defined as neuroanatomical changes occurring at any level of nervous system organization; functional changes are defined as neurochemical, neurophysiological, or behavioral effects."

Chemical substances can damage various parts of the nervous system, including the central nervous system (the brain, brain stem, spinal cord, blood brain barrier), and the peripheral nervous system. The proposed definition of the neurotoxicity hazard trait is meant to be sufficiently broad to cover the range of neurotoxicities that are considered adverse to human health by regulatory agencies such as U.S. Environmental Protection Agency and OEHHA and the medical community in addressing potential adverse effects.

In humans, neurodevelopmental effects have been observed following chemical exposure during development including exposures to ethanol, methylmercury, lead, and polychlorinated biphenyls.[277] [278]

Neurotoxicity has been used as the basis for a number of regulatory levels including the Ambient Air Quality Standard and reference levels for lead,[279] and U.S. Environmental Protection Agency and OEHHA's reference levels for mercury, manganese, xylenes, and n-hexane.[280] [281]

[276] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment, page 8.
[277] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment. Washington, D.C., U.S. Environmental Protection Agency, page 44.
[278] Moser VC, Aschner M, Richardson RJ, and Philbert MA. 2008. Toxic Response of the Nervous System, Chapter 16 in Toxicology, The Basic Science of Poisons. Klaassen CD, ed., McGraw Hill.
[279] Criteria Document for U.S. EPA National Ambient Air Quality Standards for Lead is available at: http://www.epa.gov/ttnnaaqs/standards/pb/s_pb_cr_cd.html
[280] California OEHHA RELS are available at: http://www.oehha.ca.gov/air/allrels.html
[281] U.S.EPA Reference Levels are available at: http://www.epa.gov/IRIS/

Subsection 69403.11(b) provides examples of general toxicological endpoints for the neurotoxicity hazard trait. The endpoints can reflect adverse observations in various aspects of the nervous system:

> "Neurotoxic effects can be observed at various levels of organization of the nervous system, including neurochemical, anatomical, physiological or behavioral."[282]

These general endpoints include neuropathological anatomical changes, such as alterations of the cell body, the axon, or the myelin sheath, changes in weight or volume of the whole or specific regions of the brain, and histopathological changes in neurons and glia including cell degeneration and death, presence of plaques, neurofibrillary tangles, inclusion bodies, and demyelination.[283]

The proposed endpoints in this subsection also include behavioral and neurological toxicological endpoints. These include changes in learning, memory or attention; increases or decreases in motor activity; changes in the senses; mood disorders such as anxiety or depression; alterations in sensory motor reflexes; impaired mental functioning; changes in motor coordination; limb weakness or numbness; reduced grip strength; paralysis; tremor; seizure; headache; impaired cognitive function; behavioral changes; and sexual dysfunction.

Neurochemical endpoints are also included. Animal toxicological studies of neurotransmitters, chemicals which neurons use to communicate with each other and other tissues, can assess alterations in synthesis, release, uptake and degradation of neurotransmitters. Studies that evaluate neurophysiological endpoints such as changes in the thresholds for neural activation or reduction in the speed of neurotransmission also provide evidence of neurotoxicity.

As noted by U.S. Environmental Protection Agency, "[c]linical methods are used extensively in neurology and neuropsychology to evaluate patients suspected of having neurotoxicity."[284] Nerve conduction studies, generally performed on peripheral nerves, can be useful in investigations of possible peripheral neuropathy in animals and humans. Critical variables are nerve conduction velocity, response amplitude, and refractory period. Electroencephalographic patterns are also important neurophysiological observations.

[282] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment. Washington, D.C., U.S. Environmental Protection Agency, page 9.
[283] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment. Washington, D.C., U.S. Environmental Protection Agency.
[284] US EPA Guidelines for Neurotoxicity Risk Assessment, page 13.

Evoked potential studies are electrophysiological procedures that measure the response elicited from a defined stimulus such as a tone, a light, or a brief electrical pulse. This can be studied in animals and humans. Evoked potentials reflect the function of the system under study, including visual, auditory, or somatosensory; motor, involving motor nerves and innervated muscles; or other neural pathways in the central or peripheral nervous system.

Groups of behavioral tests called functional observational batteries are used in animal neurotoxicology studies to evaluate neurobiological functions known to be affected in humans exposed to neurotoxic agents, including alterations in sensory, motor, autonomic, and cognitive function. Behavioral changes can be correlated with other test results for physiological, biochemical and pathological identification of neurotoxic injury.[285]

The general endpoints also include those of neurodevelopmental toxicity. This is an especially important type of neurotoxicity as the nervous system development is critical to the overall functioning of an organism. There are test batteries specifically designed to evaluate neurodevelopmental toxicity.[286] Toxicological endpoints for developmental neurotoxicity include behavioral effects, brain weight, and neuropathological evaluation. Toxicological testing endpoints include those indicating: nervous system symptomology; aberrant motor activity, auditory startle response, learning and memory; and pathological (and morphometric (size and shape) changes in various regions of the brain.

Subsection 69403.11(c) gives examples of other relevant data for evaluating the neurotoxicity hazard trait. Various types of *in vitro* techniques involving isolated cells, primary cell cultures, cell lines, and cloned cells, produce data for evaluating the potential for neurotoxicity.

The U.S. Environmental Protection Agency Guidelines[287] note the importance of structure activity relationships in identifying neurotoxicants:

> "The structure-activity relationships (SAR) of some chemical classes have been studied, including hexacarbons, organophosphates, carbamates, and pyrethroids. Therefore, class relationships or SAR may help predict neurotoxicity

[285] World Health Organization 2001. Environmental Health Criteria 223; Neurotoxicity risk assessment for human health: Principles and approaches. Geneva, World Health Organization**:** 1-163.
[286] TSCA developmental neurotoxicity guidelines, 1999, 40 CFR 799.9630 available as pdf at: http://edocket.access.gpo.gov/cfr_2002/julqtr/pdf/40cfr799.9630.pdf
[287] U.S.EPA 1998. Guidelines for neurotoxicity risk assessment. Washington, D.C., U.S. Environmental Protection Agency, page 47.

or interpret data from neurotoxicological studies." This procedure may be used "to evaluate the potential for neurotoxicity when little or no empirical toxicity data are available."

§ 69403.12 Ocular Toxicity

Section § 69403.12(a) defines the ocular toxicity hazard trait. This follows the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms,[288] and covers the full range of adverse effects on the eye. The definition includes the impacts of chemical substances on the functioning of the eye – the collection of light, the subsequent activation of rods and cones in the retina, and the sending of nerve impulses through the optic nerve to the brain. The definition also includes structural damage to various parts of the eye, including the cornea, lens, retina, optic nerve, and retinal ganglion cells.[289]

At least 2800 substances have been reported to be toxic to the eye.[290] Examples include: those that induce cataracts such as naphthalene and corticosteroid drugs; retinal toxicity by lead, methanol, various organic solvents, tamoxifen, and sildenafil citrate; optic nerve toxicity by methanol, acrylamide, and carbon disulfide; color vision impairment by styrene[291] and toluene;[292] and corneal damage by surfactants, solvents, and caustic substances.

Subsection 69403.12(b) provides examples of general ocular toxicity endpoints, and covers the important and commonly measured toxicological endpoints. Experimental methods may involve gross and microscopic observations of ocular tissues, measures of cellular integrity or biochemical changes, and measures of ocular function or central nervous system responses in whole animals.

Structural endpoints result from pathologic evaluation of the structures of the eyes including the cornea, lens, retina, and optic nerve.[293] Both qualitative changes and quantitative changes can be measured, typically by gross examination and by light microscopy or through clinical measurements. Common endpoints include: extent and

[288] Eaton DC and Gilbert SC Principles of Toxicology, Chapter 2, p. 11 and Gregus Z. Mechanisms of Toxicity, Chapter 3, p. 45 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008.
[289] Fox DA, Boyes WR (2008). Chapter 17 Toxic responses of the ocular and visual system. In: Cassarett and Doull's Toxicology. The Basic Science of Poisons, 7th ed. Klaassen CD, Ed. New York: McGraw-Hill.
[290] Grant WM (1986). Toxicology of the Eye, 3rd ed. Springfield, IL: CC Thomas.
[291] Campagna D, Gobba F, Mergler D, Moreau T, Galassi C, Cavalleri A, Huel G (1996). Color vision loss among styrene-exposed workers neurotoxicological threshold assessment. Neurotoxicology. 17(2):367-73.
[292] Campagna D, Stengel B, Mergler D, Limasset JC, Diebold F, Michard D, Huel G (2001). Color vision and occupational toluene exposure. Neurotoxicol Teratol. 23(5):473-80.
[293] Fox DA, Boyes WR (2008). Chapter 17 Toxic responses of the ocular and visual system. In: Cassarett and Doull's Toxicology. The Basic Science of Poisons, 7th ed. Klaassen CD, Ed. New York: McGraw-Hill.

severity of damage to the cornea, degree of opacity of the lens (cataracts), pressure inside the eye (glaucoma), detached retina, and optic nerve degeneration. A common but controversial test used in animals is the Draize test in which the chemical or a solution containing the chemical is directly put onto the corneal surface of a rabbit eye to test for eye irritation.[294] Endpoints from validated and accepted alternatives to the Draize tests are also included in this subsection.

The proposed regulation also includes functional endpoints. In humans, studies have evaluated symptoms such as irritation of the eyes in response to an airborne toxicant. Eye irritation is a commonly reported non-invasive endpoint in toxicology testing;[295] an animal's behavior (e.g., covering the eyes) can also provide a measure of response to irritating airborne chemicals. Humans can also report other symptoms such as blurred vision,[296] diminished vision including loss of peripheral vision, diminished night vision, and blindness. In the Functional Observational Battery (FOB) tests for neurotoxicity, an animal's response to an "approaching" object (e.g., a pencil) is observed, and the response of the pupil of the eye to light (papillary reflex) is observed. Other functional endpoints that can be evaluated clinically include reflex action of the pupils by light exposure, vision impairment using eye charts, and distance and close vision decrements using refractometry.after visual stimulation. The commonly used electrophysiological procedures include flash-evoked electroretinogram (ERG),[297] visual-evoked potentials,[298] and electrooculograms.[299]

Subsection 69403.12(c) describes other relevant ocular toxicity data. These are examples of data that point in the direction of potential ocular toxicity but are not necessarily definitive of this trait. For instance, elevated gene expression for inflammatory cytokines can indicate the capability of a chemical to cause inflammation in cells, including potentially those in the eye. Chemical reactivity with biological systems is itself a hazard trait in this proposed regulation (Section 69403.14) and is the basis for the identification of a number of important ocular toxicants, including those with a high oxidation/reduction (redox) potential.[300] Another example of other relevant data

[294] Draize J H, Woodward G, Calvery HO (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82, 377–90.
[295] Darley E, Middleton J, Garber M (1960). Plant damage and eye irritation from ozone-hydrocarbon reactions. Agricul Food Chem. 8(6)483-4.
[296] Guo JX, Hu L, Yand PZ, Tanabe K, Miyatalre M, Chen Y (2007). Chronic arsenic poisoning in drinking water in Inner Mongolia and its associated health effects. J Environ Sci Health A Tox Hazard Subst Environ Eng. 42(12):1853-8.
[297] Eells JT (1991). Methanol-induced visual toxicity in the rat. J Pharmacol Exp Ther. 257(1):56-63.
[298] Kutlu G, Gomceli YB, Sonmez T, Inan LE (2009). Peripheral neuropathy and visual evoked potential changes in workers exposed to n-hexane. J Clin Neurosci. 16(10):1296-9.
[299] Arden GB, Wolf JE (2000). The human electro-oculogram: interaction of light and alcohol. Invest Ophthalmol Vis Sci. 41(9):2722-9.
[300] Kise K, Kosaka H, Nakabayashi M, Kishida K, Shiga T, Tano Y (1994). Reactive oxygen species involved in phenazine-methosulfate-induced rat lens opacification. An experimental model of cataract. Ophthalmic Res. 26(1):41-50.

is a chemical substance's structural similarity to other ocular toxicants or data showing the chemical substance acts on the cell in similar ways to chemicals that are known to induce ocular toxicity.[301,302]

§ 69403.13 Ototoxicity

Subsection 69403.13(a) defines the ototoxicity hazard trait. The definition is derived from the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms,[303] and covers the full range of adverse effects on the ear, or on components of the auditory system, that may result from chemical stresses.[304 305] The definition covers chemically-induced damage to various parts of the inner ear, including the cochlea, vestibule, semicircular canals, and otoliths. The definition of the ototoxicity hazard trait is sufficiently broad to include a range of ototoxicities that are considered adverse to human health by regulatory agencies such as U.S. Environmental Protection Agency and OEHHA and the medical community in addressing potential adverse effects.

Ototoxicity came to the attention of clinical and basic scientific research in 1944 with the discovery of streptomycin,[306] an aminoglycoside antibiotic. Streptomycin can cause irreversible cochlear and vestibular damage.[307] The ototoxicity caused by drugs or toxicants can be primarily cochleotoxic, vestibulotoxic, or both. Irreversible hearing loss and balance deficiencies caused by chemical toxicants can lead to serious communication and learning impairment.

Subsection 69403.13(b) provides examples of general toxicological endpoints for the ototoxicity hazard trait. These cover important and commonly measured toxicological endpoints resulting from acute or chronic chemical exposure.

These include endpoints reflecting structural impairment. Damage to the inner ear can be measured by pathologic evaluation of the inner ear tissues. This is done typically by

[301] Gerner I, Liebsch M, Spielmann H (2005). Assessment of the eye irritating properties of chemicals by applying alternatives to the Draize rabbit eye test: the use of QSARs and *in vitro* tests for the classification of eye irritation. Altern Lab Anim. 33(3):215-37.
[302] Li Y, Liu J, Pan D, Hopfinger AJ (2005). A study of the relationship between cornea permeability and eye irritation using membrane-interaction QSAR analysis. Toxicol Sci. 88(2):434-46.
[303] Eaton DC and Gilbert SC Princiles of Toxicology, Chapter 2, p. 11, and Gregus Z. Mechanisms of Toxicity, Chapter 3, p. 45 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008.
[304] Forge A, Taylor R, Harpur ES. Ototoxicity. Chapter 56 in: General and Applied Toxicology., Ballantyne B, Marrs TC, Syversen T. eds. 2009, John Wiley and Sons.
[305] Sullivan MJ. Ototoxicity in: Encyclopedia of Toxicology, Wexler P, ed. 2nd Edition, Volume 3, p. 315-318.Elsevier, 2005.
[306] Jones D, Metzger HJ, Schatz A, Waksman SA (1944). Control of gram-negative bacteria in experimental animals by streptomycin. Science. 100(2588):103-5.
[307] Guthrie OW 2008). Aminoglycoside induced ototoxicity. Toxicology 30;249(2-3):91-6.

gross examination and by light and electron microscopy. Chemical toxicants can cause damage to various parts of the inner ear, including the cochlea, vestibule, semicircular canals, and otoliths.[308] Cochlear toxicity and hearing loss is usually the result of the damage of outer hair cells in the organ of Corti, specifically at the basal turn of the cochlea. Some ototoxic agents can cause edema of the epithelium of the stria vascularis of the cochlea. Chemical-induced ototoxicity can also result in the destruction of hair cells in vestibular end organs.[309]

This subsection also identifies endpoints reflecting functional impairment. Chemical-induced damage to structures of the auditory and balance system can lead to tinnitus, a feeling of ear fullness, hearing loss, imbalance, vertigo, vomiting, dizziness, nystagmus, inability to tolerate head movement, difficulty walking in the dark, a wide-based gait, a feeling of unsteadiness, lightheadedness, and oscillopsia during head movements.

Serial audiograms detecting changes in pure-tone thresholds are considered an important marker of ototoxic hearing loss. A high-frequency audiometry (HFA) that includes ultra-high frequencies in the testing provides data on toxicological endpoints in addition to the conventional audiometry for the detection of ototoxic changes.[310] Word recognition scores can also provide evidence of ototoxicity. In addition, objective measures such as otoacoustic emission (OAE), electrocochleography (ECochG), and auditory brainstem response (ABR) are used to determine hearing loss. Otoacoustic emission (OAE) testing is an objective test to evaluate cochleotoxicity and is specific to the condition of outer hair cells in the cochlea.[311] ECochG is a trans-tympanic test to assess cochlear and neural responses.[312] Auditory brainstem response (ABR) is a test of auditory brainstem function in response to auditory stimuli such as clicks.[313] [314]

There are a number of toxicological endpoints based on measurements that can be used to assess vestibular function, including the vesitbulo-cochlear reflex, visual vestibular interactions, postural control, dizziness, and balance difficulties from ototoxicity.[315] [316]

[308] Gagnaire F, Langlais C (2005). Relative ototoxicity of 21 aromatic solvents. Arch Toxicol. 2005 Jun;79(6):346-54.
[309] Rybak LP, Ramkumar V (2007). Ototoxicity. Kidney Int. 72(8):931-5.
[310] Jacob LC, Aguiar FP, Tomiasi AA, Tschoeke SN, Bitencourt RF (2006). Auditory monitoring in ototoxicity. Braz J Otorhinolaryngol. 72(6):836-44.
[311] de Freitas MR, de Castro Brito GA, de Carvalho JV Jr, Gomes RM Jr, Barreto Martins MJ, de Albuquerque Ribeiro R (2009). Light microscopy study of cisplatin-induced ototoxicity in rats.J Laryngol Otol. 123(6):590-7.
[312] Ferraro JA (2010). Electrocochleography: a review of recording approaches, clinical applications, and new findings in adults and children. J Am Acad Audiol. 21(3):145-52.
[313] Rebert CS, Sorenson SS, Howd RA, Pryor GT (1983). Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. Neurobehav Toxicol Teratol. 5(1):59-62.
[314] Stevens J (2001). State of the art neonatal hearing screening with auditory brainstem response. Scand Audiol Suppl. (52):10-2.
[315] Bhansali SA, Honrubia V (1999). Current status of electronystagmography testing. Otolaryngol Head Neck Surg. 120(3):419-26.
[316] Furman JM (1994). Posturography: uses and limitations. Baillieres Clin Neurol. 3(3):501-13.

Subsection 69403.13(c) provides examples of other relevant data for the ototoxicity hazard trait. Information that the chemical is similar in structure to other ototoxicants or acts on the cell in similar ways to chemicals that induce ototoxicity is relevant to assessing the ototoxicity potential of a chemical. Such information is especially important when there is little ototoxicity data to assess a chemical, which is the case for the majority of chemicals in commerce in California.

§ 69403.14 Reactivity in Biological Systems

Subsection 69403.14 (a) defines the reactivity in biological systems hazard trait. This definition derives from the observation that one common underlying trait of many chemicals that leads to toxicity is reactivity in biological systems.[317] Chemical reactivity is the tendency of a chemical to react rapidly with other molecules to produce a change in molecular structure or conformation, and in the case of biological macromolecules, altering molecular function. Chemicals may form covalent bonds with or oxidize cellular macromolecules, such as proteins and DNA.[318] A number of chemicals are capable of redox catalysis and can produce a large number of reactive radicals in the cell (e.g., superoxide anion) destroying cellular structure and function.[319] Other toxic chemicals can induce production of reactive oxygen species and reactive nitrogen species in the cell, which react with cellular components resulting in damage.[320] [321] Electrophilic chemicals can bind readily to DNA and other important biological molecules causing a number of adverse health effects including DNA mutation. [322]

Subsection 69403.14 (b) provides examples of endpoints that can be measured for the reactivity in biological systems hazard trait. These examples are meant to cover measurement *in vivo* of the results of basic chemical properties such as:

[317] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. p. 55-60.
[318] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. p. 55.
[319] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. p. 547, 51-53.
[320] Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen, EMG, Doorn JA, Slikker W. 2009. Nitrative and oxidative stress in toxicology and disease. Toxicological Sciences 112(1):4-16.
[321] Roberts RA, Smith RA, Safe S, Szabo C, Tjalkens RB, Robertson FM. (2010) Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicology 276:85-94.
[322] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. P. 56-57.

- Ability of the chemical to catalyze electron transfer from a reducing source (such as dithiothreitol or ascorbate) to oxygen to generate reactive oxygen species, and/ or oxidative stress in cells.[323]
- Ability of the chemical to catalyze generation of hydroxyl radical by Fenton reaction catalyzed by metals.[324]
- Ability of the chemical to participate in irreversible chemical reactions between its components and cellular macromolecules.

The extent of toxicity associated with chemical reactivity depends on a number of factors including the degree of macromolecular alteration and the turnover rate of new macromolecules. For example, if covalent bonds are formed by compounds reacting with proteins that have a slow synthesis time, the effects of low concentrations would be cumulative.

Subsection 69403.14(c) provides examples of other relevant data for the reactivity in biological systems hazard trait. These examples focus on *in vitro* measures of the formation of reactive species in isolated cells or cell cultures, and include structural similarity to other chemicals that have this hazard trait.

§ 69403.15 Respiratory Toxicity

Subsection 69403.15(a) defines the respiratory toxicity hazard trait. The definition is derived from the general definition of toxicology as adverse effects of chemicals on the structure and/or function of living organisms[325], and covers the full range of adverse effects on the lung, or on components of the respiratory tract such as the airways, that may result from chemical stresses.[326]

The respiratory system functions to deliver oxygen to all tissues and remove waste carbon dioxide. As such it serves a critical function, and impacts of toxic chemicals on the respiratory system are important for all organs in the body. The ubiquitous nature of air pollutants impacting the lung has large measurable adverse public health impacts.[327] It should be noted that some chemicals can damage the respiratory system when ingested.

[323] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. p. 51-52.
[324] Gregus Z. Mechanisms of Toxicity, Chapter 3 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008. p. 53.
[325] Eaton DC and Gilbert SC Principles of Toxicology, Chapter 2, p. 11, and Gregus Z. Mechanisms of Toxicity, Chapter 3, p. 45 in: Toxicology - the Basic Science of Poisons, C.D. Klaassen, Ed. 2008.
[326] Witschi HR, Pinkerton KE, VanWinkle LS, Last JA. (2008) Toxic Responses of the Respiratory System, Chapter 15 in: Toxicology, the Basic Science of Poisons; Klaassen CD, Ed. McGraw Hill.p. 609-630.
[327] Costa DL. Air Pollution, Chapter 28 in Toxicology, the Basic Science of Poisons; Klaassen CD, Ed. McGraw Hill, p 1119-1156.

Chemical toxicants can cause damage to various parts of the respiratory tract, including the upper airways or extrathoracic region (oro-nasal passages, larynx), the thoracic regions or tracheobronchial region which include the lower airways (trachea and bronchi), the bronchioles, and the pulmonary region which includes the lung parenchyma (respiratory bronchioles, alveoli and supporting interstitium).[328] The definition of the respiratory toxicity hazard trait is meant to be sufficiently broad to cover the range of respiratory toxicities that are considered adverse to human health by regulatory agencies such as U.S. Environmental Protection Agency and OEHHA and the medical community in addressing potential adverse effects.

Chemicals in the particulate phase can cause damage along the respiratory tree depending on the particle size distribution which in turn determines where the particles deposit.[329] Thus, particle size distribution is an important parameter to consider (see section on physical hazards). In addition, materials that are fibrous in nature, and respirable, such as asbestos, can deposit in the lung and cause damage along the respiratory tract. Chemicals that are gases tend to impact the upper airway if they are water soluble, and impacts can extend down to the lower airway and parenchyma if they are less water soluble.[330] In all cases, the extent of damage is dependent on the chemical concentration to which animals or people are exposed.

A number of regulatory levels are based on respiratory toxicity, including but not limited to the California Environmental Protection Agency's OEHHA Reference Exposure Levels for naphthalene, nickel, toluene diisocyanates, and crystalline silica[331],the State[332] and Federal Ambient Air Quality Standard for ozone and nitrogen dioxides[333], and regulation of a number of dusts and fibers by the U.S. Occupational Safety and Health Administration.[334]

Subsection 69403.15(b) provides examples of general toxicological endpoints for the respiratory toxicity hazard trait. These general endpoints include pathological changes

[328] Phalen RF, Prassad sb.Morphjology of the Respiratory Tract. Chapter 4 in:Concepts in Inhalation Toxicology. McClellan RO and Henderson RF, eds.Hemisphere Publishing, New York, p.123-140.
[329] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 310-313.
[330] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 310-313.
[331] CalEPA OEHHA RELs are available at: http://www.oehha.ca.gov/air/chronic_rels/index.html
[332] California Ambient Air Quality Standards available at: http://www.arb.ca.gov/research/aaqs/ozone-rs/ozone-rs.htm, and http://www.arb.ca.gov/research/aaqs/no2-rs/no2-rs.htm
[333] NO2 standard available at: http://www.epa.gov/airquality/nitrogenoxides/health.html; Ozone standard available at: http://www.epa.gov/groundlevelozone/health.html
[334] NIOSH Pocet Guide to Chemial Hazards. 2005. National Institute of Occupational Safety and Health. U.S. Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/niosh/npg/pgintrod.html#chemicalname

at the cellular and structural level in the airway and lung, as well as adverse changes in lung function.

Respiratory irritation is a commonly measured endpoint in both humans and animals, and may result in stimulation of the trigeminal nerve (sensory irritation) or in tissue damage.[335] In humans, chamber studies have evaluated symptoms such as irritation of the nose, throat, and mucous membranes in response to an airborne toxicant. In animals, irritation can be measured as a decrease in respiratory rate relative to air-exposed animals.[336] Examples of other symptoms of exposure to an airborne chemical irritant include cough, dyspnea, phlegm production, and pain on inspiration.

This subsection also includes endpoints related to structural impairment. Damage to the respiratory system from both acute and chronic chemical exposure can be measured by pathologic evaluation of the tissues of the respiratory system including the nasal, bronchial, bronchiolar and alveolar epithelium, capillary endothelium and interstitial tissues. This is done typically by gross examination and by light and electron microscopy. Examples of common endpoints include: pulmonary edema; degeneration of cells; hypertrophy; cell necrosis (death); cell proliferation; altered ratio of cells in lung tissue (generally a result of damage and subsequent repair); fibrosis; increased thickness of the alveolar and bronchiolar wall; other altered structural features including other morphometric changes.[337]

Imaging studies can also measure certain types of lung damage. Radiographic changes, such as those that can be measured using computed tomography, can be diagnostic of frank lung disease involving emphysematous and fibrotic changes, such as those caused by asbestos or crystalline silica.[338]

Another common way to measure damage to the lung in both humans and animals that is relatively non-invasive is to evaluate the composition of bronchoalveolar lavage fluid (BALF).[339] Bronchoalveolar lavage involves washing the airways with a small amount of saline and measuring the molecular composition and cell content of the fluid. BALF can be examined for the type of cells in the lumen of the alveolus and bronchiole, and

[335] Witschi HR, Pinkerton KE, VanWinkle LS, Last JA. (2008) Toxic Responses of the Respiratory System, Chapter 15 in: Toxicology, the Basic Science of Poisons; Klaassen CD, Ed. McGraw Hill.p.617-618.
[336] Kuwabara Y, Alexeeff GV, Briadwin R, Salmon AG. 2007 Evaluation and application of the RD50 for determining acceptable exposure levels of airborne sensory irritants for the general public. Environ Health Perspect. 115(11):1609-16.
[337] Witschi HR, Pinkerton KE, VanWinkle LS, Last JA. (2008) Toxic Responses of the Respiratory System, Chapter 15 in: Toxicology, the Basic Science of Poisons; Klaassen CD, Ed. McGraw Hill.p.626-627.
[338] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 326-329.
[339] Henderson R (1989) Bronchoalveolar lavage: A tool for assessing the health status of the lung. Chapter 15 in: Concepts in Inhalation Toxicology, McClellan RO and Henderson, RF (Eds.) Hemisphere Publishing Co., p. 415-441.

for the presence of biochemical indicators of inflammation and immune system response. Examples of measurements of the BALF indicative of lung injury include: increased numbers of cells from the immune system indicating immune or inflammatory response (e.g., polymorphonuclear leukocytes, lymphocytes, macrophages and monocytes and their phagocytic capabilities, and eosinophils); level of inflammatory cytokines; elevated reactive oxygen species; growth factors; arachidonate metabolites; increased enzyme activities such as lactate dehydrogenase, N-acetyl glucosaminidase, alkaline phosphatase, and lysosomal hydrolase indicating stimulated alveolar macrophages or cell damage; total protein and/or albumin content (indicating increased lung permeability and/or cell damage); elastolytic activity; presence of biochemicals associated with fibrosis (e.g., collagen, elastin, fibrin); and increased components of mucus.[340]

The presence of certain components in exhaled breath is also a non-invasive measure of inflammatory response and/or oxidative stress in the lung. Exhaled breath nitric oxide and the ratio of glutathione to reduced glutathione are two examples of exhaled breath measurements that demonstrate inflammation and oxidative stress in the lung.

This subsection includes endpoints related to functional impairment. Lung function tests are a technique used in humans and in laboratory animals to evaluate the effects of chemical exposure on the lung. Lung function testing commonly uses a spirometer to assess airway obstruction by measuring forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), the FEV1/FVC ratio, forced expiratory flow rates, and peak expiratory flow rate.[341] Other measurements of lung function used to evaluate lung function changes from chemical exposure include gas diffusion capability, total lung capacity, functional residual capacity, and residual volume. The results are compared to what is expected based on height, weight, age and gender in order to evaluate abnormality of lung function.

Another type of functional impairment associated with inhaled chemicals is bronchoconstriction or airways hyperresponsiveness.[342] Some chemicals can sensitize the immune system such that inhalation exposure results in an asthma attack characterized by constriction of the airways, hypersecretion of mucus and a resultant drop in lung function and gas exchange. Some chemical irritants also induce

[340] Henderson R (1989) Bronchoalveolar lavage: A tool for assessing the health status of the lung. Chapter 15 in: Concepts in Inhalation Toxicology, McClellan RO and Henderson, RF (Eds.) Hemisphere Publishing Co.,p. 421-438.
[341] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 311-312.
[342] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 312.

bronchoconstriction without involvement of an immune response.[343] Measurements of bronchial provocation are endpoints used to assess the impact of such chemicals.

Another lung disease that is induced by chemicals is hypersensitivity pneumonitis. This is an immunologically mediated inflammation of the lung parenchyma, which can lead to progressive interstitial fibrosis.[344]

Subsection 69403.15(c) provides examples of other relevant data useful to evaluate chemical substances for the respiratory toxicity hazard trait. A number of endpoints can be measured in isolated or cultures of various types of lung cells[345] to evaluate the effects of chemical exposure including cytotoxicity, gene expression, protein production, and metabolic changes. For instance, elevated gene expression for inflammatory cytokines in an *in vitro* assay points to the capability of a chemical to cause inflammation in cells. A chemical that has not been tested for respiratory toxicity but which has a high redox potential (ability to oxidize other molecules) may form destructive reactive oxygen species in the lung. Particle size and fiber dimension influences where in the respiratory system a particle phase chemical will deposit and influences the toxicity of the chemical. If the chemical substance has a long half-life in the lung, then the probability of adverse health impacts from toxicity increases.

§ 69403.16 Evidence for Toxicological Hazard Traits

As noted in Article 2, Section 69402.6, the distinction between strong and suggestive evidence for each of the toxicological hazard traits covered by Article 3 of the proposed regulation is provided to assist DTSC, the public and affected industries in understanding the strength of the evidence for hazards associated with chemical substances included in the Clearinghouse. It is intended to promote the inclusion of information from all well-conducted and relevant studies in the Clearinghouse, including information that is insufficient for a finding of strong or suggestive.

Subsection 69403.16(a) describes what constitutes strong evidence for the toxicological hazard traits in Article 3, based on determinations made by various authoritative bodies, or well-conducted scientific studies for chemical substances that may not have been evaluated fully by an authoritative organization.

[343] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 315-320.
[344] Balmes, J in: Occupational and Environmental Medicine, 2007; La Dou, ed., Chapter 20, Occupational Lung Diseases, p. 321-323.
[345] Witschi HR, Pinkerton KE, VanWinkle LS, Last JA. (2008) Toxic Responses of the Respiratory System, Chapter 15 in: Toxicology, the Basic Science of Poisons; Klaassen CD, Ed. McGraw Hill.p.628.

Subsection 69403.16(a)(1) provides for an authoritative organization to create strong evidence for a given chemical having one of the toxicological hazard traits described in this Article by taking one of three actions: 1. Reaching a conclusion based on well-conducted scientific studies, 2. Using the trait in a formal hazard identification, dose-response assessment or risk assessment, or 3. Listing the chemical as having the hazard trait. These three methods are common ways in which an authoritative body identifies chemical hazards.

Subsection 69403.16(a)(2) provides a method of identifying a given hazard trait in the absence of an authoritative organization finding meeting the criteria in the previous subsection. This provision is necessary because, for the vast majority of chemicals, authoritative evaluations are not available. In such cases, a finding could be based on available scientific information. A finding that a chemical substance had a certain toxicological endpoint in two or more well-conducted studies is strong evidence that the chemical substance has the hazard trait. The endpoints in the studies could be the same or closely related ones. The requirement of two or more studies is based on the need for some form of repeated finding in order to have confidence in the result. Typically, if the finding is in animals *in vivo*, it is preferred that the studies be conducted in different laboratories, by different researchers or under different protocols, in different species, or different genders of the same species.

Subsection 69403.16(b) provides example of four types of suggestive evidence that a chemical substance has a hazard trait. First, an authoritative organization finding that a chemical substance possibly has the trait, or the equivalent, constitutes suggestive evidence.

Second, suggestive evidence can be provided by a well-conducted study showing that the chemical substance has the hazard trait, preferably with a high degree of confidence in the finding.

The finding of suggestive evidence in this subsection is intended to be similar to that for limited evidence of carcinogenicity in experimental animals by IARC:

> "The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

As applied to Article 3, the hazard trait in question would not be carcinogenicity, but the general concept described above can be applied to limited data that may be available on other hazard traits.

Third, good quality mechanistic evidence can provide suggestive evidence of the hazard trait. For example, a series of *in vitro* assays demonstrating the potential for a compound to produce prolonged inflammation would be suggestive evidence for the respiratory hazard trait, among others.

Fourth, strong indications of the hazard trait can come from structure activity relationships. "[T]here is an extensive and growing range of software and literature models for predicting endocrine-related activities, in particular models for oestrogen and androgen activity."[346] Strong signals of potential for estrogen or androgen disruption, for example, would indicate the endocrine hazard trait, among others.

Article 4. Environmental Hazard Traits

All the information in the Article 4 is necessary in order for OEHHA to meet its statutory mandate [347] to specify the toxicological and environmental hazard traits, endpoints and other relevant data to be included in the Toxics Information Clearinghouse. These definitions are primarily a collection of definitions from existing documents prepared by U.S. Environmental Protection Agency and other authoritative organizations. An explanation of each of the environmental hazard traits, related endpoints and other relevant data are included in the discussion of each proposed subsection. Subsection 69404.10 of this Article provides a broad description of what constitutes "strong" versus "suggestive" evidence that a given chemical substance has a given hazard trait. As noted for the previous Articles, it is necessary for DTSC, industry and the public to be able to differentiate between the various types of information and data that may be available on the toxicity of a given chemical. OEHHA is proposing this regulation in order to provide guidance on this question.

§ 69404 General

The environmental hazard traits in this proposed regulation are based on fundamental principles of ecotoxicology, which can be broadly described as the science of contaminants in the biosphere and their effects on constituents of the biosphere.[348] Ecotoxicology is a hierarchical science, operating between several levels of biological organization (e.g., organismal, population, community and ecosystem). Significant

[346] E Lo Piparo and A Worth, 2010. Review of QSAR Models and Software Tools for predicting Developmental and Reproductive Toxicity, European Union, Joint Research Center, European Commission.
[347] Health and Safety Code section 25256.1.
[348] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Page 189.

cause and effect relationships are found by moving both “bottom-up” (mechanistic to organismal to population) and “top-down” (keystone predator) through the levels of this hierarchical science.

Ecotoxicology is focused on predicting consequences at higher levels of biological organization (population and above); however, no organizational level is more important than another in pursuit of that goal.[349] This is consistent with U.S. Environmental Protection Agency’s Guidelines for Ecological Risk Assessment,[350] which states:

> “Ecologically relevant endpoints may be identified at any level of organization (e.g., individual, population, community, ecosystem, landscape). The consequences of changes in these endpoints may be quantified (e.g., alteration of community structure from the loss of a keystone species) or inferred (e.g., survival of individuals is needed to maintain populations)”.

As defined in subsection 69401.2(j) of this Chapter, the term wildlife implies all non-human, non-domesticated organisms present in ecosystems. Although plants are technically considered wildlife, phytotoxicity is addressed separately in these proposed regulations in order to capture unique hazards such as the inhibition of photosynthesis.

Tests to assess toxicity in wildlife are described for aquatic vertebrates and invertebrates, birds, mammals, amphibians, reptiles, terrestrial invertebrates, microorganisms and plants by the US Environmental Protection Agency, other U.S. State and Federal Agencies, the Organization for Economic Co-operation and Development, and other organizations such as American Society for Testing and Materials International and the International Organization for Standardization. Testing described for human toxicity can often be applied to wildlife directly (terrestrial mammals) or indirectly (appropriate endpoints for specific toxicity, such as hepatotoxicity).

§ 69404.1 Domesticated Animal Toxicity

Subsection 69404.1(a) defines the domesticated animal toxicity hazard trait. This definition reflects the public value of livestock and pets and is separately called out in this regulation in order to address toxicities that may be specific to domesticated animals. Chemical contaminants in pet and livestock food have killed and sickened domesticated animals (e.g., melamine in cat and dog food)[351]

[349] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Page 189.
[350] U.S. EPA. (1998). Guidelines for Ecological Risk Assessment. Washington, DC: EPA/630/R-95/002F. Page 30.
[351] Dobson RLM, Motlagh S, Quijano M, et al. (2008) Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicological Sciences 106:252-262.

Subsection 69404.1(b) specifies the endpoints that indicate domesticated animal toxicity. The endpoints named are general diagnostic categories described in the Merck Veterinary Manual.[352]

Subsection 69404.1(c) provides categories of other relevant data on domesticated animal toxicity. Endpoint data described under Articles 2 and 3 of this proposed regulation, as well as those described for the impairment of wildlife development, growth, reproduction, and survival in this Article may also show the potential for effects in a pet or livestock species.

§ 69404.2 Eutrophication

Subsection 69404.2(a) defines the eutrophication hazard trait. The definition follows the U.S. Environmental Protection Agency's implementation of environmental law. The Clean Water Act requires that water quality criteria consider factors affecting rates of eutrophication.[353] The U.S. Environmental Protection Agency considers adverse effects on nutrient cycling, including eutrophication of soils, when evaluating environmental impact assessments required under the National Environmental Policy Act.[354] These endpoints are also used by the U.S. Environmental Protection Agency in ecological risk assessment.[355]

Subsection 69404.2(b) provides examples of the endpoints that point to the presence of the eutrophication hazard trait. Excessive plant growth of a subset of species that tolerate or thrive on eutrophic conditions leads to changes in species composition and diversity in aquatic and terrestrial systems. Excessive plant growth resulting from eutrophication is discussed in the U.S. Environmental Protection Agency's Generic Ecological Assessment Endpoints:

> "Eutrophication has long been a major concern of environmental managers, particularly with respect to sewage outfalls, so the models for predicting effects of nutrient additions are relatively well developed. Similarly, studies of fertilizer addition to crops, pastures, and commercial forests are numerous and provide a good basis for predicting the effects of terrestrial nutrient additions on plant

[352] Kahn, C., S. Line, et al., Eds. (2008). The Merck Veterinary Manual Online, 9th Ed. Whitehouse Station, NJ, Merck & Co., Inc.
[353] CWA §304(a)(1)
[354] U.S. EPA. (1999). Considering Ecological Processes in Environmental Impact Assessments. Section 6. Nutrient Cycling. Office of Federal Activities.
[355] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 11, Table 2-2, GEAE #9.

> production. In addition, methods for measuring plant production are well developed for both terrestrial and aquatic communities."[356]

In aquatic ecosystems, excessive plant growth associated with eutrophication leads to oxygen depletion as the plants decompose. Hypoxia, or depleted oxygen, in California waters is regulated using measurements of dissolved oxygen and biochemical oxygen demand by the Regional Water Quality Control Boards.[357]

Subsection 69404.2(c) provides general categories of other relevant data that are used by the U.S. Environmental Protection Agency to assess the impacts of eutrophication on ecosystems. As noted above methods to predict the effects of nutrient additions in aquatic and terrestrial environments are well developed.[358]

§ 69404.3 Impairment of Waste Management Organisms

Subsection 69404.3(a) defines the impairment of waste management organisms hazard trait. This definition is based on policies of the U.S. Environmental Protection Agency[359] intended to prevent chemically-induced adverse impacts on microbial sewage treatment processes. For example, the Clean Water Act "establishes responsibilities of Federal, State, and local government, industry and the public to implement National Pretreatment Standards to control pollutants which pass through or interfere with treatment processes in Publicly Owned Treatment Works or which may contaminate sewage sludge."[360]

Subsection 69404.3(b) specifies the endpoints that indicate the impairment of waste management organisms. The endpoints named are those described by the U.S. Environmental Protection Agency[361] [362] and the Organization for Economic Co-operation and Development[363] to assess the impacts of chemicals on microorganisms.

[356] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 47.
[357] San Francisco Bay Regional Water Quality Control Board. 2007. San Francisco Bay Basin Water Quality Control Plan (Basin Plan). Page 57.
[358] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 47.
[359] 40 CFR 403.5(b)(4)
[360] 40 CFR 403.1(a)
[361] U.S. EPA. (1996). Ecological Effects Test Guidelines: OPPTS 850.6800 Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals EPA 712–C–96–168.
[362] 40 CFR 136.3; Green Alga
[363] OECD, 2010. Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems. Test No. 209: Activated Sludge, Respiration Inhibition Test.

Subsection 69404.3(c) provides general categories of other relevant data used in weighing the evidence of microbial toxicity potential by the U.S. Environmental Protection Agency.[364]

§ 69404.4 Loss of Genetic Diversity, Including Biodiversity

Subsection 69404.4(a) defines the loss of genetic diversity hazard trait. This definition is adapted from the U.S. Environmental Protection Agency Generic Ecological Assessment Endpoints "taxa richness" and community-level "abundance", defined below.[365]

> "Taxa Richness: the number of native species or other taxa in an assessment community or assemblage."
>
> "Abundance: the number of individuals in an assessment community or assemblage. Total abundance or relative abundances of individual species, other taxa, trophic groups, or other ecologically defined groups may be used."

Genetic diversity is identified as important to population viability in U.S. Environmental Protection Agency ecological risk assessment guidance[366] and expert review documents.[367] The U.S. Environmental Protection Agency has also identified genetic diversity as a useful indicator of ecosystem condition and sustainability.[368]

Subsection 69404.4(b) specifies the endpoints that point to the presence of the loss of genetic diversity hazard trait. These endpoints reflect the U.S. Environmental Protection Agency's Generic Ecological Assessment Endpoints, "taxa richness" and "abundance", defined above.[369] Additionally, the California State Water Resources Control Board utilizes measures of taxa richness and abundance of individuals in determining the section 303(d) list of water bodies that do not meet the requirements of the Clean Water Act.[370] The loss of genetic diversity is used by the U.S. Environmental Protection Agency to evaluate environmental impact assessments required under the

[364] For example, to fill data gaps within the HPV Challenge program.
[365] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 14.
[366] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 24.
[367] U.S. EPA. (2009). Summary Report: Risk Assessment Forum Technical Workshop on Population-level Ecological Risk Assessment. Washington, DC: EPA/100/R-09/006. Pages 25 – 26.
[368] U.S. EPA. (2002). Genetic Diversity as an Indicator of Ecosystem Condition and Sustainability. Cincinnati, OH: EPA/600/R-03/056. See Summary, page iv.
[369] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 14.
[370] SWRCB. (2004). Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List. State Water Resources Control Board, Sacramento. Page 7.

National Environmental Policy Act and to evaluate ecosystem condition and sustainability.[371,372]

Subsection 69404.4(c) provides general categories of other relevant data for the loss of genetic diversity hazard trait.

§ 69404.5 Phytotoxicity

Subsection 69404.5(a) defines the phytotoxicity hazard trait. The definition is adapted from the following generic ecological assessment endpoints defined by the U.S. Environmental Protection Agency for use in ecological risk assessments.[373]

> "Gross anomalies: deformities, lesions, or tumors in animals; death or necrosis of plant leaves; or other overt physical injuries of organisms within an assessment population or community."
>
> "Survival, fecundity, or growth: survival (which may be reduced by direct lethality or by sublethal effects that diminish survival probabilities), fecundity (the production of viable young), and growth (increased mass or length) of some proportion of the animals or plants in an assessment population or community are the basic attributes of concern for nonhuman organisms."
>
> "Production [in populations]: the generation of biomass or individuals in an assessment population due to survival, fecundity, or growth."
>
> "Production [in communities, assemblages, or ecosystems]: the generation of biomass or individuals in an assessment community or assemblage."
>
> "Function: processes performed by ecosystems that are services to humans or other ecological entities."

Effects on plants are specifically addressed within each of these categories in the generic ecological assessment endpoints guidance document. The definition of plants is adapted from the general use of the word in the Toxic Substances Control Act and

[371] U.S. EPA. (1999). Considering Ecological Processes in Environmental Impact Assessments. Office of Federal Activities. Section 10: Genetic Diversity. Page 84.
[372] U.S. EPA. (2002). Genetic Diversity as an Indicator of Ecosystem Condition and Sustainability. Cincinnati, OH: EPA/600/R-03/056. See Summary, page iv.
[373] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Pages 13 – 15.

Federal Insecticide, Fungicide and Rodenticide Act, as described in the U.S. Environmental Protection Agency's ecological effects test guidelines for plant testing:[374]

> *"Plants* comprise vascular and nonvascular plants, algae, and fungi."

Lichen is a symbiotic association of a fungus with a photosynthetic microbe such as algae. Therefore, lichen is inferred in the definition shown above. In addition, plants may rely on interactions with microorganisms, for instance, Rhizobia are bacteria that fix nitrogen. Toxicities to such microorganisms may be included in the phytotoxicity hazard trait since they can have an adverse effect on plants.

Plants are critical to ecosystems. Plants provide food (primary productivity), oxygen and habitat, store energy and nutrients (carbon, nitrogen), modulate temperatures and water vapor, and provide natural commodities to humans.

Subsection 69404.5(b) gives examples of the endpoints that point to the presence of the phytotoxicity hazard trait. These endpoints are used by the U.S. Environmental Protection Agency to evaluate the impacts of chemicals on plants.[375,376,377,378] These endpoints are also used by the U.S. Environmental Protection Agency in ecological risk assessment.[379]

Subsection 69404.5(c) provides general categories of other relevant data that are currently used by researchers and risk assessors within the U.S. Environmental Protection Agency to evaluate the potential hazards of chemicals to plants.

§ 69404.6 Wildlife Developmental Impairment

Subsection 69404.6(a) defines the wildlife developmental impairment hazard trait. This definition is adapted from the generic ecological assessment endpoints "gross anomalies" and population "extirpation, abundance and production" identified by the U.S. Environmental Protection Agency.[380] Gross anomalies are identified as an

[374] U.S. EPA. (1996). Ecological Effects Test Guidelines: OPPTS 850.4000 Background-Nontarget Plant Testing. EPA 712–C–96–151. Page 3.
[375] U.S. EPA. (1996). Ecological Effects Test Guidelines: Nontarget Plants Test Guidelines OPPTS 850.4025 – 850.4800.
[376] U.S. EPA. (2002). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Washington, DC: EPA/821/R-02/013. Page 197.
[377] U.S. EPA. (2002). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Washington, DC: EPA/821/R-02/014. Page 332.
[378] U.S. EPA. (1995). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. Washington, DC: EPA/600/R-95/136. Page 466.
[379] U.S. EPA. (1998). Guidelines for Ecological Risk Assessment. Washington, DC: EPA/630/R-95/002F. Adverse ecological effects on plants are discussed throughout the document and used in several specific examples.
[380] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Pages 13-14.

environmental effect of regulatory concern in the Toxic Substances Control Act, the Comprehensive Environmental Response, Compensation, and Liability Act, the Clean Water Act, the Oil Pollution Act and U.S. Environmental Protection Agency guidance for state water quality control standards. U.S. Environmental Protection Agency regulation and guidance consistently acknowledge the importance of successful reproduction to population viability. DTSC also considers adverse impacts of development in ecological risk assessment.[381]

Successful development is essential to ecological fitness, which reflects the ability of an individual to produce offspring that survive and reproduce. To have high ecological fitness, offspring must survive to reproductive age, successfully reproduce, and produce offspring capable of doing the same.

Subsection 69404.6(b) provides examples of the endpoints that would point to the presence of the wildlife developmental impairment hazard trait.

These endpoints include the types of ecotoxicological data that are gathered to evaluate developmental toxicity in wildlife.[382] The U.S. Environmental Protection Agency focuses on successful reproduction as a key endpoint in ecological risk assessment:

> "Gross anomalies in birds, fish, shellfish, and other organisms are cause for public concern and have been the basis for EPA regulatory action and guidance… EPA actions to restrict the use of tributyltin as an antifoulant on boats (U.S. EPA, 1988b), as well as the restrictions imposed by the Organotin Antifouling Paint Control Act of 1988, were triggered by the observed induction of gross deformities in mollusks that threatened the marketability of oysters, reduced the fecundity of the deformed organisms, and suggested the potential for other effects."
>
> "Natural resource damage regulations for CERCLA, the CWA, and the Oil Pollution Act include gross anomalies among the designated injuries (43 CFR §11.62(f)), and deformities, erosion, lesions and tumors in fish (DELT anomalies) are used in the biocriteria of many state water quality standards and in Agency guidance (Yoder and Rankin, 1995; U.S. EPA, 1996). Changes in development, which can be manifested in physical anomalies, have been identified as an environmental effect of regulatory concern under TSCA (U.S. EPA, 1983)." [383]

[381] DTSC. (1996). Guidelines for Ecological Risk Assessment at Hazardous Waste Sites and Permitted Facilities. Department of Toxic Substance Control, Sacramento, CA. Page 18.
[382] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Chapter 10: Sublethal Effects. Pages 163-188.
[383] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Pages 31-32.

Subsection 69404.6(c) provides general categories of other relevant data that are used by researchers and risk assessors within the U.S. Environmental Protection Agency to evaluate the potential hazard of chemicals to wildlife development. Mechanistic data described for developmental toxicity under human health in subsection 69402.3(c) of this proposed regulation are also relevant to wildlife developmental toxicity. For example, toxicants shown to interact with retinoic acid receptors have implications in both human and wildlife development. Additionally, some *in vitro* screens have been specifically designed for ecotoxicological endpoints.[384] These tests investigate a chemical's potential to affect development by evaluating its interactions with proteins, metabolism, genes and gene expression.

§ 69404.7 Wildlife Growth Impairment

Subsection 69404.7(a) defines the wildlife growth impairment hazard trait. The definition is adapted from the generic ecological assessment endpoint "growth" defined by the U.S. Environmental Protection Agency as "increased mass or length of some proportion of the animal."[385] The U.S. Environmental Protection Agency report on generic ecological assessment endpoints also identifies exposure-related changes in growth rate as a potential population-level impact on species.

Animals need adequate growth in order to become reproductive, compete for mates and compete for resources. Reductions in growth rate can delay sexual maturation and thus disturb population stage-structure. This in turn can impact population growth rates and stability. Changes in growth rates can impact population dynamics, prey availability and other community functions.

Subsection 69404.7(b) provides examples of endpoints that point to the presence of the wildlife growth impairment hazard trait. Assessment of potential impacts on growth consider data on the extent and/or rate of growth in exposed wildlife. Impacts on growth affect social status, competition, predation and reproductive success, which directly affect ecological fitness, population viability and community structure.[386] Several environmental laws require testing of exposure-related perturbations of animal growth (e.g., CWA, TSCA, FIFRA). The U.S. Environmental Protection Agency states that:

> "Because the vast majority of standard toxicity tests determine effects on the

[384] Van Aggelen, G., G. T. Ankley, et al. (2009). Integrating Omic Technologies into Aquatic Ecological Risk Assessment and Environmental Monitoring: Hurdles, Achievements, and Future Outlook. Environ Health Perspect 118(1).
[385] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 13.
[386] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Chapter 10: Sublethal Effects. Pages 163-188.

survival, fecundity, and growth of organisms, direct toxic effects on this endpoint are readily predicted. In addition, extrapolation models are available that can estimate effects on this endpoint for particular organisms and exposure routes of concern on the basis of tests conducted on other species, life stages, or exposure durations or routes."[387]

Subsection 69404.7(c) provides general categories of other relevant data that are currently used by researchers and risk assessors within the U.S. Environmental Protection Agency to evaluate the potential hazard of chemicals to wildlife growth. Markers of data on growth retardation established for human health risk assessment are also relevant to wildlife reproductive toxicity. For example, markers of growth retardation identified in rodent models would also apply to mammalian wildlife.

§ 69404.8 Wildlife Reproductive Impairment

Subsection 69404.8(a) defines the wildlife reproductive impairment hazard trait. The definition is adapted from the generic ecological assessment endpoint "fecundity" defined by the U.S. Environmental Protection Agency as the "the production of viable young".[388] Many U.S. Environmental Protection Agency water quality criteria are based on measures of reproductive success.[389] The definition in Subsection 69404.8(a) is consistent with U.S. Environmental Protection Agency policy and guidance.

Subsection 69404.8(b) provides examples of endpoints that point to the presence of the wildlife reproductive impairment hazard trait. The endpoints reflect the approach used by the U.S. Environmental Protection Agency and DTSC to evaluate the hazards of chemicals to wildlife. "EPA's ecological assessments have considered effects on [fecundity] in a variety of taxa …" For example, the pesticide chlorofenapyr was not approved by U.S. Environmental Protection Agency on the basis of Agency concerns over reproductive risks to birds."[390] Several water quality criteria are based on fecundity and fertility[391]. Endpoints of endocrine disruption, including intersex, in fish and amphibians are considered in chemical reviews under the Toxic Substances Control Act.[392] Behavioral reproductive endpoints are included in U.S. Environmental Protection Agency's ecological risk assessment guidance:

[387] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 35.
[388] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 13.
[389] U.S. EPA. (1994). Water Quality Standards Handbook, 2nd edition. Washington, DC: EPA/823/B-94/005A.
[390] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 32.
[391] U.S. EPA. (1994). Water Quality Standards Handbook, 2nd edition. Washington, DC: EPA/823/B-94/005A.
[392] U.S. EPA. (2009). Endocrine Disruptor Screening Program Test Guidelines: OPPTS Series 890.

> "Measures of sensitivity may include mortality or adverse reproductive effects from exposure to toxics. Other possible measures of sensitivity include behavioral abnormalities; avoidance of significant food sources and nesting sites..."[393]

DTSC also considers impacts on reproduction and behavior to be sensitive indicators of toxicity.[394]

Subsection 69404.8(c) provides general categories of other relevant data that are used by researchers and risk assessors within the U.S. Environmental Protection Agency to evaluate the potential hazard of chemicals to wildlife reproduction. *In vitro* data described in Subsections 69402.5(c) (Reproductive Toxicity) and 69403.3(c) (Endocrine Toxicity) for human health in this proposed regulation are also relevant to wildlife reproductive toxicity. For example, toxicants shown to bind the estrogen receptor have implications in both humans and wildlife. *In vitro* screens have been specifically designed for ecotoxicological endpoints. Examples include screens for reactivity within the hypothalamic–pituitary–gonadal axis, agonism of the aryl hydrocarbon receptor, and binding or disruption of the function of the estrogen or androgen receptors.[395]

§ 69404.9 Wildlife Survival Impairment

Subsection 69404.9(a) defines the wildlife survival impairment hazard trait. The definition is adapted from the generic ecological assessment endpoint "survival," which is defined by the U.S. Environmental Protection Agency to include the reduction of survival "by direct lethality or by sublethal effects that diminish survival probabilities".[396] This definition is also supported by the general understanding of survival impairment in the field of ecotoxicology. For example, Newman and Clements[397] state that sublethal effects are likely to "play a crucial role in the local extinction of exposed populations". The definition in the proposed regulation describes sublethal effects as "disease or other biological impairment", which is more understandable and specific than the term sublethal effects.

[393] U.S. EPA. (1998). Guidelines for Ecological Risk Assessment. Washington, DC: EPA/630/R-95/002F. Page 33.
[394] DTSC. (1996). Guidelines for Ecological Risk Assessment at Hazardous Waste Sites and Permitted Facilities. Department of Toxic Substance Control, Sacramento, CA. Page 21.
[395] Van Aggelen, G., G. T. Ankley, et al. (2009). Integrating Omic Technologies into Aquatic Ecological Risk Assessment and Environmental Monitoring: Hurdles, Achievements, and Future Outlook. Environ Health Perspect 118(1).
[396] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 13.
[397] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Page 207.

Populations, communities and ecosystems are dependent on the viability of their constituents. Survival impairment (or reduced viability) can reduce population size, change population dynamics (e.g., carrying capacity of habitat) and increase the probability of population extinction.

Subsection 69404.9(b) provides examples of endpoints that would point to the presence of the wildlife survival hazard trait. Endpoints of wildlife survival impairment measured in non-human organisms include:

- Lethality
- Disease susceptibility (including immunological function, disease endpoints described in Articles 2 and 3 of the proposed regulation, disease specific to wildlife)
- Organ system toxicity (organ system endpoints described in Articles 2 and 3 of the proposed regulation, adverse effects on organ systems specific to wildlife)
- Non-specific toxicity (narcosis, uncoupled oxidative phosphorylation, generalized stress)
- Survival-dependent behavior (predator avoidance, hunting, foraging, migration)
- Population viability (changes in population trajectory or stability)

The relationship between the sublethal endpoints described above and impacts on survival potential and population viability are widely acknowledged in the U.S. Environmental Protection Agency's approach to ecological risk assessment. Physiological status, disease or debilitation, avoidance behavior and migratory behavior are identified as important to population viability in the U.S. Environmental Protection Agency's Generic Ecological Assessment Endpoints.[398] The U.S. Environmental Protection Agency also recognizes the value of using population modeling to predict the impact of factors that regulate populations, such as disease.[399] The connection between sublethal impacts and population viability is a central theme in the field of ecotoxicology.[400]

Subsection 69404.9(c) provides general categories of other relevant data that reflect the approach used by a leading ecotoxicology division within the U.S. Environmental Protection Agency, the Mid-Continent Ecology Division, in performing hazard rankings of chemicals with insufficient data sets. Quantitative structure-activity relationships (QSARs) are available for many chemicals in the U.S. Environmental Protection

[398] U.S. EPA. (2003). Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. Washington, DC: EPA/630/P-02/004F. Page 24.
[399] U.S. EPA. (2009). Summary Report: Risk Assessment Forum Technical Workshop on Population-level Ecological Risk Assessment. Washington, DC: EPA/100/R-09/006.
[400] Newman, M. and W. Clements (2008). Ecotoxicology: a comprehensive treatment, CRC. Chapter 10: Sublethal Effects. Pages 163-188.

Agency's environmental database ASTER (ASsessment Tools for the Evaluation of Risk). *In vitro* testing is commonly utilized in evaluating potential toxicity pathways of chemicals with limited datasets. *In vitro* data described for the human health hazard traits in the proposed regulation may also be relevant to wildlife toxicity. For example, toxic modes of action that impact the heart would be expected to affect terrestrial mammals, birds, fish and other wildlife.

§ 69404.10 Evidence for Environmental Hazard Traits

As noted in Article 2, Section 69402.6, the distinction between strong and suggestive evidence for each of the environmental hazard traits covered by Article 4 of the proposed regulation is provided to assist DTSC, the public and affected industries in understanding the strength of the evidence for hazards associated with chemical substances included in the Clearinghouse. It is intended to promote the inclusion of information from all well-conducted and relevant studies in the Clearinghouse, including information that is insufficient for a finding of strong or suggestive.

Subsection 69404.10(a) describes what constitutes strong evidence for the toxicological hazard traits in Article 4, based on determinations by authoritative bodies or well-conducted scientific studies for chemical substances that may not have been evaluated fully by authoritative organizations.

Subsection 69404.10(a)(1) describes three categories of findings from authoritative organizations that constitute strong evidence that a chemical substance has an environmental hazard trait. In the first category, an authoritative body draws the direct conclusion that a chemical has the hazard trait based on its review of well-conducted scientific studies. Examples of this type of evidence would be findings by the U.S. Environmental Protection Agency made in toxicological reviews performed under FIRFA or TSCA for a new chemical or new use of a chemical. A specific example would be the finding that chlorfenapyr is a reproductive toxicant in birds.[401] Such a finding would represent strong evidence for the wildlife reproductive impairment hazard trait for the use of chlorfenapry in consumer products (other than pesticides, which are excluded by statute from DTSC consideration under the Green Chemistry Program).

Several authoritative organizations provide direct conclusions on the hazards of chemicals to human health endpoints. When such findings are based on laboratory animal data (e.g., rats or mice), and do not depend on a large interspecies extrapolation uncertainty factor, they may also be relevant to terrestrial mammalian wildlife.

[401] U.S. EPA. 2000. Decision memorandum. Denial of registration of chlorfenapyr for use on cotton. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington D.C. Pages 2–4.

The second category of strong evidence from an authoritative organization consists of broader scientific evaluations of chemicals that are based, at least partly, on endpoints of a given hazard trait. An example would be the development of a wildlife toxicity reference value or environmental screening level based on a hazard trait included in this Article. A specific example is the U.S. Environmental Protection Agency's development of a pentachlorophenol soil screening level for mammals based on both growth and reproductive impairment.[402] This finding would constitute strong evidence that pentachlorophenol has the wildlife growth impairment and the wildlife reproductive impairment hazard traits.

The third category of findings that represent strong evidence of a hazard trait consists of regulatory actions that identify a chemical as having a hazard trait. An example of this would be the chemicals included in the priority pollutant list of the Clean Water Act. A specific example is the priority pollutant acrolein, which is regulated on the basis of survival effects on amphibians, fish and aquatic invertebrates.[403] This finding would constitute strong evidence that acrolein has the wildlife survival impairment hazard trait.

Subsection 69404.10(a)(2) is a provision for identifying the hazard trait in the absence of an authoritative organization finding. For the vast majority of chemicals, authoritative evaluations are not available. In such cases, a finding that a chemical substance had a certain toxicological endpoint in two or more well-conducted studies is strong indication that the chemical substance has the hazard trait. The endpoints could be the exact endpoints described in this regulation, or closely related ones. The requirement of two or more studies is based on the need for some form of repeated finding in order to have confidence in the result. Studies include, but are not limited to, standard aquatic and terrestrial toxicity testing to determine a lethal or effective dose (or concentration) for the purpose of hazard screening or criteria development, as well as research-based investigations of ecotoxicological endpoints.

Subsection 69404.10(b) provides four types of suggestive evidence that a chemical substance has a hazard trait. First, an authoritative organization finding that a chemical substance may have the trait constitutes suggestive evidence.

Second, suggestive evidence may be found in a well-conducted study showing that the chemical substance has the hazard trait, preferably with a high degree of confidence in the finding. The finding of suggestive evidence contemplated in the proposed regulation is similar to that used for limited evidence of carcinogenicity in experimental animals by the International Agency for Research on Cancer:

[402] U.S. EPA. 2005. Ecological soil screening levels for pentachlorophenol. Interim Final. OSWER Directive 9285.7-58. U.S. Environmental Protection Agency. Office of Solid Waste and Emergency Response. Washington, DC. Revised April 2007. Pages 9 – 13.

[403] U.S. EPA. 2009. Ambient aquatic life water quality criteria for acrolein (CAS registry number 107-02-8). U.S. Environmental Protection Agency. Office of Water. Washington D.C. EPA-822-F-09-004. Pages 5 – 8.

> "The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

The hazard trait being identified would not be carcinogenicity, but the general concept that the evidence is limited and a definitive evaluation of the hazard trait cannot be made would hold.

Third, good mechanistic evidence can be suggestive of the hazard trait. For example, a series of assays demonstrating the potential for a compound to inhibit estrogen binding to the estrogen receptor (estrogen antagonism) would be suggestive evidence for the wildlife reproductive impairment hazard trait.[404]

Fourth, structure activity relationships can provide suggestive evidence of the hazard trait. Quantitative structure-activity relationships (QSAR) are incorporated into the U.S. Environmental Protection Agency's expert system ASTER (ASsessment Tools for the Evaluation of Risk) for use in developing comprehensive ecological risk assessments.[405] Several QSAR models are available for ecotoxicological application.[406,407] Guidance on the development and application of QSAR models in ecotoxicology is available from several sources.[408,409,410]

[404] For a review of this mechanism see: Ankley, G.T., et al., Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem, 2010. 29(3): p. 730-41.
[405] Russom, C. L., E. B. Anderson, et al. (1991). ASTER: an integration of the AQUIRE data base and the QSAR system for use in ecological risk assessments. The Science of The Total Environment 109-110: 667-670.
[406] Moore, D. R. J., R. L. Breton, et al. (2003). A comparison of model performance for six quantitative structure-activity relationship packages that predict acute toxicity to fish. Environmental Toxicology and Chemistry 22(8): 1799-1809.
[407] European Centre for Ecotoxicology and Toxicology of Chemicals. 2003. (Q)SARs: Evaluation of the commercially available software for human health and environmental endpoints with respect to chemical management applications. Technical Report 89. Brussels, Belgium.
[408] Walker, J. D., J. Jaworska, et al. (2003). Guidelines for developing and using quantitative structure-activity relationships. Environmental Toxicology and Chemistry 22(8): 1653-1665.
[409] Comber, M. H. I., J. D. Walker, et al. (2003). Quantitative structure-activity relationships for predicting potential ecological hazard of organic chemicals for use in regulatory risk assessments. Environmental Toxicology and Chemistry 22(8): 1822-1828.
[410] European Centre for Ecotoxicology and Toxicology of Chemicals. 1998. QSARs in the assessment of the environmental fate and effects of chemicals. Technical Report 74. Brussels, Belgium.

Article 5. Exposure Potential Hazard Traits

All the information in the Article 5 is necessary in order for OEHHA to meet its statutory mandate[411] to specify the toxicological and environmental hazard traits, endpoints and other relevant data to be included in the Toxics Information Clearinghouse. These definitions are primarily a collection of definitions from existing documents prepared by the U.S. Environmental Protection Agency and other authoritative organizations. An explanation of each of the exposure potential hazard traits, related endpoints and other relevant data are included in the discussion of each proposed subsection.

§ 69405 General

The exposure potential hazard traits capture properties of chemicals that increase exposure of humans and wildlife once those chemicals are released into the environment. Regulatory agencies both nationally and internationally regard chemicals that persist in the environment, bioaccumulate, or are mobile in the environment as problematic for human and ecological receptors (e.g., wildlife) exposures. For example, the particle size and fiber dimension hazard trait identifies the increased availability for exposure of chemicals in the form of small particles and fibers. These may enter the lung and cause damage to the lung, or if small enough cross biological membranes of the lung, gut or skin into the systemic circulation. Another example is pre-natal and early postnatal exposures to chemicals because they can cross the placenta or get into breast milk. Finally, the exposure potential hazard traits also include traits of chemical that result in increased exposure to ozone formed in the atmosphere, to ultraviolet light by depletion of stratospheric ozone, and that contribute to heat exposure by increasing global warming.

§ 69405.1 Ambient Ozone Formation

Subsection 69405.1(a) defines ambient ozone formation as a hazard trait. The definition is based on the established science of ozone formation: Ozone is formed by a series of photochemical reactions from precursors such as oxides of nitrogen, volatile organic compounds, and some other pollutants, including carbon monoxide.[412]

There are strong correlations between ambient ozone concentrations and concentrations of ozone forming chemicals such as oxides of nitrogen and volatile organic compounds. Scientific studies show that exposure to ozone can reduce lung

[411] Health and Safety Code section 25256.1

[412] NRC (2008). Ambient ozone and related pollutants. Estimating Mortality Risk Reduction and Economic Benefits from Controlling Ozone Air Pollution, Committee on Estimating Mortality Risk Reduction Benefits from Decreasing Tropospheric Ozone Exposure, National Research Council, National Academy of Sciences: 48-74.

function and increase respiratory symptoms, airway hyper reactivity and inflammation, premature death, hospitalization for cardiopulmonary causes, emergency room visits for asthma, and restrictions in activity. For certain respiratory effects, children may be more affected due to effects on the developing lung.[413] The California Air Resources Board and the U.S. Environmental Protection Agency have adopted ozone ambient standards based on health and environmental effects. The Air Resources Board regulates volatile organics in consumer products, among other sources, and nitrogen oxides from combustion sources to control ambient ozone formation.[414]

Subsection 69405.1(b) describes evidence that indicates a given chemical has the capacity for ozone formation. For example, the Maximum Incremental Reactivity Scale is a key measure in the method used by the Air Resources Board to regulate ozone forming materials. The Maximum Incremental Reactivity method measures the maximum change in weight of ozone formed by adding a compound to the "Base ROG [Reactive Oxygen Gas] Mixture" per weight of compound added. Maximum Incremental Reactivity Scale values for individual compounds and hydrocarbon solvents are specified in Title 17, Cal. Code of Regulations, sections 94700 and 94701. The Air Resources Board has established methods for estimating values that may be applied to chemicals that are not included in the regulation.

§ 69405.2 Bioaccumulation

Subsection 69405.2(a) defines the bioaccumulation hazard trait. The definition proposed in the regulation is based on a U.S. Environmental Protection Agency regulation which has been primarily applied to aquatic organisms:[415]

> "**Bioaccumulation —**The accumulation of contaminants in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, pore water, or dredged material."

The phrase "or by biomagnification up the food chain" was added to the U.S. Environmental Protection Agency's definition to create the proposed definition to add emphasis for the general applicability to all organisms, including terrestrial organisms. The bioaccumulation hazard trait describes the capacity for a chemical substance to concentrate in organisms at levels higher than the surrounding environment or medium. The definition includes the sequestration of a chemical substance in a tissue at a higher

[413] OEHHA and ARB, 2005,Review of the California Ambient Air Quality Standard For Ozone Staff Report Initial Statement of Reasons for Proposed Rulemaking March 11, 2005, OEHHA and ARB 81-91.
[414] Title 17, California Code of Regulations, Section 94509.
[415] U.S. EPA, Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment. Status and Needs. EPA-823-R-00-001. Office of Water, February 2000, pp 53-55.

concentration than the body as a whole. The definition accounts for the uptake of chemicals from the environment from any route or pathway. A non-exclusive list of common sources of contaminated material that may directly contact aquatic organisms through various pathways (e.g., water, sediment residues) has been provided by the U.S. Environmental Protection Agency. This is given as an example. The proposed regulation is intended to cover all organisms and exposure routes and pathways.

Chemical concentration in the organism from bioaccumulation depends on: the rate of uptake, the mode of uptake (e.g., through the gills of a fish, ingesting contaminated food, contact with skin), how quickly the substance is eliminated from the organism, transformation of the substance by metabolic processes, the lipid (fat) content of the organism, the hydrophobicity of the substance, environmental factors, and other biological and physical factors. In biomagnification, a type of bioaccumulation, tissue concentrations of chemicals increase as the chemical passes from one organism to another, through two or more trophic levels. The term implies an efficient transfer of chemical from food to consumer, so that residue concentrations increase systematically through the food chain. In order for biomagnification to occur, the pollutant is generally long-lived, mobile, and fat soluble.

Bioaccumulation is an important hazard trait, because as a toxic substance becomes more concentrated, tissue exposures increase and the substance has the greater potential to cause harm to the organism, particularly near the top of the food chain, where tissue concentrations can be greatest.[416]

Subsection 69405.2(b) describes the type of evidence that indicates whether a chemical substance has the bioaccumulation hazard trait. For many chemical substances, particularly organic chemical substances, the potential to bioaccumulate can be quantified by measuring or predicting the chemical substance's bioaccumulation factor, bioconcentration factor, or log octanol water partition coefficient. For example, under its Toxics Release Inventory Program, the U.S. Environmental Protection Agency adopted a criterion for reporting for bioaccumulation/bioconcentration factors of 1,000.[417] This is the value included in proposed Subsection 69405.2(b) of the regulation. A log octanol water coefficient of 5 is proposed in this regulation. It has been used by the Canadian government's Environment Canada and the United Nation Environment Programme to define substances that are persistent and bioaccumulative[418] [419]. Some chemicals may have low bioaccumulation factors

[416] U.S. EPA, Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment. Status and Needs. EPA-823-R-00-001. Office of Water, February 2000. Pg xvii.
[417] For the purposes of section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) and section 6607 of the Pollution Prevention Act of 1990; Federal Register, 64(209):58665-58753.
[418] CEPA 1999 Section 73: Ecological Categorization Criteria and Process. Available at: http://www.ec.gc.ca/substances/ese/eng/dsl/cat_criteria_process.cfm.

predicted based on their physical-chemical properties, but may bioaccumulate if the chemical causes inhibition of an efflux transporter.[420] Thus evidence of such inhibition is also included as a criterion for evaluating bioaccumulation in Subsection 69405.2(b). Evidence from studies showing bioaccumulation in animal or human tissues also provides evidence for finding that a chemical substance has the bioaccumulation hazard trait. If a chemical met any of these criteria, it would be evidence of the bioaccumulation hazard trait.

§ 69405.3 Environmental Persistence

Subsection 69405.3(a) defines the hazard trait of environmental persistence. The proposed regulation is consistent with the U.S. Environmental Protection Agency's discussion of persistence: [421]

> "A chemical's persistence refers to the length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes."

Environmental persistence is an important determinant of exposure potential because the longer the chemical remains unchanged in the environment, the greater the chance of human or wildlife exposure. Environmental persistence is recognized as a hazard by many regulatory agencies such as the U.S. Environmental Protection Agency, European Union, and Canada.[422]

Subsection 69405.3(b) identifies sources of evidence indicating whether or not a chemical substance has the environmental persistence hazard trait. The proposed regulation is based on criteria adopted by U.S. Environmental Protection Agency and the European Union. The U.S. Environmental Protection Agency established persistence criteria for the Toxics Release Inventory Program of half-lives of 2 months in water, soil, and sediment and 2 days in air and these values are included in Subsection 69405.3(b) as examples. The criteria used by the European Union is a half-life in marine water that is greater than 60 days, or a half-life in fresh or estuarine water that is greater than 40 days.[423]

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[419] United Nations Environmental Programme (UNEP). *Stockholm Convention on Persistent Organic Pollutants (POPs*); 2001; http://www.pops.int/.
[420] Epel, D., Stevenson, C.A.,. MacManus-Spencer, L. A, Luckenbach T., and T. Smital. 2008. Efflux Transporters: Newly Appreciated Roles In Protection Against Pollutants. Environmental Science & Technology. 42:3914-3920.
[421] U.S. Environmental Protection Agency, 40 CFR Part 372. Persistent Bioaccumulative Toxic (PBT) Chemicals; Final Rule. Federal Register, 64(209):58665-58753.
[422] Section 77 Canadian Environmental Protection Act 1999.
[423] European Parliament (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

These criteria are similar to the persistence screening values for the Stockholm Convention, which are 2 months in water or 6 months in soil or sediment, with a two-day screening criterion for air transport.[424]

§ 69405.4 Global Warming Potential

Subsection 69405.4 (a) defines the global warming potential hazard trait. It is a synthesis of the definition of greenhouse gases and greenhouse effect adopted by the Intergovernmental Panel on Climate Change:[425]

> "Greenhouse gases effectively absorb thermal infrared radiation, emitted by the Earth's surface, by the atmosphere itself due to the same gases, and by clouds. Atmospheric radiation is emitted to all sides, including downward to the Earth's surface. Thus, greenhouse gases trap heat within the surface-troposphere system. This is called the greenhouse effect."

Subsection 69405.4 (b) provides examples of the types of evidence that indicate whether or not a chemical would be a significant contributor to global warming, based on U.S. Environmental Protection Agency and the California Air Resources Board criteria. The U.S. Environmental Protection Agency and the California Air Resources Board use internationally accepted "global warming potential" values for greenhouse gases, as provided by the Intergovernmental Panel on Climate Change. In Title 17 of the California Code of Regulations, section 94508(a), the California Air Resources Board has adopted the following definition:

> "Global Warming Potential Value" or "GWP Value" means the global warming potential value of a chemical or compound as specified in the IPCC: 1995 Second Assessment Report (SAR), Table 2.14, in Climate Change 2007: The Physical Sciences Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, which is incorporated by reference herein. If Table 2.14 does not contain a SAR 100-year GWP Value for a specific chemical or compound, then the 100-year GWP Value in Table 2.14 for that chemical or compound shall be used. If there is no 100-year GWP Value for a chemical or compound listed in Table 2.14 or GWP Value listed in Table 2.15, then the GWP Value is assumed to be equal to the GWP limit of the applicable product category."

[424]The Foundation for Global Action on Persistent Organic Pollutants: A United States Perspective Office of Research and Development Washington, DC 20460 EPA/600/P-01/003F NCEA-I-1200 March 2002.
[425] Intergovernmental Panel on Climate Change , IPCC Fourth Assessment Report: Climate Change 2007. World Health Organization and United Nations Environment Programme, Annex II Glossary.

If a chemical meets these criteria it provides evidence that the chemical has the global warming potential hazard trait.

§ 69405.5 Lactational or Transplacental Transfer

Subsection 69405.5 (a) defines the lactational and transplacental transfer hazard trait. Lactational and transplacental transfer result in exposure to the infant and fetus.[426] Early in life exposure to chemical substances can result in developmental toxicity. That is, different toxicities can occur or greater toxicity can occur relative to adult exposure. This is a well-recognized scientific concept.[427]

Subsection 69405.5 (b) provides examples of the types of evidence that indicate whether or not a chemical substance has this hazard trait. Analytical methods that measure the concentration of a chemical in breast milk or in the placenta can provide evidence that chemicals exhibit this hazard trait. If the chemical possesses physicochemical properties that are associated with transport or diffusion into breast milk or across the placenta, it is likely to travel from mother to fetus or infant, particularly if the chemical is stored in the mother's body. Both lipophilic and nonlipophilic chemicals can accumulate in the mother's tissues and then actively or passively transfer to breast milk and pass to an infant during breast feeding.[428] [429] Chemicals can also be transferred across the placenta actively or passively.[430] Poorly metabolized, nonvolatile, lipophilic chemicals are most likely to be transferred in significant quantities.

§ 69405.6 Mobility in Environmental Media

Subsection 69405.6 (a) defines the mobility in environmental media hazard trait. Movement of a chemical through and between environmental soil, ground water, air and other environmental media is a function both of the composition of the media (e.g. soil organic content) and of the physicochemical characteristics of the individual substance. For example, chemicals that are water soluble and have low partitioning into carbon material in soil can move through soil or fractured rock with water; they are not

[426] Lehman-McKeemam, LD. Absorption, distribution, and exrcretion of toxicants. Chapter 5 in: Toxicology, The Basic Science of Poisons. Klaassen CD, ed. Seventh Edition, 2008, p. 149-150, 156.
[427] Klaassen CD (2001) Casarett &Doull's Toxicology The Basic Science of Poisons. pp 351-386, McGraw-Hill:New York; OEHHA and ARB, 2005, Review of the California Ambient Air Quality Standard For Ozone Staff Report Initial Statement of Reasons for Proposed Rulemaking March 11, 2005, OEHHA and ARB 81-91.
[428] Somogyi, A and Beck H. Nurturing and breast-feeding: Exposure to chemicals in breast milk. Environ. Health Perspectives Supplem 101:45-52, 1993.
[429] Li P.-J., Sheng Y.-Z., Wang Q.-Y., Gu L.-Y. and Wang Y.-L. (2000). Transfer of lead via placenta and breast milk in human. Biomed Environ Sci 13(2): 85-89.
[430] Slikker W Jr, Miller RK. Placental Metabolism and Transfer. Role in Developmental Toxicology. Developmental Toxicology, C. A. Kimmel and J. Buelke-Sam, Editors; Raven Press, Ltd., New York, second edition, pages 245-283, 320 references, 1994

adsorbed onto charged solid surfaces to any appreciable degree. Chemicals that are volatile or semi-volatile at environmental temperatures can be transported in air as gases. Semi-volatile chemicals can condense out of the atmosphere as particles in distant places and cause toxicity in humans and ecological receptors.[431]

Subsection 69405.6 (a) gives examples of evidence that indicate whether or not a chemical substance has this hazard trait. It includes direct evidence from scientific studies showing environmental mobility or physico-chemical characteristics that enable movement through environmental compartments.

§ 69405.7 Particle Size or Fiber Dimension

Subsection 69405.7(a) defines the particle size or fiber dimension hazard trait. The proposed regulation recognizes that a chemical substance can either be manufactured as a small particle or form into a small particle with use or release.

The size dimension used in the proposed regulation is derived from the known physical characteristics that render a particle respirable[432] – and includes the potential for particles of 1 µm or less to pose an exposure potential hazard through multiple exposure pathways.[433] Particles that are 10 µm or less in diameter will end up in the lung when inhaled, where local irritation, inflammation and more severe toxicity can occur. If small enough, the particles may cross into the systemic circulation. Further, chemical constituents of particles may solubilize in lung fluids and cause local or systemic toxicity. Very small particles can also cross the walls of the gastrointestinal tract and the skin into the systemic circulation.[434]

Fiber dimension is an important factor in determining whether the fiber can be inhaled and get into the deep lung. An example of a problematic fiber is asbestos, which causes lung disease. A fiber is different than a particle; a particle is approximately spherical, where a fiber is long. So, a fiber much longer than 10 µm can still be inhaled deeply if it is narrow enough. Fibers that are considered respirable[435] are generally

[431] White Paper on Methods for Assessing Ecological Risks of Pesticides with Persistent, Bioaccumulative and Toxic Characteristics Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs Environmental Fate and Effects Division Washington, D.C. October 7, 2008.
[432] California Ambient Air Quality Standard for Particulate Matter, available at: http://www.arb.ca.gov/research/aaqs/std-rs/pm-final/pm-final.htm
[433] Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology.Oberdörster G.J Intern Med. 2010 Jan;267(1):89-105.
[434] Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology.Oberdörster G.J Intern Med. 2010 Jan;267(1):89-105.
[435] National Research Council. Asbestiform Fibers, Nonoccupational Health Risks, National Academy Press, Washington D.C., 1984.

defined as having an aspect ratio (length to width) of 3:1, and a width less than or equal to 3 µm.

The U.S. Environmental Protection Agency and the California Air Resources Board have adopted regulations intended to reduce air exposure to particles 10 micrometers or less and 2.5 um or less because of known, serious health effects.[436]

Subsection 69405.7(b) provides that measurement of particle size is evidence of whether or not a chemical substance has the hazard trait.

§ 69405.8 Stratospheric Ozone Depletion Potential

Subsection 69405.8(a) defines the stratospheric ozone depletion hazard trait. .Ozone-depleting chemical substances degrade in the upper atmosphere to generate reactive compounds that break apart stratospheric ozone molecules. These chemicals deplete stratospheric ozone, and thereby contribute to higher levels of ultraviolet B radiation reaching the earth's surface. This can increase human cancer and cataracts and impact wildlife.[437]

Subsection 69405.8(b) provides examples of evidence that indicate a chemical has the hazard trait. .Under the Clean Air Act [438] the United States banned the production and import of stratospheric ozone-depleting substances, in compliance with the 1987 Montreal Protocol on substances that deplete the ozone layer. The U.S. Environmental Protection Agency maintains a list of acceptable and unacceptable substitutes under its Significant New Alternatives Policy program, which is based on its evaluation of chemicals and technologies. In Section 612(c) of the Clean Air Act, the U.S. Environmental Protection Agency is authorized to identify and publish lists of acceptable and unacceptable substitutes for class I or class II ozone-depleting substances. Under Section 608 of the Clean Air Act, the U.S. Environmental Protection Agency has established federal regulations (40 CFR Part 82, Subpart F). The European Chemicals Agency has regulations concerning labeling of ozone-depleting chemicals.[439] Under the proposed regulation, evidence from sources other than the U.S. EPA may also indicate the hazard trait.

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[436] U.S. EPA National Ambient Air Quality Standard for Particulate Matter, Criteria Document available at: http://www.epa.gov/ttnnaaqs/standards/pm/s_pm_cr.html; California Ambient Air Quality Standard for Particulate Matter, available at: http://www.arb.ca.gov/research/aaqs/std-rs/pm-final/pm-final.htm

[437] Vol. 57 No. 147 Thursday, July 30, 1992 p 33754 (Rule) 1/5857 Environmental Protection Agency 40 CFR Part 82 [FRL-4158-2] Protection of Stratospheric Ozone Agency: Environmental Protection Agency (EPA).

[438] 42 U.S.C.A. § 7401 et seq.

[439] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (1) Official Journal of the European UnionL 353 Volume 51 31 December 2008. http://eurolex.europa.eu/JOHtml.do?uri=OJ:L:2008:353:SOM:EN:HTML

Article 6. Physical Hazard Traits

All the information in the Article 6 is necessary in order for OEHHA to meet its statutory mandate [440] to specify the toxicological and environmental hazard traits, endpoints and other relevant data to be included in the Toxics Information Clearinghouse. These proposed regulations are primarily a collection of definitions from existing documents prepared by U.S. Environmental Protection Agency and other authoritative organizations. An explanation of each of the physical hazard traits, related endpoints and other relevant data are included in the discussion of each proposed subsection.

§ 69406 General

Section 69406 explains that the purpose of this proposed Article is to identify physical hazard traits. The primary basis for Article 6 is the United Nations' Globally Harmonized System of Classification and Labeling of Chemicals,[441] commonly known as the "GHS."

Sections 69406.1 through 69406.3 of this proposed regulation define three general physical hazard traits combustion facilitation, explosivity, and flammability. These hazard traits are determined by intrinsic properties of chemical substances. The GHS criteria provide useful guidance and can be applied to evaluate whether a chemical has a physical hazard trait.

The GHS was developed to harmonize systems for the classification and labeling of chemicals worldwide. The U.S. Department of Transportation and a number of industries in the United States participated in the development of the GHS. The GHS provides general guidance that other national and international organizations can adopt or adapt to suit their particular needs. As stated in the GHS:[442]

> "The harmonized elements of the GHS may thus be seen as a collection of building blocks from which to form a regulatory approach. While the full range is available to everyone, and should be used if a country or organization chooses to cover a certain effect when it adopts the GHS, the full range does not have to be adopted."

[440] Health and Safety Code section 25256.1.
[441] Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third Revised Edition, United Nations, 2009, ST/SG/AC.10/30/Rev.3, available at http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html
[442] GHS, Third Revised Edition, United Nations, 2009, section 1.1.3.1.5.3.

The European Union incorporated GHS criteria into European Community law in regulation 1272/2008 on classification, labeling and packaging of substances and mixtures.[443]

The third revised edition of the GHS was consulted in drafting this proposed regulation. Evaluation of the evidence regarding whether or not a chemical has a physical hazard trait under the proposed regulation should consider the most current edition of the GHS.

Classification of a chemical substance as having a hazard trait does not depend upon its potential uses. For instance, fuel meeting the criteria for a flammable liquid would have the flammability physical hazard trait regardless of its intended use.

§ 69406.1 Combustion Facilitation

Subsection 69406.1(a) defines the combustion facilitation hazard trait as an umbrella term to cover hazards due to chemical substances, such as oxidizers, that can cause or contribute to the combustion of another material. This definition is similar to, and includes chemical substances, that meet the definitions of oxidizing gases, liquids, and solids in the GHS.[444]

Subsection 69406.1(b) provides an example of evidence that indicates whether or not a chemical substance has the combustion facilitation hazard trait using GHS criteria. Evidence for this hazard trait includes meeting the criteria specified by the GHS for oxidizing gases, liquids, or solids.

§ 69406.2 Explosivity

Subsection 69406.2(a) defines the explosivity hazard trait. The definition is adapted from the GHS definition of explosives.[445]

Subsection 69406.2(b) provides an example of evidence that indicates whether or not a chemical substance has the explosivity hazard trait using the GHS criteria.

[443] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, Official Journal of the European Union, L 353 Volume 51 31, December 2008, http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2008:353:SOM:EN:HTML
[444] GHS, Third Revised Edition, United Nations, 2009, sections 2.4, 2.13, 2.14.
[445] GHS, Third Revised Edition, United Nations, 2009, section 2.1.

§ 69406.3 Flammability

Subsection 69406.3(a) defines the flammability hazard trait. The definition in the proposed regulation includes several classifications used by the GHS [446] under the common heading of "flammability."

Subsection 69406.3(b) provides an example of the evidence that indicates whether or not a chemical substance has the flammability hazard trait using GHS criteria. Evidence for this hazard trait includes meeting the criteria specified by several sections of the GHS.[447]

[446] GHS, Third Revised Edition, United Nations, 2009, sections 2.2, 2.3, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.15.
[447] GHS, Third Revised Edition, United Nations, 2009, sections 2.2, 2.3, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.15.

Appendix Table. Key Sources for Definitions of Green Chemistry Hazard Traits

Toxicological Hazard Traits	
Carcinogenicity	World Health Organization's International Agency for Research on Cancer, *Preamble,* 2006
Cardiovascular Toxicity	*Toxicology: The Basic Science of Poisons,* Chapter 4
Dermatotoxicity	*Toxicology: The Basic Science of Poisons,* Chapter 19
Developmental Toxicity	U.S. Environmental Protection Agency *Guidelines for Developmental Toxicity Risk Assessment*; World Health Organization International Programme for Chemical Safety *Principles for Evaluating Health Risks to Reproductive Associated with Exposures to Chemicals*
Endocrine Toxicity	U.S. Environmental Protection Agency, European Union, *Toxicology: The Basic Science of Poisons, Chapter 21*
Epigenetic Toxicity	U.S. National Institutes of Health
Genotoxicity	*Toxicology: The Basic Science of Poisons,* Chapter 9
Hematotoxicity	*Toxicology: The Basic Science of Poisons,* Chapter 11
Hepatotoxicity and Digestive System Toxicity	*Toxicology of the Gastrointestinal Tract,* Chapter 6. *Toxicology: The Basic Science of Poisons,* Chapter 13
Musculoskeletal Toxicity	General and Applied Toxicology., Ballantyne B, Marrs TC, Syversen T. eds. 2009, Chapter 61 and 62
Nephrotoxicity	American Society of Nephrology; *Toxicology: The Basic Science of Poisons,* Chapter 14
Neurotoxicity	U.S. Environmental Protection Agency *Guidelines for Neurotoxicity Risk Assessment*
Ocular Toxicity	*Toxicology: The Basic Science of Poisons,* Chapter 17
Ototoxicity	General and Applied Toxicology., Ballantyne B, Marrs TC, Syversen T. eds. 2009, Chapter 56; Encyclopedia of Toxicology, Wexler P, ed. 2nd Edition, Volume 3, p. 315-318.Elsevier, 2005.
Reactivity in Biological Systems	*Toxicology: The Basic Science of Poisons,* Chapter 3
Reproductive Toxicity	U.S. Environmental Protection Agency *Guidelines for Reproductive Toxicity Risk Assessment*
Respiratory Toxicity	*Toxicology: The Basic Science of Poisons,* Chapter 15

Environmental Hazard Traits	
Domesticated Animal Toxicity	Kahn, C., S. Line, et al., Eds. (2008). The Merck Veterinary Manual Online, 9th Ed. Whitehouse Station, NJ, Merck & Co., Inc.
Eutrophication	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Impairment of Waste Management Organisms	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Loss of Genetic Diversity, Including Biodiversity	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Phytotoxicity	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Wildlife Developmental Impairment	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Wildlife Growth Impairment	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Wildlife Reproductive Impairment	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Wildlife Survival Impairment	U.S. Environmental Protection Agency *Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment*
Exposure Potential Hazard Traits	
Ambient Ozone Formation	National Academy of Sciences' National Research Council *Estimating Mortality Risk Reduction and Economic Benefits from Controlling Ozone Air Pollution*
Bioaccumulation	U.S. Environmental Protection Agency *Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment*
Environmental Persistence	U.S. Environmental Protection Agency *Persistent Bioaccumulative Toxic (PBT) Chemicals. Final Rule*

Global Warming Potential	Intergovernmental Panel on Climate Change, *IPCC Fourth Assessment Report*
Lactational or Transplacental Transfer	Lehman-McKeemam, LD. Absorption, distribution, and exrcretion of toxicants. Chapter 5 in: Toxicology, The Basic Science of Poisons. Klaassen CD, ed. Seventh Edition, 2008, p. 149-150, 156.; Slikker W Jr, Miller RK. Placental Metabolism and Transfer. Role in Developmental Toxicology. Developmental Toxicology, C. A. Kimmel and J. Buelke-Sam, Editors; Raven Press, Ltd., New York, second edition, pages 245-283, 1994
Mobility in Environmental Media	U.S. Environmental Protection Agency, White Paper on Methods for Assessing Ecological Risks of Pesticides with Persistent, Bioaccumulative and Toxic Characteristics Office of Pesticide Programs, Washington, D.C., 2008.
Particle Size or Fiber Dimension	California Ambient Air Quality Standard for Particulate Matter National Research Council. Asbestiform Fibers, Nonoccupational Health Risks, National Academy Press, Washington D.C., 1984
Stratospheric Ozone Depletion	Environmental Protection Agency 40 CFR Part 82 [FRL-4158-2] Protection of Stratospheric Ozone[448]
Physical Hazard Traits	
Explosivity	United Nation's Globally *Harmonized System for Classification and Labelling*
Flammability	United Nation's Globally *Harmonized System for Classification and Labelling*
Combustion Facilitation	United Nation's Globally *Harmonized System for Classification and Labelling*

[448] Federal Register Vol. 57 No. 147, page 33754