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International Regulatory Requirements for Skin Sensitization Testing

Amber B. Daniel^a, Judy Strickland^a, David Allen^a, Silvia Casati^b, Valérie Zuang^b, João Barroso^b, Maurice Whelan^b, M.J. Régimbald-Krnel^c, Hajime Kojima^d, Akiyoshi Nishikawa^d, Hye-Kyung Park^e, Jong Kwon Lee^e, Tae Sung Kim^e, Isabella Delgado^f, Ludmila Rios^g, Ying Yang^h, Gangli Wangⁱ, and Nicole Kleinstreuer^j

^aILS, P.O. Box 13501, Research Triangle Park, NC 27709, USA

^bEuropean Commission, Joint Research Centre (JRC), Via E. Fermi, 2749, 21027 Ispra (VA), Italy

^cEnvironmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, K1A 0K9 Canada

^dJapanese Centre for the Validation of Alternative Methods, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, Japan

^eKorean Centre for the Validation of Alternative Methods, National Institute of Food and Drug Safety Evaluation, 187 Osongsaengmyeong 2(i)-ro, Osong-eup, Heungdoek-gu, Cheongju-si, Chungcheongbuk-do, 28159, Korea

^fNational Institute of Quality Control in Health, Oswaldo Cruz Foundation (Fiocruz), Avenida Brasil, 4365 - Manguinhos, 21045-900 - Rio de Janeiro, RJ, Brazil

^gBrazilian Health Regulatory Agency (ANVISA), Setor de Indústria e Abastecimento (SIA) -Trecho 5, Área Especial 57, Lote 200, 71205-050 - Guará/DF, Brazil

^hGuangdong Provincial Center for Disease Control and Prevention, Qunxian Road 160, Panyu strict, Guangzhou, China 510430

ⁱNational Institutes for Food and Drug Control, Tiantan Xili Road 2, Beijing, China 100050

^jNational Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709, USA

Abstract

Skin sensitization test data are required or considered by chemical regulation authorities around the world. These data are used to develop product hazard labeling for the protection of consumers

*Correspondence to: Judy Strickland, ILS, P.O. Box 13501, Research Triangle Park, NC 27709, USA. strick12@niehs.nih.gov. Phone 1-919-281-1110, ext 245.

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or workers and to assess risks from exposure to skin-sensitizing chemicals. To identify opportunities for regulatory uses of non-animal replacements for skin sensitization tests, the needs and uses for skin sensitization test data must first be clarified. Thus, we reviewed skin sensitization testing requirements for seven countries or regions that are represented in the International Cooperation on Alternative Test Methods (ICATM). We noted the type of skin sensitization data required for each chemical sector and whether these data were used in a hazard classification, potency classification, or risk assessment context; the preferred tests; and whether alternative non-animal tests were acceptable. An understanding of national and regional regulatory requirements for skin sensitization testing will inform the development of ICATM's international strategy for the acceptance and implementation of non-animal alternatives to assess the health hazards and risks associated with potential skin sensitizers.

Keywords

skin sensitization testing; alternative approaches; non-animal methods; regulatory requirements; defined approaches

1. Introduction

The United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) defines a skin sensitizer as a substance or mixture that will cause an allergic response following contact with skin (UN, 2017). Skin sensitization testing identifies the potential for a chemical to cause allergic contact dermatitis, a local skin reaction characterized by redness, swelling, and itching (Murphy et al., 2012). Chemical regulatory authorities require skin sensitization test data for hazard classification and labeling of products to alert workers and consumers to potential hazards and for potency-based risk assessment to determine acceptable human exposure limits. Testing for skin sensitization potential has traditionally been accomplished using animal tests such as the murine local lymph node assay (LLNA), the guinea pig maximization test (GPMT), and the Buehler test (OECD, 1992; 2010a). However, several factors have prompted focused activities around the development of alternatives to animal testing for this endpoint, including ethical concerns about the use of animals, scientific concerns about the relevance of animal test results to humans, and legislative mandates to eliminate or reduce animal testing (EC, 2006; 2009b). Aided by a detailed adverse outcome pathway for skin sensitization (OECD, 2012), these activities have yielded a number of validated non-animal tests (EURL ECVAM Scientific Advisory Committee, 2016a; b; Joint Research Centre of the European Union, 2013; 2014; 2015). Internationally recognized test guidelines have been adopted by the Organization for Economic Co-operation and Development (OECD) (OECD, 2015a; b; 2017a), and additional tests are under consideration for test guideline development (OECD, 2017b). However, none of the currently adopted non-animal tests is recommended as a stand-alone replacement for the animal tests. Therefore, efforts have focused on the development of integrated strategies, referred to as defined approaches by OECD (OECD, 2016b), that use multiple testing (*in vitro* and *in chemico*) and non-testing (*in silico*) information sources to derive predictions that are expected to be sufficiently

predictive to replace animal tests for hazard assessment and potency classification (OECD, 2016c).

To date, defined approaches have not gained routine regulatory acceptance, despite demonstrating comparable or superior performance to the animal tests (Ezendam et al., 2016; Kleinstreuer et al., 2018; OECD, 2016c). To promote regulatory acceptance of integrated non-animal approaches to skin sensitization assessment, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) convened the International Cooperation on Alternative Test Methods (ICATM) *Workshop on the International Regulatory Applicability and Acceptance of Alternative Non-animal Approaches to Skin Sensitization Assessment of Chemicals* on October 4-5, 2016, in Ispra, Italy. The workshop was attended by 36 experts representing international regulatory authorities from 14 countries, test method validation authorities, and supporting organizations, such as OECD and the Scientific Committee on Consumer Safety. Workshop participants reviewed the performance of multiple non-animal integrated strategies for skin sensitization hazard assessment, discussed regulatory requirements for skin sensitization testing among various global regions by chemical sector, discussed obstacles to implementing non-animal approaches, and planned the path forward for evaluating and accepting integrated approaches in lieu of animals for skin sensitization testing (Casati et al., 2018).

In order to identify opportunities for regulatory uses of non-animal replacements for skin sensitization tests, the needs and uses for skin sensitization test data must first be clarified. As a follow-up activity to the workshop, ICATM partner organizations agreed to produce this summary of regulatory requirements for skin sensitization testing in countries and regions represented by ICATM participants. ICATM includes governmental organizations from seven countries and regional authorities who work together to promote enhanced international cooperation and coordination on the scientific development, validation, and regulatory use of alternative approaches.

The regulatory requirements discussed in this review apply to the countries or regions of the following ICATM partner organizations: EURL ECVAM, Health Canada, Japanese Center for the Validation of Alternative Methods, Korean Center for the Validation of Alternative Methods, U.S. Interagency Coordinating Committee on the Validation of Alternative Methods, the Brazilian Center for the Validation of Alternative Methods, the Guangdong Provincial Center for Disease Control and Prevention, and the Chinese National Institutes for Food and Drug Control. For each chemical sector, this review notes the type of skin sensitization data required (i.e., hazard and/or potency), the preferred tests to address these requirements, and whether alternative tests are acceptable in lieu of traditional animal tests. This review will inform the development of an international strategy for implementing non-animal approaches to assess skin sensitization hazard and risk.

2. Skin Sensitization Testing Requirements by Sector

The following sections contain regulatory information regarding skin sensitization testing requirements, including statutes/regulations and their applications, current skin sensitization

testing requirements, and preferred testing methods for various chemical sectors. We have attempted to provide a comprehensive characterization of the regulatory requirements across all regions and chemical sectors wherever the information was available; gaps in coverage indicate that there was no published documentation or applicable regulatory statute. Certain regulated sectors may be included under another chemical sector depending on differences in how chemicals are regulated in the different countries or regions. Chemical sectors, and countries or regions, are organized alphabetically.

2.1. Cosmetics and Personal Care Products

2.1.1. Brazil—The re-edition of the “Guidance for Safety Evaluation of Cosmetic Products” (ANVISA, 2012) recommends criteria for safety evaluation of cosmetics commercialized in Brazil and provides information about testing strategies. Regarding skin sensitization assessment, the Guidance does not require any specific test, but recommends the LLNA. The GPMT, Buehler test, and clinical studies such as the human repeat insult patch test (Politano and Api, 2008) are also described. In Brazil, a hazard assessment must be performed for classification of cosmetic ingredients as skin sensitizers or nonsensitizers. Alternative methods are accepted, in accordance with the specific limitations of each method and its regulatory application (ANVISA, 2015b). For example, *in silico* methods, including (quantitative) structure-activity relationships, are accepted when used as elements of integrated approaches to testing and assessment.

The technical regulations of cosmetic products intended for children’s use are provided by Collegiate Board of Directors Resolution (RDC) 15 (ANVISA, 2015a), which requires the submission of toxicological test data for skin sensitization for most cosmetic categories, e.g. lipstick and eye shadow. RDC 19 (ANVISA, 2013b) provides technical regulations for registering cosmetic insect repellents. Under both RDCs, a hazard assessment should classify the product as either skin sensitizer or nonsensitizer; only the nonsensitizers are acceptable for commercial use. No particular test method is specified. Sanitary absorbents, like other disposable hygienic products, are exempt from registration under RDC 10 (ANVISA, 1999). Marketing of these products is subject to notification and compliance with the provisions of Ordinance n. 1480 (Brazilian Ministry of Health, 1990). This ordinance, published by the Brazilian Ministry of Health, requires that the GPMT be used for testing these products. However, a skin sensitization assay is not necessary if the product is known to cause skin irritation. Disposable body hygienic products must be classified as skin sensitizers or nonsensitizers, and only those products determined to be nonsensitizers are acceptable for commercial use. It should be noted that RDC 10 (ANVISA, 1999) and the Ordinance n. 1480 (Brazilian Ministry of Health, 1990) are currently under revision.

2.1.2. Canada—The Consumer Product Safety Directorate of the Healthy Environments and Consumer Safety Branch of Health Canada, the authority responsible for the regulation of cosmetics in Canada, operates under a post-market regime. Under the *Food and Drugs Act* (Minister of Justice Canada [2017]-b) and its associated *Cosmetic Regulations* (Minister of Justice Canada [2007]), there are no requirements for companies to submit animal testing data (including skin sensitization) in order to market a cosmetic product.

In order to quantify risks from cosmetic products and/or define acceptable exposure limits, Health Canada may use skin sensitization results from the open literature. Generally, LLNA, GPMT and Buehler test results are considered acceptable for this purpose, although the LLNA is preferred. Additionally, *in silico*, *in chemico*, or *in vitro* sensitization data can be considered.

If necessary, Health Canada can require manufacturers to provide evidence of safety under the authority of the *Cosmetic Regulations* (Minister of Justice Canada [2007]). With respect to skin sensitization, Health Canada does not recommend specific tests for this purpose; however, various animal or alternative tests may be considered acceptable.

2.1.3. China—“Regulations Concerning the Hygiene Supervision over Cosmetics” (Ministry of Health, No. 3/1989) and “Detailed Rules for the Implementation of the Regulation on the Hygiene Supervision over Cosmetics” (Ministry of Health, No. 13/1991), both issued by the Ministry of Health, provide the management requirements for cosmetics registration in China. As of 2016, China Food and Drug Administration’s “Technical Safety Standard for Cosmetics” replaced the Ministry of Health’s “Hygienic Standard for Cosmetics” (CIRS, 2016). This mandatory guideline requires skin sensitization testing for new special-purpose cosmetics, including new cosmetic ingredients and newly imported cosmetics. Special-purpose cosmetics include products for hair growth, removal, color, or curl; weight loss; breast enhancement; sun block; deodorizing and spot removal (CFDA, Undated). The Buehler test or GPMT are the recommended test methods. Clinical studies are also recommended.

The results of skin sensitization tests are used to assign a potency classification of non-sensitive, weak-, light-, medium-, strong-, or very strong-sensitive. Only the non-sensitive and weak-sensitive products are considered “non-sensitizing” and acceptable for commercial use.

2.1.4. European Union—The European Regulation on Cosmetic Products (EC, 2009b) is the main regulatory framework for placing finished cosmetic products on the market in the European Union. It applies to all substances used as ingredients in cosmetic products regardless of the amount manufactured or imported into the European Union. The regulation contains the same provisions of the replaced Cosmetics Directive and its amendments (EC, 1976; 2003) in relation to animal testing. Specifically, it prohibits (1) testing finished cosmetic products and cosmetic ingredients on animals and (2) marketing finished cosmetic products and ingredients in the European Union that have been tested using animals after certain dates.

In order for a substance to be used in cosmetics, skin sensitization information must be provided as part of the minimum safety information requirement. As animal testing is no longer allowed, alternative methods should be utilized to test for skin sensitization. Relevant information on the different aspects of testing and safety evaluation of cosmetic substances in Europe is provided in the European Commission Scientific Committee on Consumer Safety’s (SCCS) guidance (SCCS, 2016). It must be noted, however, that animal data generated for the purpose of other regulations, such as Registration, Evaluation,

Authorization and Restriction of Chemicals (REACH; EC, 2006b), is also acceptable. As part of the evaluation, hazard assessment must be performed. If a cosmetic ingredient has skin sensitization potential, potency information is needed. The potency, the ingredient's concentration in the finished product, and the predicted human exposure scenario are then evaluated for the purpose of guaranteeing the safe use of cosmetic products.

2.1.5. Japan—There is currently no formal Japanese legislation that requires skin sensitization testing of cosmetics, quasi-drugs, or medicated cosmetics, including some personal care products (Inomata, 2014), if these products use existing ingredients. However, the Pharmaceuticals and Medical Devices Agency (PMDA) requires skin sensitization data for new ingredients in quasi-drugs to determine skin sensitization hazard and potency, and prefers that this data be generated using the GPMT, Buehler test, or LLNA.

Japanese cosmetics companies have independently ceased testing cosmetic ingredients on animals since 2011 subsequent to the EU Cosmetics Directive banning such tests. Therefore, in January 2018, PMDA began accepting an integrated strategy with three alternative *in vitro* methods: Direct Peptide Reactivity Assay (DPRA) (OECD, 2015a), ARE-Nrf2 Luciferase Test Method (OECD, 2015b), and Human Cell Line Activation Test (OECD, 2017a), in a “bottom-up” approach to identify non-sensitizing ingredients for cosmetics and quasi-drugs (PMDA, 2018).

2.1.6. South Korea—The regulatory requirements for skin sensitization testing of cosmetic products in South Korea are provided by the Cosmetics Act (Act No. 14027) (Ministry of Food and Drug Safety, 2016). The Ministry of Food and Drug Safety upholds this act and enforces testing requirements set forth in the “Regulation on the Examination of Functional Cosmetics.” These regulations require data from skin sensitization tests used for hazard classification to be submitted for review when new substances are used for functional cosmetics (Ministry of Food and Drug Safety, 2015b). As of 2016, South Korean law does not permit animal testing for ingredients used solely in cosmetics. A ban on marketing cosmetics containing ingredients tested on animals went into effect in the same year. OECD test guidelines are currently accepted in Korea for safety evaluation of cosmetics. The DPRA, the ARE-Nrf2 Luciferase Test Method, and the Human Cell Line Activation Test have been used since 2017 for the safety evaluation of cosmetics.

2.1.7. United States—No skin sensitization testing or data are required for cosmetics in the United States. Companies and individuals who manufacture or market cosmetics are legally responsible for ensuring the safety of their products. The U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition advises that the safety of a product can be adequately substantiated through (1) reliance on available toxicological test data for individual ingredients and for product formulations that are similar in composition to the product of interest, and (2) performance of additional toxicological and other tests that are appropriate in light of existing data and information (FDA, 1975). In cases where the safety of a cosmetic product has not been determined, 21 CFR 740.10 addresses the appropriate labeling that FDA may require (FDA, 2016a).

2.2. Household Products

2.2.1 Canada—The Consumer Product Safety Directorate of the Healthy Environments and Consumer Safety Branch of Health Canada operates under a post-market regime, and is responsible for consumer products, including household products and art materials under the *Canada Consumer Product Safety Act* (CCPSA) (Minister of Justice Canada [2016]-a). Under the CCPSA, the onus is on industry to ensure the safety of consumer products that they manufacture, import, advertise or sell in Canada. Health Canada does not require industry to provide pre-market skin sensitization test results for consumer products. In order to verify compliance, Health Canada could require this information using authorities provided by the CCPSA. Reviews are generally limited to the data in the open literature. Health Canada does not recommend specific skin sensitization tests for this purpose; however, the LLNA, GPMT and Buehler test may be considered acceptable. If no data from animal tests are available, *in silico*, *in chemico*, or *in vitro* sensitization data can be considered. In cases where a consumer product contains an ingredient of concern, Health Canada may conduct a quantitative risk assessment using data from the open literature, preferably from the LLNA.

2.2.2. Japan—As far as new designations of harmful substances are concerned, information or testing for skin sensitization potential is not specifically required. When skin sensitization information is needed, the test method may be selected on a case-by-case basis. The acceptance of non-animal alternative methods is undetermined.

2.2.3. United States—The Federal Hazardous Substances Act of 1964 is administered by the Consumer Product Safety Commission (CPSC) and applies to hazardous household products, excluding heating, cooking, and refrigeration fuels stored in containers. Substances that are subject to the Food, Drug, and Cosmetic Act or the Federal Insecticide, Fungicide, and Rodenticide Act are also excluded. The Federal Hazardous Substances Act does not require animal testing, but it does require appropriate labeling of strong sensitizers, which are defined as those substances having a significant potential for causing hypersensitivity (16 CFR 1500.13). CPSC has identified a number of substances as strong sensitizers based on the frequency and severity of skin sensitization reactions from multiple information sources. Human data are emphasized, but animal data are also considered.

Before considering animal testing, strong sensitizers should be identified using a weight-of-evidence approach that includes existing data from clinical and diagnostic studies, epidemiological studies, animal studies, *in vitro* tests, cross-reactivity data, and case histories (16 CFR 1500.3). If data are available and methods are validated, CPSC may also give consideration to quantitative structure-activity relationships, *in silico* data, or other potency and sensitizer information. If animal tests are necessary, CPSC recommends that these tests use the most humane procedures possible and the fewest animals necessary to provide reliable results. Currently, the traditional LLNA, the reduced LLNA, and non-radiolabeled modifications are the preferred methods of testing (OECD, 2010a; b; c). The Buehler test and GPMT are acceptable substitutes (OECD 1992). Although no non-animal methods are currently recognized as complete replacements, they may be accepted on a case-by-case basis (CPSC, 2012).

The CPSC also enforces the Labeling of Hazardous Art Materials Act of 1988, which mandates the appropriate labeling of known sensitizers that are present in art materials in sufficient amounts to cause skin sensitization (16 CFR 1500.14). These products should be assigned classifications of either nonsensitizer or sensitizer. The policy on animal testing and alternative methods is the same as described above for the Federal Hazardous Substances Act.

2.3. Industrial Chemicals

2.3.1. Canada—The *Canadian Environmental Protection Act*, 1999 (Minister of Justice Canada [2017]- a), was introduced to help prevent pollution and to control toxic substances by providing a regulatory framework for chemical risk assessment. This legislation requires numerous activities not covered by other legislation, including screening of all chemicals on the Canadian Domestic Substances List that were in commerce between 1984 and 1986, just prior to the original *Canadian Environmental Protection Act* legislation of 1988. These substances are referred to as “existing substances.” The 1999 legislation also requires the screening of all chemicals that were not on the Domestic Substances List, which are considered new to Canada. This legislation covers substances also found in cosmetics and in products available to consumers including household products and art materials.

The initial categorization of the substances on the Domestic Substances List, which did not specifically include skin sensitization, identified priority chemicals to undergo a screening level risk assessment as part of the Chemicals Management Plan. Under the Chemicals Management Plan, the Existing Substances Risk Assessment Bureau (ESRAB) within the Healthy Environments and Consumer Safety Branch of Health Canada co-leads risk assessments of substances on the Domestic Substances List alongside Environment and Climate Change Canada. The assessments are consistent with internationally accepted guidelines set forth by the World Health Organization and OECD. ESRAB uses published literature as the primary source of data. The LLNA, GPMT, and, in some cases, Buehler tests (OECD, 1992; 2010a) are generally considered preferred methods. Additional skin sensitization testing is not required since ESRAB does not perform premarket assessments; however, data from non-animal alternative methods may be submitted to ESRAB on a voluntary basis.

The skin sensitization potential of chemicals or polymers not on the Domestic Substances List is assessed by the New Substances Assessment and Control Bureau (NSACB) within the Healthy Environments and Consumer Safety Branch of Health Canada under the *New Substances Notification Regulations* issued in 2005. Import or manufacture of substances above a certain volume requires notifiers to provide additional information that includes skin sensitization data. All testing must be compliant with Good Laboratory Practices and OECD test guidelines. The preferred skin sensitization method is the traditional LLNA (OECD, 2010a); however, the GPMT and Buehler guinea pig methods (OECD, 1992) and LLNA methods that do not require radiolabeled probes are also considered acceptable (OECD, 2010b; c). Properly documented human patch tests yielding positive or negative responses may be acceptable alternatives to animal testing, and NSACB occasionally receives human repeat insult patch test data (Politano and Api, 2008) that can be used as part of a weight-of-

evidence approach. Anecdotal information from persons exposed to the substance is not an acceptable alternative (Government of Canada, 2006). NSACB has not yet received any skin sensitization data from *in silico* or *in vitro* alternative methods, but will consider them on a case-by-case basis provided a quantitative risk assessment can be performed using the data. The potency of a substance is considered in determining skin sensitization potential, and the EC3 value (the estimated concentration expected to produce a stimulation index of three, the threshold value for a positive response) obtained from an LLNA is currently considered the most reliable method of potency determination.

2.3.2. China—The Chinese Ministry of Health regulates synthetic and natural extracts of chemicals intended for industrial or domestic use exclusive of substances covered under statutes that regulate drugs, cosmetics, food additives, or other specific types of substances. The guidances “Management Standards for Toxicity Identification of Chemicals” (MOH No. 69/2015) and “Regulations of Safety Management of Dangerous Chemicals (Chinese State Council No. 591/2011) require a human hazard evaluation to determine occupational risk. “Technical Standards for Toxicity Identification of Chemicals” (MOH No. 272/2005) provides the associated technical requirements, which include skin sensitization testing for chemicals with a high likelihood of repeated dermal exposure. The Buehler test and GPMT are the accepted test methods, and the LLNA is an optional method; however, the testing requirement is waived for chemicals known to cause strong skin irritation or corrosion.

New chemicals are regulated by the Ministry of Environmental Protection under Environment Management Measures for New Chemical Substances (No. 7/2010). This regulation, which is comparable to the European Union’s REACH regulation, provides the environmental management requirements for new chemicals imported or used in research, production, and processing in quantities of one ton or more per year. Hazard classification and warning label information must be submitted to satisfy the information requirements for registration. The Buehler test and GPMT are the standard protocols for deriving skin sensitization data; the LLNA is an optional method.

Appropriate labeling of hazardous chemicals is mandatory in China. According to Part 21 of the Compulsory National Standards for chemical classification (GB 30000-2013), product labeling must indicate when a known skin sensitizer is present in sufficient amounts to elicit a sensitization effect. These standards are aligned with the GHS System. No specific test methods are associated with this standard.

2.3.3. European Union—For the classification of chemicals, the European Union is aligned to the GHS system via the Classification, Labeling, and Packaging (CLP) Regulation No. 1272/2008 (EC, 2008b). Under CLP, manufacturers of industrial chemicals and mixtures must alert customers of potential hazards by ensuring that products are appropriately classified, labeled, and packaged before distribution within the European Union. All materials that are determined to be skin sensitizers are classified into Category 1. Subcategorization into Category 1A or 1B is not mandatory, but the option to subcategorize, when the appropriate data are available, was introduced in the fourth revision of CLP in 2013. Only human or animal data are currently used to subcategorize sensitizers. The

preferred method for new testing is the LLNA; however, the Buehler test or the GPMT are acceptable with accompanying justification if the LLNA is not appropriate.

The CLP Regulation is closely linked with the REACH Regulation (EC) No 1907/2006 (EC, 2006). REACH applies to all chemical substances, including those used in industrial processes and those contained in mixtures and articles (such as books, laptops, toys) supplied to professional users and consumers. REACH establishes requirements for the registration of substances manufactured or imported in the European Union in a quantity of one or more tons per year on their own or in mixtures, and substances that are released from articles in a quantity of one or more tons per year. The classification of a substance according to CLP is a mandatory part of the REACH registration process.

REACH standard information requirements for skin sensitization are detailed in Annex VII of the Regulation (EC, 2006; 2016) and they apply to all tonnage levels. The information required should allow a determination on whether the substance is a skin sensitizer or not. Furthermore, if the substance is assessed to be a skin sensitizer, information is required to enable a conclusion on whether the substance can be presumed to have the potential to produce significant sensitization in humans (GHS Category 1A) and to allow risk assessment where required (EC, 2006; 2016). Information on exposure, use, and risk management measures should also be collected and evaluated in order to ensure that potential risks are identified and adequate risk management measures are taken (ECHA, 2017).

The registrant should begin by gathering and evaluating all existing available information before considering further testing. This includes structural information, physicochemical properties, (quantitative) structure-activity relationships, information from structurally similar substances, *in vitro/in chemico* data, animal studies, and human data (ECHA, 2017).

If the available data are inadequate for hazard classification (or risk assessment, where required), new data need to be generated with *in vitro/in chemico* test method(s) laid down in the Commission Test Methods Regulation (EC, 2008a) or recognized by the Commission or ECHA as being appropriate (e.g., OECD test guidelines). These *in vitro/in chemico* test methods must address each of the following key events of skin sensitization: (1) molecular interaction with skin proteins; (2) inflammatory response in keratinocytes; and (3) activation of dendritic cells. *In vivo* testing may be pursued only if the available *in vitro/in chemico* test methods are not applicable to the substance or if the results obtained from those methods are not adequate for classification and risk assessment. The preferred method of *in vivo* testing is the LLNA, unless another method can be justified. The testing requirements can be waived if the substance is classified as a GHS Category 1 corrosive, if the substance has a pH less than or equal to 2.0 or greater than or equal to 11.5, or if the substance is spontaneously flammable at room temperature. According to the general adaptation rules set out in Annex XI of REACH, waiving the standard testing regime is also possible when other available information is sufficient for classification and risk assessment. Available historical human data or a weight-of-evidence assessment using several independent sources of information could be used to request such a waiver.

2.3.4. Japan—As far as new designations of harmful substances are concerned, information regarding skin sensitization potential is not specifically required. When skin sensitization information is needed, the test method may be selected on a case-by-case basis. The acceptance of non-animal alternative methods is undetermined.

2.3.5. South Korea—The Act on the Registration and Evaluation of Chemicals (Act No. 13891) (MOE, 2016) is similar to the European Union's REACH regulation. Under this Act, the South Korean Ministry of Environment requires skin sensitization testing and a hazard assessment for all chemical substances registered in quantities of one ton or more. OECD test guidelines for the traditional and non-radiolabeled LLNA methods (OECD, 2010a; b; c) have already been accepted in Korea. DPRA (OECD, 2015a) and the ARE-Nrf2 Luciferase Test Method (OECD, 2015b) have been used since 2017 for the safety evaluation of industrial chemicals.

2.3.6. United States—The Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxics evaluates the hazards of industrial chemicals to ensure that they do not pose an unreasonable risk to human health or the environment. These evaluations are carried out under the Toxic Substances Control Act (TSCA) (15 U.S.C. §§2601-2692 [1976]), which applies to any new and existing industrial chemical manufactured or imported into the United States that is not covered by other statutes (e.g., pesticides, cosmetics, etc.). The Frank R. Lautenberg Chemical Safety for the 21st Century Act amended TSCA in 2016 to broaden EPA's authority to require data on poorly characterized chemicals and to require EPA to develop a plan to promote the implementation of scientifically valid methods that reduce, refine, or replace vertebrate animal testing, such as computational approaches, read-across, and *in vitro* testing (EPA, 2016a).

No skin sensitization testing is required under TSCA, but chemical sponsors are required to submit all available toxicology information to EPA prior to manufacture or import of new chemicals or initiating a new use of an existing chemical. If no data are available, EPA performs a read-across evaluation using data for similar substances. If the data are insufficient to conduct a read-across assessment, EPA may request new toxicity data. A tiered-testing approach is required by the amended TSCA; all available information must be evaluated before animal testing is required by EPA (EPA, 2016a). Data generated according to EPA and OECD test guidelines, as well as non-guideline tests, are accepted. EPA currently accepts and uses non-animal approaches for skin sensitization hazard assessments; however, additional information may be required to address this endpoint for quantitative risk assessments.

2.4. Pesticides

2.4.1. Brazil—In Brazil, active ingredients in pesticide products, inert ingredients, or finished products for plant protection are evaluated by three different federal institutions before registration: the Brazilian Health Regulatory Agency (ANVISA), the Ministry of Agriculture, Livestock and Supply, and the Brazilian Institute of Environment and Renewable Natural Resources. ANVISA is responsible for the toxicological analysis of these products, and the Ministry of Agriculture, Livestock and Supply is responsible for

agronomic evaluation and granting registration (Brazil, 1989). Other types of pesticides, such as those used as household products, in urban areas, or in hospitals, are evaluated and registered exclusively by ANVISA and regulated by other legislation (Brazil, 1976).

Skin sensitization testing is one of the mandatory requirements for toxicological evaluation of active ingredients, inert ingredients, and finished products for plant protection in accordance with Public Consultation Act 260 (ANVISA, 2016a). ANVISA's Act 260 establishes criteria for the toxicological evaluation of ingredients and finished products, and recommends the application of validated and internationally recognized alternative methods for regulatory use. All toxicological tests must be performed in accordance with internationally accepted guidelines, such as those issued by OECD or EPA. Public Consultation Act 262 (ANVISA, 2016b) establishes criteria for the toxicological classification according to GHS (UN, 2017). Based on skin sensitization test data, the ingredients and finished products must be classified as nonsensitizers or sensitizers (GHS Category 1) for hazard assessment, classification, and labeling of products to alert handlers. To evaluate the equivalence of new sources of technical ingredients, (quantitative) structure-activity relationships are used to predict skin sensitization. Negative predictions with a sufficient weight of evidence, are considered nonsensitizers, without the need for animal studies.

For registration of microbial pesticides with insecticidal properties, skin sensitization testing is a mandatory requirement under the following regulations: ANVISA's RDC 34 (ANVISA, 2010a) and MERCOSUR Common Market Group n. 18 (MERCOSUR, 2010a). A hazard assessment must be performed to classify finished products as sensitizers or nonsensitizers, but RDC 34 does not specify the toxicological test methods to be used.

Skin sensitization testing is also mandatory for registration of a new active ingredient of an antimicrobial pesticide for general use, according to RDC 14 (ANVISA, 2007) and to MERCOSUR Common Market Group n. 50 (MERCOSUR, 2006), as well as for hospital use, according to regulation RDC 35 (ANVISA, 2010b) and MERCOSUR Common Market Group n. 19 (MERCOSUR, 2010b). Under both RDCs, hazard assessment must classify new active ingredients as either skin sensitizers or nonsensitizers. The GPMT and Buehler test are the methods of choice; alternative methods are accepted in accordance with the specific limitations of each method and their regulatory applications (ANVISA, 2015b).

Normative Resolution 18 of 2014 (CONCEA, 2014) and Normative Resolution 31 of 2016 (CONCEA, 2016) of the National Council for the Control of Animal Experimentation (CONCEA) recognize the traditional and non-radiolabeled LLNA test methods as well as the DPRA and the ARE-Nrf2 Luciferase Test Method for skin sensitization assessment (OECD, 2010a; b; c; 2015a; b). Normative Resolutions 18 and 31 state that the specific applications of each method as described in the OECD test guidelines must be respected.

RDC 35 (ANVISA, 2015b) accepts the regulatory application of the alternative methods previously recognized by CONCEA. In this context, all ANVISA's regulations that require animal testing for marketing authorization should accept validated and internationally recognized alternative methods.

2.4.2. Canada—The Pest Management Regulatory Agency (PMRA) of Health Canada is responsible for ensuring adherence to the *Pest Control Products Act*, which provides for the regulation of pest control products. Under this act, *Pest Control Product Regulations* makes provisions for pesticide registration and labeling (Minister of Justice Canada [2017]-e). Skin sensitization data are required for active ingredients and for finished products (Health Canada, 2016a).

The traditional LLNA (OECD, 2010a) is the preferred method for animal assays, but the GPMT and Buehler test (OECD, 1992) are also acceptable (Health Canada, 2016a). Alternative non-animal assays conducted according to OECD test guidelines are acceptable for submission to address dermal sensitization. Note that multiple alternative assays may be necessary to satisfy the data requirement. No single assay or combination of assays is preferred at this time. Information submitted from non-guideline studies are assessed on a case-by-case basis. Data are not necessary for substances that are corrosive to skin or have a pH less than 2.0 or greater than 11.5; however, testing of the finished product may be requested if the conditions of use call for dilution. Data must be sufficient to classify products as sensitizers or nonsensitizers. Quantitative risk assessment methods are being developed to use potency information from threshold doses for sensitization induction or elicitation. Such risk assessments would be useful for unlabeled products that contain a sensitizing chemical for which exposures could potentially be high (e.g., material preservatives).

It should be noted that a dermal sensitization study may not be required when the criteria provided in the PMRA guidance for waiving or bridging of mammalian acute toxicity tests for pesticides are met (Health Canada, 2013). The PMRA also recognizes the criteria as established in the equivalent OECD technical guidance (OECD, 2016a).

2.4.3. China—Regulations on Pesticide Administration (Chinese State Council, Decree No. 326/2001) and Rules for the Implementation Methods of Pesticide Management Regulations (Ministry of Agriculture, No. 9/2007) provide the management requirements for pesticide registration in China. These regulations apply to new pesticides being manufactured or imported into China.

Toxicological testing is required for these products. The sponsor must submit all available toxicology data for review, including skin sensitization data. The Ministry of Agriculture has implemented “Toxicological Test Methods for Pesticide Registration” (National Standard of the People’s Republic of China, 1995), which specifies the Buehler test as the method of choice. As is the case with cosmetics, the results are used to assign a classification of non-sensitive, weak-, light-, medium-, strong-, or very strong-sensitive. Only the non-sensitive and weak-sensitive products are considered “non-sensitizing” and are acceptable for commercial use.

2.4.4. European Union—In the European Union, pesticidal substances are categorized either as plant protection products, which are used on crops, or biocides, which include disinfectants, preservatives, and non-crop pest control agents. The marketing and use of plant protection products (EC, 2009a; 2013a; b) and biocides (EC, 2012) are regulated by

different pieces of legislation. However, the plant protection product and the biocides regulations each require an assessment of skin sensitization for both the active ingredients and the final product marketed, independent of the tonnage level.

2.4.4.1. Biocidal Products: For biocidal products, Regulation No. 528/2012 (EC, 2012) mandates an assessment of skin sensitization for active ingredients and mixtures. First, all available human, animal, and alternative data are evaluated. If the available data fail to provide sufficient evidence of skin sensitization potential, *in vivo* testing should be considered. The LLNA, including, where appropriate, the reduced variants of the assay, is the method of choice. If another skin sensitization test is used, justification should be provided.

It should be noted that *in vivo* testing is not necessary if the available information can classify the substance as a skin sensitizer or corrosive, or if the substance has a pH less than 2.0 or greater than 11.5. Furthermore, *in vivo* testing is not necessary for mixtures when there is enough available data on each component to allow classification according to Directive 1999/45/EC and Regulation (EC) No. 1272/2008, and when synergistic effects between any of the components are unlikely (EC, 2012).

2.4.4.2. Plant Protection Products: Skin sensitization testing is mandatory unless the active ingredient or co-formulants are known sensitizers or the applicant can justify an alternative approach under the CLP Regulation (EC, 2008b). Under CLP, classification and labeling of mixtures may be possible based on the skin sensitization properties of the components of the mixture. In this case, skin sensitization properties of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the sensitizing potential of the total mixture.

The LLNA is the preferred test method, including where appropriate the reduced variants of the assay, but GPMT or Buehler assays are accepted with justification.

2.4.5. Japan—The Ministry of Agriculture, Forestry, and Fisheries provides the toxicity testing requirements for pesticides in Japan under the Agricultural Chemical Regulation Law (MAFF, 2000). Skin sensitization testing is required for active ingredients and finished products to inform a hazard assessment to classify these substances as sensitizers or nonsensitizers. The preferred tests are the GPMT and the Buehler test, but other methods such as LLNA may be acceptable. The acceptance of non-animal alternative methods is undetermined.

2.4.6. South Korea—The Pesticide Control Act (Act No. 13403) (Ministry of Agriculture Food and Rural Affairs, 2015) gives authority to the Rural Development Administration of the National Institute of Agricultural Sciences to regulate the registration of pesticide products in South Korea. Skin sensitization testing is required for active ingredients and finished products. A hazard assessment should be performed for classification of a product as a sensitizer or nonsensitizer. Guidance is detailed in “Standard for the Registration of Pesticides and Active Substances” (Rural Development Administration, 2016). Acceptable

test methods include the LLNA, GPMT and Buehler test; however, alternative methods may be considered on a case-by-case basis.

2.4.7. United States—The Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. §136 et seq. [1996]), administered by the EPA Office of Pesticide Programs (OPP), is the legislation that supports U.S. pesticide regulation. Pesticide products must be classified as sensitizers or nonsensitizers, and skin sensitization data are required for active ingredients as well as final products. However, data are not required if (1) repeat dermal exposure is not likely to occur under conditions of use; or (2) the test material is corrosive to skin or has a pH less than 2.0 or greater than 11.5 (EPA, 2015a; b).

The OPP guidance for skin sensitization testing is detailed in “OPPTS 870.2600, Health Effects Test Guidelines for Skin Sensitization” (EPA, 2003), which recognizes test guidelines published by the OECD as the standard methods for skin sensitization testing and lists the traditional LLNA as the preferred method. The reduced LLNA, which uses a smaller number of animals, is acceptable when potency assessment is not required (EPA, 2011). In situations where the LLNA is inappropriate for the test article, the GPMT and Buehler tests are acceptable (EPA, 2003; 2016b). In addition to the testing exceptions mentioned above, requests for skin sensitization test waivers may be considered in the following circumstances: (1) the test material is a pesticidal paint that contains strong dyes or pigments, making dermal evaluation impossible; (2) the product design prevents dermal exposure; or (3) the technical active ingredient is a known dermal sensitizer (EPA, 2012). OPP may also accept bridging of data for similar formulations with reduced hazard potential. Though non-animal skin sensitization tests have not yet been formally accepted by OPP, some are currently being considered as alternatives to animal tests.

2.5. Pharmaceuticals

2.5.1. Brazil—The “Guidance for Development of Non-Clinical Studies to Assess Safety Pharmacology and Toxicology of Drugs” (ANVISA, 2013a) recommends a skin sensitization test for hazard assessment for topical pharmaceuticals to classify them as sensitizers or nonsensitizers. Products classified as sensitizers are not registered. The skin sensitization test data should involve humans, in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines, or experimental animals, in agreement with OECD test guidelines. The traditional LLNA is the method of choice, followed by the Buehler test or GPMT. Alternative methods are accepted in accordance with the specific limitations of each method and their regulatory applications (ANVISA, 2015b).

2.5.2. Canada—The Therapeutic Products Directorate, which is part of the Health Products and Food Branch of Health Canada, is the Canadian authority that regulates prescription pharmaceutical drugs and medical devices for human use under the *Food and Drugs Act* (Minister of Justice Canada [2017]-b), the *Food and Drugs Regulations* (Minister of Justice Canada [2017]-c), and the *Medical Devices Regulations* (Minister of Justice Canada [2017]-d). Stand-alone skin sensitization tests are not required prior to clinical trials of prescription pharmaceuticals. Rather, skin sensitization is often incorporated into repeat-

dose toxicity studies using animals, which are required for topical drug products. Guideline M3(R2) of the ICH is followed with respect to nonclinical data requirements to support use in clinical trials (Health Canada, 2016b). The information from the repeat-dose studies is applied in the planning of the clinical trial to assess the acceptability of the proposed dose in humans and the proposed duration of use, to define inclusion and exclusion criteria for trial subjects, and to suggest patient monitoring parameters. The same standard testing methods are applied for pre-market authorization of prescription pharmaceuticals.

For medical devices, skin sensitization tests are recommended for patient contacting devices as outlined in the International Organization for Standardization ISO 10993-10: Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization (ISO, 2010). Sensitization testing typically involves the LLNA and the GPMT. While test protocols are similar to those specified in OECD Test Guideline 406 - Skin Sensitization (OECD 1992), testing on medical devices is usually conducted using the finished product or components and not on specific chemical constituents as is typically done for pharmaceuticals or chemicals. The Therapeutic Products Directorate knows of no validated non-animal alternatives for skin sensitization testing of medical devices; however, these would be acceptable as they become available.

The principal regulatory application of skin sensitization test data for topical non-prescription disinfectant and antiseptic drugs is hazard labeling. These products are regulated by the Natural and Non-prescription Health Products Directorate (NNHPD), part of the Health Products and Food Branch of Health Canada (Health Canada, 2016c). Skin sensitization tests of non-prescription and disinfectant drugs should be conducted according to OECD test guidelines for the GPMT, Buehler test, and LLNA (OECD, 1992; 2010a). The LLNA is the preferred animal method. Skin sensitization studies for disinfectant drugs are acceptable if they were carried out using any of these three methods per OPPTS 870.2600 (EPA, 2003). If skin sensitization hazard information already exists for a formulation similar to that of the disinfectant drug in question, NNHPD may consider reviewing that in lieu of additional testing. Available data should contain information regarding the potential toxicity of both the active and inert ingredients (Health Canada, 2014). Any submissions of data from alternative tests should be accompanied by a justification.

For new antiseptic drugs intended for dermal application, the current data requirements to determine hazard labeling specify skin sensitization testing. It is preferable that these tests be conducted in humans according to the 2009 Human Use Antiseptics Guidance Document (Health Canada, 2009), although no particular human test is specified.

2.5.3. European Union—Within the European Union, requirements and procedures for the marketing authorization for medicinal products for human use are provided primarily in Directive 2001/83/EC of the European Parliament and the Council (EC, 2001). Skin sensitization testing should be conducted in compliance with the European Medicines Agency's Guideline on Non-Clinical Local Tolerance Testing of Medicinal Products (EMA, 2015). Under this guideline, the sensitizing potential of drug products (active substances and excipients) applied to the skin or mucosae should be evaluated in a hazard assessment before administration in humans (EMA, 2015). In the absence of an accepted *in vitro* integrated

testing strategy, the evaluation should be conducted in at least one approved animal model. The selection of the animal model should be appropriate for the physicochemical properties of the test article. For example, hydrophilic compounds and metals or metal salts should be tested in a guinea pig assay.

2.5.4. Japan—The regulation of pharmaceuticals and medical devices in Japan falls under the purview of PMDA. Together with the Ministry of Health, Labour, and Welfare’s Pharmaceutical and Food Safety Bureau, PMDA conducts scientific reviews of marketing authorization applications for pharmaceuticals to monitor their post-market safety. The International Organization for Standardization guidelines are used to establish testing requirements for medical devices (FDA, 2016b). The GPMT, Buehler test, and LLNA are acceptable for skin sensitization testing of medical devices. However, PMDA has not yet accepted alternative non-animal tests for pharmaceuticals or medical devices.

2.5.5. South Korea—The South Korean Pharmaceutical Affairs Act (Act No. 13655) (Ministry of Food and Drug Safety, 2015a) addresses many aspects of pharmaceutical affairs within the country. Under the Ministry of Food and Drug Safety in South Korea, the National Institute of Food and Drug Safety Evaluation oversees hazard and potency assessments for pharmaceuticals intended for human use. These include evaluations of sensitization potential for medicines, quasi-drugs (i.e., non-prescription products with minimal to moderate pharmacologic activity), herbal medicines, and biological products applied to the skin or mucosae.

The “Standard for Toxicity Study of Pharmaceuticals” provides guidance for skin sensitization testing using the GPMT; however, it notes several other acceptable test methods, including Adjuvant and Patch test, Buehler test, Draize test, Freund’s Complete Adjuvant test, Open Epicutaneous test, Optimization test, and Split Adjuvant test (Ministry of Food and Drug Safety, 2015c).

Topical toxicity test data are also required for animal medicines that could accidentally or intentionally come into contact with human skin or mucosae. These test requirements are mandated by the Ministry of Agriculture, Food and Rural Affairs (animal medicine) or the Ministry of Oceans and Fisheries (aquatic animal drugs). A hazard assessment should be executed for classification of these substances as well.

2.5.6. United States—The Federal Food, Drug, and Cosmetic Act (21 U.S.C. §301 et seq. [1938]) authorized FDA to oversee the safety of new drugs, drugs intended for a new use or route, and medical devices. Pharmaceutical companies seeking to market a drug in the United States must provide FDA with evidence that the drug is reasonably safe for human use. FDA accepts any test for local toxicity as well as information on all metabolites in animals and in humans, associated pharmacokinetics for all *in vivo* doses, and information regarding impurities.

FDA’s Center for Drug Evaluation and Research is responsible for reviewing the submitted evidence for pharmaceuticals (FDA, 2014). For topically applied drugs, both local and systemic toxicity are of interest. FDA prefers that submissions include skin sensitization

screening tests, which may use any scientifically valid and predictive approach including animal or non-animal assays, to support pivotal studies. Pivotal studies to assess skin sensitization for topically applied drugs test the clinical formulation in human subjects and are used to make regulatory decisions. Potency and incidence data are preferred. No specific skin sensitization test method is recommended for screening; however, most screening information on local toxicity can be obtained from repeat-dose toxicity studies in animals.

FDA's Center for Devices and Radiological Health is responsible for reviewing data that applies to medical devices and radiation-emitting products (FDA, 2017). Assessment of skin sensitization potential of medical devices that may come into contact with tissue is recommended (FDA, 2016b). Chemical characterization and risk assessment tools can be used to determine whether known sensitizers contained in medical devices may cause skin sensitization during clinical use. If testing is necessary, FDA recommends use of the GPMT. The Buehler test is recommended only for topical medical devices. Data from the traditional and non-radiolabeled LLNA (OECD, 2010a; b; c) can be accepted in some cases but not for new materials or devices that contact deep tissues or broken skin surfaces. FDA does not currently accept data from alternative methods because FDA is not aware of any validated non-animal method for mixtures of potential skin sensitizers that are commonly found in medical devices. Clinical studies are recommended for labels claiming reduced potential for sensitizing users to rubber chemical additives or reduced potential for causing a reaction in users already sensitized (FDA, 2008).

2.6. Workplace Chemicals

Regulatory approaches to “workplace chemicals” are discussed in this section. “Workplace chemicals” refers to products containing chemical substances or mixtures that workers may be exposed to in their place of employment, regardless of the type of product it is. Products may contain, for example, pharmaceutical ingredients, industrial chemicals, or cosmetics ingredients. The sale and use of workplace products are subject to regulatory measures for the protection of the health and safety of workers. These measures include chemical classification, labeling, and chemical handling and information requirements.

2.6.1. Canada—The Workplace Hazardous Materials Bureau of Health Canada enforces the *Hazardous Products Act* as it applies to chemicals in the workplace (Minister of Justice Canada [2016]-b). Enabled by this act, Part 8.4 of the *Hazardous Products Regulations* entails classification of substances or mixtures as skin sensitizers based on human or animal data from scientifically validated methods. Skin sensitizers should be classified into the GHS potency subcategories if data are sufficient (Minister of Justice Canada [2015]). Product testing is not a regulatory requirement; data needed for classification is usually taken from peer-reviewed public literature. Acceptable sources of human data are patch testing, epidemiological studies, and experimental studies. Accepted animal test methods include the LLNA, GPMT, and Buehler test (OECD, 1992; 2010a). Non-animal data are not accepted as per current *Hazardous Products Regulations*; however, these may be revised in the future to allow for consideration of such data.

2.6.2. China

China does not have any mandatory statutes or regulations that specifically address workplace chemicals; however, Parts 7 and 30 of the Ministry of Health's "Procedures and Test Methods for Toxicological Evaluation of Chemicals" both provide guidance on skin sensitization test methods. This publication provides the National Occupational Health Standard method for the allergic reactions of chemicals and recommends the Buehler, GPMT, and/or LLNA assays be utilized in safety evaluations of workplace chemicals.

2.6.3. Japan—As far as new designations of harmful substances are concerned, information regarding skin sensitization potential is not specifically required. When skin sensitization information is needed, the test method may be selected on a case-by-case basis. Acceptance of non-animal alternative methods is undetermined.

2.6.4. South Korea—In accordance with the Enforcement Rule of the Occupational Safety and Health Act (Act No. 13906) (Ministry of Employment and Labor, 2016), substances that cause allergic reactions when coming into contact with the skin are classified as hazards to human health and the environment. Hazard and risk assessment are required for chemicals in the workplace. When skin sensitization information is needed, the test method may be selected on a case-by-case basis. Acceptance of non-animal alternative methods is undetermined.

2.6.5. United States—The Occupational Safety and Health Act (29 U.S.C. §651 et seq. [1970]) addresses the safety of chemicals in the workplace. The Occupational Safety and Health Administration requires that chemical manufacturers, importers, distributors, and employers using hazardous chemicals perform a hazard assessment, supply safety data sheets, and apply appropriate product labeling aligned with the GHS. Skin sensitization information is needed, but testing is not required. Data needs are not limited to data derived from animal studies; non-standard methods and information for structurally similar analogs may be considered as part of a weight-of-evidence approach for classification on a case-by-case basis.

Sensitizers should be classified into GHS potency categories, if data are sufficient. In general, high-quality human data are preferable for classification purposes. In some cases, however, well-designed animal studies are preferred to human patch test studies, because human patch test studies are typically not controlled experiments but are more often performed to confirm the absence of sensitization in animal studies. The quality and reliability of each dataset should be considered on a case-by-case basis. The LLNA, Buehler test, and GPMT are the suggested methods for *in vivo* assays when testing is necessary.

3. Discussion

The appropriate management of exposures to chemical skin allergens is necessary to minimize the occurrence of allergic contact dermatitis. Regulatory requirements for skin sensitization testing support efforts to protect public health by ensuring that skin sensitization data are available for chemical substance classification, warning label information, and potency evaluations for risk assessment. However, regulatory requirements

for skin sensitization data vary across chemical sectors and across countries and political regions.

This survey of specific regulatory needs and uses for skin sensitization information for seven countries and regions highlights the difficulty in obtaining unambiguous information on whether regulatory agencies use skin sensitization data and whether the need is for hazard or potency information or risk assessment. ICATM partners or participants, who are experts in chemical regulatory matters, collected this information from the regulatory authorities in their respective regions. Table 1 provides a summary of the regulatory requirements for hazard evaluation, potency classification, and risk assessment for the countries and regions reviewed by chemical sector. The chemical sectors listed for each country and region are not the same. This is due either to a lack of information on each chemical sector or due to regional differences in the manner in which chemicals are regulated. Some regulated sectors are included in another chemical sector. For example, household substances and workplace chemicals are covered under the industrial chemical legislation in the European Union but are separate chemical sectors in the United States. These cases are noted in the table.

The chemical sectors that were best documented regarding needs for hazard classification, potency, or risk assessment were pesticides and cosmetics. Needs across the countries and regions were the most uniform for pesticides. Six of the seven countries or regions require hazard information only. Skin sensitization data requirements for pharmaceuticals in Japan and for workplace chemicals in China were not specifically described as being used for hazard, potency, or risk assessment; these are indicated as “not specified” for the endpoint in Table 1. No skin sensitization testing or data are required for cosmetics in the United States or for household substances, industrial chemicals, or workplace chemicals in Japan.

Acceptance of test methods to fulfill regulatory requirements is affected by the OECD Mutual Acceptance of Data agreement. This states that data generated using OECD test guidelines in any OECD member country must be accepted for review in other OECD member countries (CPSC, 2012). However, the other member countries are not required to accept such data as fulfillment of regulatory requirements; they may require additional data to satisfy the requirement. In general, where such data are accepted, non-animal alternative method data generated using OECD test guidelines (or equivalent) and non-guideline data (e.g., scientific literature, mode of action data) are considered with all available data using a weight-of-evidence approach. Many countries in the EU are also members of the OECD. Of the countries included in this review, Canada, Japan, South Korea, and the United States are members, but Brazil and China are not.

Most regulatory authorities among the countries and regions reviewed prefer or suggest animal data and some require animal data (Table 1) to fulfill regulatory requirements for skin sensitization testing. Only animal test data are accepted by Chinese regulatory authorities for all chemical sectors reviewed. Japanese regulatory authorities accept only animal data for two chemical sectors: pharmaceuticals and medical devices. Other countries and regions accept only animal data for fewer sectors. The preferred animal methods in most cases are the LLNA, GPMT, and Buehler test when conducted according to accepted test guidelines, such as those of OECD or equivalents. Cosmetics and personal care products is

the only sector for which animal tests have been banned in some countries or regions (i.e., the European Union and South Korea). Where such data are banned, animal data are acceptable only if the tests were performed to meet other regulatory requirements.

Although the majority of these national and regional regulatory authorities prefer or suggest animal data to assess skin sensitization potential, many are flexible in their consideration and acceptance of non-animal alternative methods (Table 1). European Union authorities accept non-animal alternatives for all of the chemical sectors reviewed except pesticides and plant protection products. In fact, REACH legislation, which applies to all chemicals in commerce within the European Union, requires non-animal alternative tests for skin sensitization to be used before animal testing is considered (ECHA, 2017). In Canada, regulatory authorities accept non-animal alternatives for all chemical sectors except workplace chemicals (as per current regulations). Brazil accepts alternative methods as long as they have regulatory application in accordance with RDC 35 of 2015. Regulatory authorities for all sectors except medical devices in the United States accept non-animal methods on a case-by-case basis with justification. China is the only country or region that does not accept alternatives for any of the chemical sectors reviewed.

While a number of regulatory authorities accept non-animal alternatives for skin sensitization, few provide a list of acceptable alternative methods (Table 1). Clarity on the specific non-animal methods accepted by regulatory authorities is needed to improve the implementation of these methods. The skin sensitization requirements for REACH are more advanced in this respect (ECHA, 2017). If existing data are inadequate to determine skin sensitization potential and to assess whether the chemical can be presumed to have the potential to produce significant sensitization in humans (GHS Category 1A), then additional information should be generated using *in vitro* methods and the animal test considered only as a very last resort. Although validated and internationally accepted methods such as the DPRA, ARE-Nrf2 Luciferase Test Method, and Human Cell Line Activation Test are preferred, other validated non-animal methods that are not yet described in OECD test guidelines may also be acceptable when used in a weight-of-evidence approach together with other relevant information and in accordance to the provisions of REACH Annex XI. South Korean authorities are also specific about the non-animal test methods that are acceptable for skin sensitization assessments of cosmetic ingredients (DPRA, ARE-Nrf2 Luciferase Test Method, and Human Cell Line Activation Test) and industrial chemicals (DPRA and ARE-Nrf2 Luciferase Test Method) (Table 1). In addition, Japanese authorities began accepting DPRA, ARE-Nrf2 Luciferase Test Method and Human Cell Line Activation Test to identify non-sensitizing ingredients for cosmetics and quasi-drugs in January 2018.

ICATM partners and participants are working with regulatory agencies to evaluate non-animal alternatives for skin sensitization testing and identify those with acceptable performance. The *ICATM Workshop on the International Regulatory Applicability and Acceptance of Alternative Non-animal Approaches to Skin Sensitization Assessment of Chemicals Used in a Variety of Sectors* was a milestone in this effort. As a result of the workshop, ICATM partners proposed the development of a performance-based test guideline for non-animal skin sensitization test methods to the OECD Test Guidelines Programme (Casati et al., 2018). In April 2017, the project was accepted for inclusion into the Test

Guidelines Programme work plan. The proposed test guideline will describe performance criteria for application to new non-animal methods, based on the reproducibility and predictivity of the most widely used animal method, the LLNA. This effort will provide specific integrated strategies and stand-alone methods that meet the criteria to be accepted as replacements to the animal tests. This activity with OECD is important for the acceptance of such methods because OECD's internationally adopted test guidelines are viewed by the regulatory community as reliable standards for the conduct of chemical testing and are covered by Mutual Acceptance of Data.

Another goal set by ICATM as a follow-up to the workshop was development of this manuscript for the purpose of documenting skin sensitization data needs and testing requirements for regulatory authorities relevant to the countries and regions represented by ICATM partners. An understanding of national and regional regulatory requirements for skin sensitization testing is critical to achieve ICATM's goal of acceptance and implementation of non-animal alternatives for meeting regulatory testing requirements. We expect that this information will be used to align the skin sensitization data needs for the various chemical sectors and regulatory authorities with acceptable non-animal test methods from the OECD test guidelines effort. Regulatory authorities can then provide clear information on the acceptable non-animal methods or approaches that are appropriate for their respective applications of hazard assessment, potency classification, or risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANVISA	Brazilian Health Regulatory Agency
CCPSA	<i>Canada Consumer Product Safety Act</i>
CLP	Classification, Labelling, and Packaging
CONCEA	National Council for the Control of Animal Experimentation (Brazil)
CPSC	Consumer Product Safety Commission (U.S.)
DPRA	direct peptide reactivity assay
EPA	Environmental Protection Agency (U.S.)
ESRAB	Existing Substances Risk Assessment Bureau (Canada)

EU	European Union
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FDA	Food and Drug Administration (U.S.)
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GPMT	guinea pig maximization test
ICATM	International Cooperation on Alternative Test Methods
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LLNA	murine local lymph node assay
NNHPD	Natural and Non-prescription Health Products Directorate (Canada)
NSACB	New Substances Assessment and Control Bureau (Canada)
OECD	Organisation for Economic Co-operation and Development
OPP	EPA Office of Pesticide Programs
PMDA	Pharmaceuticals and Medical Devices Agency (Japan)
PMRA	Pest Management Regulatory Agency
RDC	Collegiate Board of Directors Resolution (ANVISA)
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals (EU)
TSCA	Toxic Substances Control Act (U.S.)

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Highlights

- We reviewed regulatory requirements for skin sensitization testing, by chemical sector, of seven countries or regions.
- This review summarizes data needs for hazard classification, potency classification, and risk assessment.
- We identify preferred test methods and note whether non-animal alternative test methods are acceptable.
- This effort will inform an international strategy for implementing non-animal approaches for skin sensitization assessment.

Table 1

Regulatory Skin Sensitization Endpoints and Test Methods Accepted for Regulatory Use as of 2017

Region/country	Chemical Sector	Endpoint ^a	Accepted <i>In Vivo</i> Methods ^b	Non-animal Alternative Accepted?
Brazil	Cosmetics and Personal Care Products	Hazard	Clinical studies ^c LLNA ^c and its modifications Buehler GPMT	Yes, as part of an integrated strategy
	Pesticides and Plant Protection Products	Hazard	LLNA ^c and its modifications Buehler GPMT	Yes, as part of an integrated strategy
	Pharmaceuticals	Hazard	Clinical studies ^c LLNA ^c and its modifications Buehler GPMT	Yes, as part of an integrated strategy
Canada	Cosmetics	Risk ^d	Data submitted upon request only. Results from open literature are acceptable (e.g., LLNA, GPMT, Buehler)	Yes
	Household Products and Art Materials	Risk ^d	LLNA (preferred if risk assessment is needed) GPMT Buehler	Yes, if animal data are unavailable
	Industrial Chemicals (on Domestic Substances List)	Potency, risk	Data submission not required; Health Canada uses data from accepted methods (LLNA, GPMT, Buehler)	Yes (in voluntary submission)
	Chemicals Not Listed on Domestic Substances List	Potency, risk	LLNA ^c GPMT Buehler LLNA methods that do not require radiolabeled probes	Considered on a case-by-case basis
	Medical Devices	Hazard	GPMT LLNA	Yes, if validated
	Pesticides	Hazard ^e	LLNA ^c GPMT Buehler	Yes, as part of an integrated strategy
	Prescription Pharmaceuticals	Hazard, risk	Not specified	Yes, with justification
	Topical Nonprescription Pharmaceuticals	Hazard	LLNA ^c GPMT Buehler Clinical studies (e.g., human repeat insult patch test) are preferred for antiseptic drugs	Yes, with justification
	Workplace Chemicals	Hazard, potency	New testing not required; data used must be from humans or accepted animal method (e.g., LLNA, GPMT, Buehler)	No ^f
	China	Cosmetics	Potency	Buehler ^c GPMT ^c

Region/country	Chemical Sector	Endpoint ^a	Accepted <i>In Vivo</i> Methods ^b	Non-animal Alternative Accepted?
European Union			Clinical studies LLNA (optional)	
	Industrial Chemicals	Hazard, potency, risk	Buehler ^c GPMT ^c LLNA (optional)	No
	Pesticides	Potency	Buehler	No
	Workplace Chemicals	Not specified	Buehler GPMT LLNA	No
	Biocides	Hazard	LLNA ^c (only required if available data are insufficient) Buehler GPMT	Yes
	Cosmetics	Hazard, potency, risk	Banned (only existing animal data or data generated for other regulatory purposes are accepted)	Yes
	Household Chemicals ^g	Hazard, potency, risk	LLNA ^c Buehler GPMT	Yes; OECD TGs 442C, D, and E are preferred before <i>in vivo</i> testing ^h
	Industrial Chemicals	Hazard, potency, risk	LLNA ^c Buehler GPMT	Yes; OECD TGs 442C, D, and E are preferred before <i>in vivo</i> testing ^h
	Pharmaceuticals	Hazard	Not specified (method should be justified)	Yes
	Plant Protection Products (i.e. Pesticides)	Hazard	LLNA ^c , including the reduced LLNA Buehler GPMT	No
Japan	Workplace Chemicals ^g	Hazard, potency, risk	LLNA ^c Buehler GPMT	Yes; OECD TGs 442C, D, and E are preferred before <i>in vivo</i> testing ^h
	Cosmetics and Personal Care Products	Hazard, potency	GPMT Buehler LLNA	Yes; OECD TG 442C, D and E ^h in an integrated strategy (as of January 2018)
	Household Products	Not required	Selected on a case-by-case basis	Undetermined
	Industrial Chemicals	Not required	Selected on a case-by-case basis	Undetermined
	Medical Devices	Hazard, potency, risk	GPMT Buehler LLNA	No
	Pesticides	Hazard	GPMT ^c Buehler ^c LLNA	Undetermined
	Pharmaceuticals	Not specified	Not specified	No
	Workplace Chemicals	Not required	Selected on a case-by-case basis	Undetermined
South Korea	Cosmetics	Hazard, risk	Banned (unless justified)	Yes; OECD TG 442C, D, and E ^h

Region/country	Chemical Sector	Endpoint ^a	Accepted <i>In Vivo</i> Methods ^b	Non-animal Alternative Accepted?
United States	Industrial Chemicals	Hazard, risk	GPMT Buehler Other justified methods	Yes; OECD TG 442C and D ^h
	Pesticides	Hazard	LLNA GPMT Buehler Other justified methods	Considered on a case-by-case basis
	Pharmaceuticals	Hazard, potency	GPMT ^c Adjuvant and Patch test Buehler Draize Freund's Complete Adjuvant test Open Epicutaneous test Optimization test Split Adjuvant test	No
	Workplace Chemicals	Hazard, risk	Selected on a case-by-case basis	Undetermined
	Cosmetics	Not required	Not applicable	Not applicable
	Household Products and Art Materials	Hazard, potency	LLNA and its modifications GPMT Buehler	Considered on a case-by-case basis
	Industrial Chemicals	Hazard, risk	Testing not required	Considered on a case-by-case basis
	Medical Devices	Hazard	GPMT ^c Buehler test (topical devices only) LLNA (case-by-case)	No
	Pesticides	Hazard ^e	LLNA ^c Reduced LLNA GPMT Buehler	Waivers only
	Pharmaceuticals	Potency ⁱ	Not specified	As a screen on a case-by-case basis
Workplace Chemicals	Hazard, potency	LLNA GPMT Buehler	Yes, if scientifically validated	

^aRefers to hazard assessment, potency categorization, or risk assessment

^bOECD test guidelines (or equivalent)

^cPreferred assays

^dSkin sensitization data are typically not required, but risk assessment may be performed for ingredients of concern

^eWhile not a regulatory requirement, a quantitative risk assessment may be conducted on a case-by-case basis

^fThe *Hazardous Products Regulation* may be revised in the future to allow for the consideration of non-animal data

^gThese sectors are covered by REACH, the industrial chemical legislation in Europe

^hOECD Test Guidelines 442C, D, and E include the DPRA, ARE-Nrf2 Luciferase Test Method, and Human Cell Line Activation Test, respectively

ⁱFDA prefers potency information from human tests