ChemSkin Reference Chemical Database for the Development of an In Vitro Skin Irritation Test

Juhee Han 1,†, Ga-Young Lee 1,†, Green Bae 1, Mi-Jeong Kang 2,* and Kyung-Min Lim 1,∗

Abstract: Since the animal test ban on cosmetics in the EU in 2013, alternative in vitro safety tests have been actively researched to replace in vivo animal tests. For the development and evaluation of a new test method, reference chemicals with quality in vivo data are essential to assess the predictive capacity and applicability domain. Here, we compiled a reference chemical database (ChemSkin DB) for the development and evaluation of new in vitro skin irritation tests. The first candidates were selected from 317 chemicals (source data \( n = 1567 \)) searched from the literature from the last 20 years, including previous validation study reports, ECETOC, and published papers. Chemicals showing inconsistent classification or those that were commercially unavailable, difficult or dangerous to handle, prohibitively expensive, or without quality in vivo or in vitro data were removed, leaving a total of 100 chemicals. Supporting references, in vivo Draize scores, UN GHS/EU CLP classifications and commercial sources were compiled. Test results produced by the approved methods of OECD Test No. 439 were included and compared using the classification table, scatter plot, and Pearson correlation analysis to identify the false predictions and differences between in vitro skin irritation tests. These results may provide an insight into the future development of new in vitro skin irritation tests.

Keywords: skin irritation test; reference chemical; alternative test method; chemical database

1. Introduction

Cosmetics and toiletries are the main sources of human exposure to potentially dangerous chemicals among the general public [1]. Therefore, it is essential to evaluate the toxicity of chemicals used in these products before product release into market. Most of the toxicity items required for the safety evaluation of chemicals use experimental animals, but more than 35 countries have banned animal tests for cosmetics since 2013 [2,3]. Various alternatives have been developed to replace animal testing for cosmetics, including tests in nonvertebrate animals and in vitro, and in chemico and in silico methods [4–6]. For the regulatory body to accept the data produced from methods other than the standard test method, the methods should be verified in their ability to address the toxic endpoint with relevance and reliability to the same extent as the existing standard test method through officially endorsed procedures. This validation procedure is clearly described in OECD Guidance Document 34 [7].

Relevance, also called predictive capacity, is evaluated by comparing the concordance of the prediction for reference chemicals made by the new test method with that from the existing standard test method, generally an in vivo animal test [8,9]. Reference chemicals with quality in vivo data are, therefore, important for evaluating the relevance of new test methods [10]. A reference chemical database for in vivo eye irritation test sensitization has been well established [11]. However, to the best of our knowledge, a reference chemical
database for the skin irritation test with in vivo and in vitro evidence of sufficient quality is lacking.

The in vivo skin irritation test has been the primary test to replace, since rabbits, the test subject animal, experience an enormous level of pain and discomfort from the confinement and hair-shaving [12]. The Draize skin irritation test (OECD TG 404) [13] was developed by John Draize in 1944 [14]. In this test, the dorsal hair is shaved, and on the following day a test chemical (0.5 g solid or 0.5 mL liquid) is applied on a small area (~6 cm²); the treated site is covered with a patch to prevent the animal from licking off the test chemical. The patch is removed after 4 h and skin reactions are scored for signs of erythema and edema at 1, 24, 48, and 72 h. Erythema and edema are scored with grades from 0 to 4 depending on the severity. Based on the severity and reversibility, skin corrosion and irritation are categorized into Category 1, with sub-categories of 1A, 1B, and 1C (corrosive, mean scores above 4.0); Category 2 (irritant, mean scores of 2.3~4.0 for erythema or for edema in at least 2 of 3 tested animals); Category 3 (mild irritant, mean scores of 1.5~2.3 for erythema or for edema in at least 2 of 3 tested animals); or No category (below 1.5) according to the UN GHS classification. There have been multiple classification standards for skin irritation, such as the four levels of irritancy classification on primary irritation index (PII), EU Dangerous Substances Directive/Dangerous Preparations Directive (EU DSD/DPD), and EU Classification, Labelling, and Packaging Regulation (EU CLP) systems. Importantly, these classification systems are not compatible with UN GHS classification (Figure 1), which leads to uncertainty on the irritancy decision of some borderline chemicals. Therefore, it is necessary to provide both in vivo Draize scores and corresponding UN GHS classifications for reference chemicals [15–17].

The OECD Test No. 439: In vitro Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method [18] was developed to identify No category chemicals from other chemicals in accordance with the UN GHS classification. The OECD test guideline (TG) 439 provides an in vitro procedure using RhE to predict the skin hazard of irritant chemicals (substances and mixtures) based solely on the cell viability value obtained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Three validated reference methods (VRMs), the EpiDerm™ skin irritation test (SIT), EpiSkin™ SIT, and SkinEthic RhE SIT, were originally approved for OECD TG 439 in 2010. Following the performance standards of TG 439, four me-too models were additionally approved for OECD TG 439 in 2021 [19] and new models like USP-RhE [20] are under development for inclusion in TG 439. However, TG 439 has several limitations. TG 439 does not classify chemicals to the optional UN GHS Category 3 (mild irritants). In addition, TG 439 RhE test methods cannot resolve between UN GHS Categories 1 and 2; thus, further information on skin corrosion is required to decide on the final classification of certain chemicals. To resolve these limitations, inclusion of an IL-1α assay was considered [21,22], but it failed to
exceed the result of MTT assay [23]. Therefore, a novel skin irritation test method is still in demand to overcome the limitations of the current OECD TG 439.

One of the major problems for TG 439 is that incongruent sets of reference chemicals have been used to evaluate the predictive capacity of the individual RhE methods in TG 439, suggesting a need to establish a well-characterized reference chemical database for the evaluation of novel skin irritation test methods [24] that will overcome the current TG 439. Here, we compiled a reference chemical database (ChemSkin DB) for the development and evaluation of alternative methods for in vivo skin irritation test. The first candidates were selected from 317 chemicals (source data \( n = 1567 \)) searched from the literature for the last 20 years, including previous validation study reports, ECETOC, and published papers. Chemicals without GHS category information or only with the information on past EU classification criteria were removed. In addition, chemicals showing inconsistent classification results and those without both in vivo and in vitro test results were excluded, leaving a total of 100 chemicals. Supporting references, in vivo Draize scores, UN GHS/EU CLP classifications, and commercial sources in the manuscripts were compiled. Furthermore, test results produced by OECD TG 439 (RhE Test Method) were included if available, to provide an insight into the future development of alternatives to the in vivo skin irritation test.

2. Materials and Methods

2.1. Classification Criteria for the Skin Irritancy of Chemicals

2.1.1. Classification Based on Primary Irritation Index (PII)

As described in OECD TG 404 [13], the in vivo skin irritation test (Draize test) is conducted and erythema and edema scores at 24- and 72-h post-exposure (or 24, 48, and 72 h, if available) are combined and averaged into a PII. According to PII, the skin irritancy of a test chemical is classified as described in Table 1 [25].

| Classification     | Primary Irritation Index (PII) |
|--------------------|--------------------------------|
| Negligible         | 0–0.4                          |
| Slight Irritation  | 0.5–1.9                        |
| Moderate irritation| 2–4.9                          |
| Severe irritation  | 5–8                            |

2.1.2. Classification Based on EU DSD/DPD and EU CLP

According to the EU DSD/DPD criteria, the skin irritancy of a test chemical is classified as follows: an in vivo score of 2.0 or higher is classified as R38 (irritant) and a score less than 2.0 is classified as No label (non-irritant).

As a replacement for the EU DSD/DPD regulations, the CLP regulation was created as a new system that reflects the UN GHS classification and labeling regulation. This is referred to as the EU CLP or UN GHS/CLP, which is different from the current UN GHS. Although the CLP regulation was defined in 2008 as Regulation (EC) No. 1272/2008 [16], it has been used interchangeably with the classification criteria of the previous EU DSD/DPD [15]. Chemical classification and labeling systems have been completely replaced. According to this UN GHS/CLP classification, the skin irritancy of a test chemical is classified as follows: scores < 2.3 indicate No category (non-irritant) and scores > 2.3 indicate Category 2 (irritant).

2.1.3. Classification Based on the Current UN GHS

In the revised version of the UN GHS 2019 [17], the skin irritancy of a test chemical is classified as follows: No category (non-irritant) in cases of <1.5, Category 3 (mild irritant) in cases of \( \geq 1.5 \) and <2.3, and Category 2 (irritant) in cases of \( \geq 2.3 \) and \( \leq 4.0 \). Category 3 is newly adopted but only a few authorities employ it. OECD TG 439 defines Category 3 as a non-classified chemical [19].
2.2. Classification of Edema/Erythema of 100 Reference Chemicals Based on the In Vivo Draize Test

The in vivo scores of 100 reference chemicals of the ChemSkin DB were obtained through the literature search. ChemSkin DB chemicals were newly classified according to the in vivo classes and in vivo categories described above.

2.3. Comparison of In Vivo and In Vitro Data of ChemSkin DB Reference Chemicals

In vivo scores and in vitro viability values obtained from OECD TG 439 were compared for ChemSkin DB reference chemicals. The skin irritancy was identified based on in vivo scores of 2.3 or higher or with a viability cut-off of 50%. The in vivo score was compared with the viability data of VRMs and the KeraSkin™ model. The VRMs used in the analysis are EpiSkin™, SkinEthic™, RhE, EpiDerm™, and LabCyte-EPI. The results of the test methods were plotted as a scatter plot to show the data distribution, and the cut-off values were displayed to easily demonstrate the incorrect values.

The predictive capacity of VRMs and the KeraSkin™ was calculated by indexes of sensitivity, specificity, and accuracy. According to the OECD TG 439 performance standard, a sensitivity of ≥80%, specificity of ≥70%, and accuracy of ≥75% must be satisfied.

The Pearson correlation coefficient was obtained to confirm the correlation between in vivo score and viability. The Pearson correlation coefficient is a measure of the degree of correlation between two variables, and it is one of the most widely used measures of relationships [26,27]. It has a range of values from −1 to +1; the closer the coefficient is to the absolute value of 1, the greater the association between the two variables. Correlation coefficients ≤0.35 are generally considered to represent low or weak correlations, those of 0.36 to 0.67 have a modest or moderate correlation, those of 0.68 to 1.0 indicate a strong or high correlation, and correlation coefficients ≥0.90 reflect a very high correlation [28].

3. Results and Discussion

3.1. Chemical Selection for the Establishment of ChemSkin DB

To establish the ChemSkin DB, the reference chemicals were searched from the literature over the last 20 years and reviewed for the quality of in vivo data and availability of in vivo scores, which is critical for the classification of optional Category 3 as recently stated by UN GHS. In addition to in vivo results, human patch test results and the in vitro results produced by four validated reference methods of OECD 439 were included. The final ChemSkin DB was completed by adding the source literature information or official review reports (SCCS, SCCP, CIR, etc.). Through this procedure, 100 reference chemicals were included (source data n = 1567) in the final version of ChemSkin DB. The composition of 100 chemicals is shown in Table 2 and Figures 2 and 3.
## Table 2. Information of major reference chemicals among substances included in ChemSkin DB.

| Chemical | CAS No. | Physical State | In Vivo Category | In Vivo Score | Human In Vivo Result | In vivo Class | In Vivo Score Data Source References | KeraSkin™ SIT | VRMs | Vendor | EpiSkin | SkinEthic | EpiDerm | LabCyte | VRM References |
|----------|---------|----------------|------------------|---------------|----------------------|--------------|--------------------------------------|---------------|------|---------|---------|-----------|---------|---------|----------------|
| 1-Decanol | 112-30-1 | Liquid | Cat 2 | 2.3 | NC | I | [29–32] | 9.3 | Sigma | 7.3 | 1.4–2.4 | 6.0 | 32.3 | [23,30,33,34] |
| Cyclamen aldehyde | 103-95-7 | Liquid | Cat 2 | 2.3 | - | I | [30–32] | 1.7 | TCI | 24.4 | 1.6–1.8 | 10.5 | 30.1 | [23,30,33–37] |
| 1-Bromohexane | 111-25-1 | Liquid | Cat 2 | 2.7 | I | I | [29–32] | 18.9 | Sigma | 18.4 | 1.1–1.7 | 20.7 | 39.9 | [23,30,33,34] |
| 2-Chloromethyl-3,5-dimethyl-4-methoxypyridine HCl | 86604-75-3 | Solid | Cat 2 | 2.7 | - | I | [30–32] | 3.7 | Sigma | 5.7 | 0.7–0.8 | 6.7 | 12.7 | [23,30,33,34] |
| Di-n-propyl disulphide | 629-19-6 | Liquid | Cat 2 | 3.0 | NC | I | [9,29–32] | 26.8 | Sigma | 52.0 | 1.2–2.4 | 89.3 | 71.7 | [23,30,33,34] |
| Potassium hydroxide (5% aq.) | 1310-58-3 | Liquid | Cat 2 | 3.0 | - | I | [23,33,34] | -0.5 | Sigma | 37.7 | 0.1–0.6 | 4.3 | 3.0 | [23,30,33,34] |
| Benzenethiol, 5-(1,1-dimethylethyl)-2-methyl | 7340-90-1 | Liquid | Cat 2 | 3.3 | - | I | [23,33,34] | 12.7 | ACROS | 17.3 | 1.1–1.5 | 27.6 | 15.2 | [23,30,33,34] |
| 1-Methyl-3-phenyl-1-piperazine | 5271-27-2 | Solid | Cat 2 | 3.3 | - | ci | [29,30,32] | 5.5 | AK Scientific | 9.5 | 9.2 | 7.4 | 15.2 | [23,30,33,34] |
| Heptanal | 111-71-7 | Liquid | Cat 2 | 3.4 | I | I | [29–32] | 2.5 | Sigma | 29.4 | 1.1–1.5 | 6.4 | 17.3 | [23,30,33,34] |
| Tetrachloroethylene | 127-18-4 | Liquid | Cat 2 | 4.0 | I | I | [30–32] | 14.1 | Sigma | 26.2 | 0.6–1.5 | 4.8 | 17.0 | [23,30,33,34] |
| 1-Bromo-4-chlorobutane | 6940-78-9 | Liquid | NC | 0 | NC | NI | [30–32] | 88.1 | Sigma | 95.3 | 86.8–93.1 | 86.4 | 87.6 | [23,30,33,34] |
| 1-Bromo-4-chlorobutane | 6940-78-9 | Liquid | NC | 0 | NC | NI | [30–32] | 88.1 | Sigma | 95.3 | 86.8–93.1 | 86.4 | 87.6 | [23,30,33,34] |
| Diethyl phthalate | 112-61-8 | Solid | Cat 2 | 1.0 | NC | NI | [30–32] | 93.4 | Sigma | 103.3 | 99.4–108.2 | 98.7 | 104.4 | [23,30,33,34] |
| Naphthalene acetic acid | 7493-74-5 | Liquid | Cat 2 | 2.0 | - | NC | [30–32] | 77.8 | Sigma | 96.5 | 67.9–92.0 | 92.6 | 82.3 | [23,30,33,34] |
| Allyl phenoxyacetate | 67-63-0 | Liquid | Cat 2 | 3.3 | - | NC | [30–32] | 76.6 | Sigma | 97.5 | 91.3–104.1 | 90.1 | 84.6 | [23,30,33,34] |
| Isopropanol | 67-63-0 | Liquid | Cat 2 | 4.0 | I | I | [9,29,31,32] | 8.2 | Sigma | 51.5 | 2.8–4.2 | 7.4 | 22.6 | [23,30,33,34] |
| Methyl stearate | 68-67-2 | Liquid | Cat 2 | 1.7 | NC | NI | [9,29–31] | 17.1 | Sigma | 8.9 | 2.8–4.2 | 17.1 | 70.9 | [23,30,33,34] |
| Heptyl butyrate | 5870-93-9 | Liquid | Cat 2 | 3.0 | NC | I | [9,29–31,33] | 24.6 | Sigma | 11.3 | 1.1–3.7 | 11.6 | 24.5–33.6 | [30,33,36] |
| Butyric acid | 107-92-6 | Liquid | Cat 1B | - | - | NI | [33,38] | 2.2 | Sigma | 2.1–4.5 | 0.4–1.1 | - | - | [33,38] |
| N,N-Dimethylisopropylamine | 112-05-0 | Liquid | Cat 2 | 4.0 | I | I | [9,29,30] | 43.6 | Sigma | 3.1 | <10 | 5.5 | 16.1–17.6 | [30,34,35,39] |
| Decanoyl acid (capric acid) | 97-88-1 | Liquid | Cat 2 | 3.0 | NC | I | [33,38] | 24.6 | Sigma | 11.3 | 1.1–3.7 | 11.6 | 24.5–33.6 | [30,33,36] |
| Polyethylene glycol 400 (PEG-400) | 25322-68-3 | Liquid | NC | 0 | NC | NI | [29,30,34,39] | 96.1 | TCI | 101.4 | >80 (93.4) | 99.9 | 98.2–106.6 | (98.2) | [30,34,39,44] |

**Notes:**
- ChemSkin™ SIT: Keratinocyte Skin Toxicity Predictive Framework
- EpiSKin: EpiSkin Human Skin Toxicity Prediction Framework
- SkinEthic: SkinEthic Human Skin Toxicity Prediction Framework
- EpiDerm: EpiDerm Human Skin Toxicity Prediction Framework
- LabCyte: Laboratory Cyte Human Skin Toxicity Prediction Framework
- VRMs: Validation Reference Materials
Table 2. Cont.

| Chemical | CAS No. | Physical State | In Vivo Category | In Vivo Score | In vivo Class | In vivo Score | Data Source References | KeraSkin™ SIT | VRMs | VRM Data Source | Vendor | VRM Data Source |
|----------|---------|----------------|------------------|---------------|--------------|--------------|------------------------|-------------|------|----------------|---------|----------------|
| 3-(Chloropropyl)trimethoxysilane (Silane A-1430) | 2530-87-2 | Liquid | NC | 0 | - | NI | [31,35,45] | 3.3 | Sigma | 36.0–94.6 (10.8) | 80.7 | 61.7–98.6 | – | [33,35,37,38] |
| 3,3′-Dithiodipropionic acid | 31 | Solid | NC | 0 | - | NI | [30,31,45] | 96.3 | Sigma | 96.7–107.5 | 102.4–117.1 | 98.0 | 89.9–100 | [30,33,34,38] |
| 2-Phenylethanol (phenylethyl alcohol) | 60-12-8 | Liquid | NC | 1.0 | - | NI | [31,35] | 0.3 | Sigma | 63.1–104.4 (91.7) | 5.6 | 45.7–92.9 | – | [30,35,37,38] |
| Benzyl salicylate | 118-58-1 | Liquid | NC | 0.3 | NC | NI | [29,30,45] | 98.5 | Sigma | 121.37 (96.4) | 86.5–113.3 | 89.5 | 93.6–99.9 | [31,33,34,37] |
| 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)urea | 101-20-2 | Solid | NC | 0 | - | NI | [46,47] | 102.3 | Sigma | 111.2 | – | – | – | [46] |
| 3,3-Dimethylpentane | 562-49-2 | Liquid | NC | 0 | - | NI | [30,34] | 88.3 | ACROS | – | – | – | – | [30,34] |
| 4,4′-Methylenebis(2,6-di-tert-butylphenol) | 118-82-1 | Solid | NC | 0 | - | NI | [30,31] | 97.3 | Sigma | 85.8–111.2 | 86.5–113.3 | 96.9 (94.4) | 100.0–101.3 | [30,31,33,34,38] |
| Dodecanoic acid (lauric acid) | 143-07-7 | Liquid | NC | 0.3 | NC | NI | [29–31] | 84.8 | Sigma | 102.5–127.6 | 90.0 (102.5) | – | 94.0–110.0 | [30,31,33,34,38] |
| Benzyl benzoate | 120-51-4 | Liquid | NC | 0 | - | NI | [30,31] | 98.3 | Sigma | 110.0 | 84.3–104.1 | 93.4 (100.1) | 99.6–105.7 | [30,31,33,34] |
| 4-Amino-4H-1,2,4-triazole (4-amino-1,2,4-triazole) | 43028-69-9 | Solid | NC | 0 | - | NI | [31,45] | 100.9 | Sigma | 89.0–95.8 | 108.0 | 97.8–105.7 | – | [31,35] |
| Sodium bisulphite | 7631-90-5 | Solid | NC | 1.0 | - | NI | [30,34,45] | 9.4 | Sigma | 108.0 | 79.1–99.7 | 56.1 | 11.1–74.7 | [30,33,34] |
| 4-Acetoxy-2,5-dimethyl-3(2H)-furanone (2,5-dimethyl-4-oxo-4,5-dihydrofuran-3-yl acetate) | 4166-20-5 | Liquid | NC | 0 | - | NI | [31,35,45] | 4.6 | TCI | 98.2–114.3 | 102.3 | 80.3–91.2 | – | [31,35] |
| 4-Amino-4H-1,2,4-triazole (4-amino-1,2,4-triazole) | 584-13-4 | Solid | NC | 0 | - | NI | [30,31,45] | 89.7 | Sigma | 102.2–106.0 | 91.0 (92.1) ** | 97.4–101.0 | – | [30,31,24] |
| Dipropylene glycol | 25265-71-8 | Liquid | NC | 0 | NC | NI | [9,30,31,45] | 94.9 | Sigma | 81.5–115.1 | 95.9–103.5 | 93.5 | 92.9–109.9 | [30,33,34,38] |
| 2-Formylamin-3-thiophencarboxylic acid | 29911-28-2 | Liquid | NC | 0 | - | NI | [31,35,45] | 13.9 | Sigma | 85.0–113.9 | 97.4 | 102.7 | – | [33,35,38] |
| Sodium bicarbonate | 112-84-5 | Solid | NC | 0 | - | NI | [30,31,45] | 103.1 | Sigma | 97.8–106.2 | 95.1–107.7 | 102.7 | 90.0–102.5 | [30,33,35,38] |
| Triethylene glycol | 1-6 | Liquid | NC | 0 | NC | NI | [29,39,48,49] | 84.5 | Sigma | 90.7–116.9 | 101.9 (101.9) | 101.4 | 95.8–95.3 | – | [31,33,35,37,38] |
| Triethylene glycol | 112-27-6 | Liquid | NC | 0 | - | NI | [31,35,45] | 76.6 | Sigma | 92.0–104.2 | 94.7–113.3 | 90.9 | 99.6–100.3 | [30,33,38] |
| Isopropyl palmitate | 144-55-8 | Solid | NC | 0 | - | NI | [30,31,45] | 91.0 | Sigma | 93.9–104.1 | 100.7–115.1 | 93.0 | 102.5–115.9 | [30,33,34,38] |
| Isopropyl myristate | 142-91-6 | Liquid | NC | 1.0 | NC | NI | [29,30,45] | 106.0 | Sigma | 102.8–118.6 | 98.6–110.5 | 97.5 | 97.2–107.9 | [30,33,34,38] |
| 2-Ethylhexyl-4-methoxyceinnamate (octinoxate) | 5466-77-3 | Liquid | NC | - | - | NI | [50,51] | 106.5 | Sigma | 83.3–109.7 | – | – | – | [50] |
Table 2. Cont.

| Chemical Description | CAS No. | Physical State | In Vivo Category | In Vivo Score | Human In Vivo Result | In Vivo Class | In Vivo Score Data Source References | KeraSkin™ TM SIT | Vendor | EpiSkin | SkinEthic | EpiDerm | LabCyte | VRMs | VRM References |
|-----------------------|---------|----------------|------------------|---------------|----------------------|--------------|--------------------------------------|-----------------|--------|---------|-----------|---------|---------|------|----------------|
| Tetramethylammonium chloride solution (in D.W.) (citrinum chloride) | 112-02-7 | Liquid | Cat 2 | - | - | I | [52,54,55] | 1.1 | Sigma | 9.5 | - | - | - | [52] |
| 25% Cetyltrimethylammonium chloride solution | 131-57-7 | Solid | NC | - | - | NI | [52,54,56] | 99.3 | Sigma | 104.1 | (64-119)** | - | - | - | [52,54] |
| 2-Hydroxy-4-methoxybenzophenone, (Benzophenone-3) | 131-57-7 | Solid | NC | - | - | NI | [52,54,56] | 99.3 | Sigma | 104.1 | (64-119)** | - | - | - | [52,54] |
| Cyclohexadecanone | 2550-52-9 | Solid | NC | 1.7 | - | NI | [31,45] | 9.7 | Symrise | 6.0 | 7.9–113.6 | - | [31] |
| Methyl laurate | 111-82-0 | Liquid | Cat 3 | 2.0 | NC | NI | [29,31,45] | 91.2 | Sigma | 76.0 | 73.9–82.1 | - | [31,37,39] |
| Capryl-isostearate | 209802-43-7 | Liquid | NC | 1.0 | - | NI | [31,45] | 9.1 | Chemi-cal | 96.0–102.3 | - | 99.5–108.0 | - | [31] |
| 2-Ethylhexyl-4-aminoazobenzene | 26218-04-2 | Solid | NC | 0.7 | - | NI | [31,45] | 10.3 | Santacruz | 90.9–111.4 | - | 91.9–107.3 | - | [31] |
| 2-Phenylhexanenitrile | 3508-98-3 | Liquid | Cat 3 | 1.7 | - | NI | [31,45] | 78.0 | ACROS | 86.7–116.2 | - | 73.9–82.1 | - | [31] |
| Barium sulfate | 7727-43-7 | Solid | NC | - | - | NI | [31,45] | 9.4 | Nippon Fine Chemicals | 96.0–102.3 | - | 99.5–108.0 | - | [31] |
| Disopropyl sebacate | 7491-02-3 | Liquid | NC | - | - | NI | [31,45] | 9.3 | 2-Ethylhexyl-4-aminoazobenzene | 90.9–111.4 | - | 91.9–107.3 | - | [31] |
| 10% Xanthan gum (in D.W.) | 11138-66-2 | Gel | NC | - | - | NI | [44,57,58] | 9.2 | Sigma | 101.3 | 103.9 | 95.3 | 99.4 | 92.9 | [44] |
| 3-Chloro-4-fluoronitrobenzene | 592-02-7 | Liquid | NC | 1.0 | - | NI | [31,45] | 80.7 | Sigma | 110.5–104.9 | (3.0)** | 101.6 | 53.2–101.7 | - | [31,35,37] |
| 1,5-Hexadiene | 56-81-5 | Liquid | NC | 0.7 | - | NI | [30,34] | 8.0 | Sigma | - | - | 100.4 | 85.6–95.6 | - | [30,34] |
| Glycerol | 140-11-4 | Liquid | NC | 1.0 | - | NI | [30,34] | 8.1 | Sigma | - | - | 99.7 | 98.1–125.7 | - | [30,34] |
| Benzyl acetate | 107-75-5 | Liquid | NC | 1.0 | NC | NI | [9,30,45] | 14.7 | Sigma | 92.1–104.0 | (31.5)** | <10 | 23.2–45.9 | - | [30,31,34,38] |
| n-Butyl propionate | 590-01-2 | Liquid | NC | 1.0 | - | NI | [30,31,45] | 72.5 | Sigma | <10 | <10 | 1.25 | 79.6 | 86.8–101.2 | [30,31,34,38] |
| allyl heptanoate | 142-19-8 | Liquid | Cat 3 | 1.7 | - | NI | [31,33,38] | 9.2 | Sigma | 88.6–112.5 | (101.1)*** | 74.4–101.7 | 97.0–102.1 | (99.2)*** | 95.6–108.9 | [30,31,33,34,38] |
| 2-Ethoxy ethyl methacrylate | 2370-63-0 | Liquid | Cat 3 | 1.7 | - | NI | [31,38,45] | - | Sigma | 65.3–116.3 | (6.7)** | 9.8 | 6.9 | (99.0)*** | 29.3–74.4 | [30,31,33,34,38,46] |
| Linalyl acetate | 115-95-7 | Liquid | Cat 3 | 2.0 | NC | NI | [29,31] | - | Sigma | 50.0–103.8 | (43.2)** | 1.25 | 79.6 | 86.8–101.2 | [30,34,35,38] |
Table 2. Cont.

| c   | Chemical                  | CAS No. | Physical State | In Vivo Category | In Vivo Score | Human In Vivo Result | In Vivo Class | In Vivo Score Data Source References | KeraSkin™ SIT | VRMs | Vendor | VRM References |
|-----|---------------------------|---------|----------------|------------------|---------------|----------------------|---------------|---------------------------------------|---------------|------|---------|----------------|
| 76  | Terpinyl acetate          | 80-26-2 | Liquid         | Cat 3            | 2.0           | -                    | NC            | NI [29–31]                            | [29,31,33,45] | Sigma | 4.9–75.4 | [30,31,34,38] |
| 77  | Linalool                  | 78-70-6 | Liquid         | Cat 3            | 2.0           | -                    | NI            | - [30,31,45]                          | [30,34]       | Sigma | 14.7    | [31,40,59]    |
| 78  | D-Limonene                | 5989-27-5 | Liquid        | Cat 3            | 2.0           | -                    | NI            | - [31,45]                             | [30,34]       | Sigma | 1.1     | [30,34]       |
| 79  | Eugenol                   | 97-53-0 | Liquid         | Cat 3            | 2.0           | NC                   | NI            | [29–31,38]                            | [30,33,34,38] | Sigma | 5.1–8.3 | [30,34]       |
| 80  | Methyl palmitate****      | 112-39-0 | Liquid        | Cat 2            | 3.0           | NC                   | I             | [29,31,33,45]                        | [31,33,38]    | Sigma | 70.9–110.6| [30,34]       |
| 81  | 1,1,1-Trichloroethane     | 71-55-6 | Liquid         | Cat 2            | 4.0           | -                    | I             | [30,31,45]                            | [30,33,34,38] | Sigma | 36.6     | [30,34,39]    |
| 82  | SLS (50% aq.)             | 151-21-3 | Liquid        | Cat 2            | 4.0           | -                    | I             | [31,33]                              | [31,33,37,38,40] | Sigma | 13.1–34.5 | [31,33,34,38] |
| 83  | SLS (20% aq.)             | 151-21-3 | Liquid        | Cat 2            | 4.0           | I                    | I             | [29–31,33]                           | [30,31,33,34,38] | Sigma | 5.8***  | [30,34,44]    |
| 84  | SLS (5% aq.)              | 151-21-3 | Liquid        | Cat 2            | 4.0           | -                    | I             | [30,34]                              | [30,33,34,38] | Sigma | 2.1     | [30,34,44]    |
| 85  | Tri-isobutyl phosphate    | 126-71-6 | Liquid         | Cat 3            | 2.0           | -                    | NI            | [24,30,36,38,45]                     | [30,31,36,38] | Sigma | 4.4–8.3 | [30,31,36,38] |
| 86  | 10-Undecenoic acid        | 112-38-9 | Solid         | (Liquid at room temp.) | 2.0           | -                    | NI            | [29,31,45,60]                        | [30,38,40,59,60] | Sigma | 6.0–15.3 | [31,39,59,61] |
| 87  | dl-Citronellol             | 106-22-9 | Liquid         | Cat 3            | 2.0           | NC                   | NI            | [29,31,45]                            | [30,31,33,45,60] | Sigma | 5.9–11.4 | [31,39,59,61] |
| 88  | 33% Sodium undecylenate (in aqueous solution) | 3398-33-2 | Liquid        | Cat 3            | 2.0           | -                    | NI            | [33,42]                              | [31,40,59,61] | Sigma | 9.4–28.0| [31,39,59,61] |
| 89  | 2-Methoxyphenol (guaiacol) | 90-05-1 | Liquid         | Cat 3            | 2.0           | -                    | NI            | [33,40,60]                            | [31,39,59,61] | Sigma | 0.7      | [31,39,59,61] |
| 90  | 1,9-Decadiene             | 1647-16-1 | Liquid        | Cat 3            | 2.0           | -                    | NI            | [33,42,60]                            | [31,39,59,61] | Sigma | 17.2–20.0| [31,39,59,61] |
| 91  | 2-tert-Butylphenol        | 88-18-6 | Liquid         | Cat 1B/1C        | 2.0           | -                    | I             | [38,42,62,63]                        | [31,39,59,61] | Sigma | 3.1–5.3 | [31,39,59,61] |
| 92  | Carvacrol                 | 499-75-2 | Liquid        | Cat 1B/1C        | 2.0           | -                    | I             | [38,42,62]                            | [31,39,59,61] | Sigma | 4.5–5.6 | [31,39,59,61] |
| 93  | Cyclohexylamine           | 108-91-8 | Liquid        | Cat 1B/1C        | 2.0           | -                    | I             | [33,38,64]                            | [31,39,59,61] | Sigma | 4.3–6.6 | [31,39,59,61] |
| 94  | Lactic acid               | 50-21-2 | Liquid        | Cat 1B/1C        | 2.0           | -                    | I             | [29,33,60,63]                        | [31,39,59,61] | Sigma | 8.4      | [31,39,59,61] |
| 95  | Ethanolamine              | 141-43-5 | Liquid        | Cat 1B           | 2.0           | -                    | I             | [33,60]                              | [31,39,59,61] | Sigma | 3.4      | [31,39,59,61] |
| Chemical                                      | CAS No.          | Physical State | In Vivo Category | In Vivo Score | Human In Vivo Result | In vivo Class | In Vivo Score Data Source References | KeraSkin™ SIT | Vendor | VRMs | SkinEthic | EpiDerm | LabCyte | VRM References |
|-----------------------------------------------|------------------|----------------|------------------|---------------|----------------------|---------------|--------------------------------------|----------------|--------|------|-----------|---------|---------|----------------|
| 96. Boron trifluoride acetic acid complex      | 373-61-5         | Liquid         | Cat 1B           | -             | I                    | [33,38]       | -                                    | Sigma          | 3.4–4.5 | 0.4–1.0 | –          | –       | –       | [33,38]       |
| 97. Propionic acid                            | 79-09-4          | Liquid         | Cat 1B           | -             | I                    | [33,38,60]    | -                                    | Sigma          | 3.0–5.5 | (2.9)* | 0.6–0.7  | –       | –       | [33,38,60]    |
| 98. N,N-Dimethylbenzylamine                   | 103-83-3         | Liquid         | Cat 1C           | >4            | I                    | [33,38,60,65]| -                                    | Sigma          | 4.7–6.8 | (0.3)* | 0.5–0.9  | –       | –       | [33,38,60]    |
| 99. Maleic anhydride                          | 108-31-6         | Solid          | Cat 1C           | -             | I                    | [36,60,64]    | -                                    | Sigma          | 4.7–7.5 | (6.1)* | 0.4–0.5  | –       | –       | [33,38,60]    |
| 100. 48% Fluoroboric acid (in D.W.)           | 16872-11-0       | Liquid         | Cat 1C           | -             | I                    | [33,60]       | -                                    | Sigma          | 2.4     | 0.6–1.1 | –          | –       | –       | [33,60]       |

VRMs: Validated reference methods; KeraSkin: KeraSkin™ SIT; EpiSkin: EpiSkin™ SIT; SkinEthic: SkinEthic™ RHE; EpiDerm: EpiDerm™ SIT; LabCyte: LabCyte EPI-MODEL 24 SIT; PII: primary irritation index; SLS: sodium dodecyl sulfate. Abbreviations: VRMs = in vitro cell viability value, % of control; NC = No category, skin non-irritant; I = skin irritant; Cat 2 = UN GHS Category 2, skin irritant; Cat 1B = UN GHS Sub-category 1B, skin corrosive; Cat 1B/1C = combination of UN GHS sub-category 1B and 1C, skin corrosive. Cat 3 = UN GHS optional Category 3, skin mild irritant (in vivo score 1.5 ≤ Cat 3 < 2.3). OECD TG 439 (in vitro skin irritation: RhE test methods) does not classify chemicals to the optional Category 3 [19,32]. Under this test guideline, Cat 3 is considered as no category (non-irritant). * Twenty reference chemicals are performance standards (PS) based on the Series on Testing and Assessment No. 220 [32]. These PS are now available related to the present OECD TG 439. PS are available to facilitate the validation and assessment of similar and modified RhE-based test methods, in accordance with the principles of OECD Guidance Document No. 34 [7]. ** 1-Decanol (a borderline reference chemical) and di-n-propyl disulphide (a false negative of the VRM) are non-irritants in humans, although being identified as irritants in the rabbit test. Since RhE models are based on cells of human origin; they may predict these reference chemicals as non-irritants (UN GHS No category). *** According to the OECD TG 439, 1-methyl-5-phenyl-1-piperazine and 1-bromooxane can have variable results in different laboratories dependent on the supplier. **** According to the Series on Testing and Assessment No. 137 [31], methyl palmitate is a false negative in EpiSkin™, modified EpiDerm™, and SkinEthic™ RHE. This chemical is also a non-irritant to humans based on the human 4-hr patch test. * EpiSkin™ data produced in China [37]. ** Data based on the Series on Testing and Assessment No. 137 [31]. *** Data reported by Sugiyama et al., 2018 [44]; **** Data reported by Alépée et al., 2016 [52]; ***** SCCS Memorandum (addendum) data [54] on the in vitro EpiSkin™. * Data reported by Kandárová et al., 2006 [40]; ** Data reported by Alépée et al., 2010 [36]; *** Data reported by Kandárová et al., 2009 [30]; **** Data reported by Alépée et al., 2015b [33].
The reference chemical group included 46 No category chemicals, 18 optional Category 3 chemical, 22 Category 2 chemicals, 5 Category 1B chemicals, 3 Category 1C chemicals, and 6 Category 1B/1C chemicals (Figure 2). Among the total chemical group, there were 76 liquids, 23 solids, and 1 gel (Figure 3). A total of 51 studies were reviewed to establish ChemSkin DB; 41 were used for in vivo data, consisting of 22 published papers, 14 reports, and 5 government documents. In vivo Draize scores and UN GHS/EU CLP classification information were also added. In addition, the in vitro data produced by the RhE test methods of OECD TG 439 were sourced from 16 published papers, 3 reports, and 4 government documents.

3.2. In Vivo Draize Scores of 86 Chemicals in ChemSkin DB

Scatter plot is widely used to analyze the data distribution across categories [66]. In vivo Draize scores of 86 chemicals, excluding the 14 Cat 1 chemicals, are plotted as Figures 4 and 5. Plotted chemicals based on irritant or non-irritant classification showed that some irritant chemicals have scores just above the threshold of 2.3, suggesting that they may be determined as false negatives (Figure 4). The mean ± SD of the in vivo score
for each in vivo class was 3.27 ± 0.62 (20 irritants) for irritants (excluding Cat 1 chemicals, not stated values) and 0.78 ± 0.81 (55 non-irritants) for non-irritants (excluding not stated values) (irritant ≥ 2.3, non-irritant < 2.3), respectively. When Cat 3 is considered, the in vivo score of Cat 2 was 3.27 ± 0.62, Cat 3 was 1.92 ± 0.14 and No Cat was 0.36 ± 0.47 (Figure 5), which conforms to the current UN GHS classification criteria (UN GHS classification criteria: No Cat < 1.5, 1.5 ≤ Cat 3 < 2.3, 2.3 ≤ Cat 2 ≤ 4.0).

Figure 4. Scatter plot of edema/erythema in vivo Draize scores of 86 chemicals excluding Cat 1 chemicals according to irritant and non-irritant classification. The scatter plot shows the distribution of the in vivo Draize test scores. The value of 2.3 on the Y-axis indicates the in vivo score classification cut-off. The in vivo score 1.5 is marked to identify Cat 3.

Figure 5. Scatter plot of edema/erythema in vivo scores of 86 chemicals excluding Cat 1 chemicals according to irritancy category (NC, Cat 3, and Cat 2).
The scatter plot indicates the irritation signs for the Draize scoring. The in vivo score 2.3 is the cut-off for Cat 2 and 1.5 is the cut-off for Cat 3. Scores of NC chemicals without an in vivo score value available were set to 0.

3.3. Comparison of Prediction Results Produced by OECD TG 439 Test Methods for ChemSkin DB Substances

In the comparison of the results of the four approved methods of OECD TG 439 and in vivo scores, EpiSkin™ showed 3 false positives and 1 false negative, SkinEthic HCE™ showed 6 false positives, EpiDerm™ showed 5 false positives and 1 false negative, LabCyte EPI-MODEL24 showed 6 false positives and 1 false negative, and KeraSkin™ showed 4 false positives (Figure 6). Interestingly, compared with other models, KeraSkin™ and SkinEthic™ showed viability values near either 0% or 100%, reflecting that these models show a type of ‘all-or-none’ responses, while other models showed a number of borderline results around the cut-off.

![Figure 6](image)

Figure 6. Relationship between in vivo scores and viability data of OECD TG 439. The cut-off of cell viability was defined as 50%, and the cut-off in vivo score was defined as 2.3. The red dots are incorrectly predicted. In vitro irritant (I) = a, c area; in vitro non-irritant (NI) = b, d area; in vivo irritant (I) = a, b area; in vivo non-irritant (NI) = c, d area.

We calculated the mean ± SD viability of irritants and non-irritants. KeraSkin™ showed 10.57 ± 9.12% and 77.55 ± 33.46% for irritants and non-irritants, respectively. EpiSkin™ showed 20.21 ± 14.16% and 88.23 ± 33.03%; SkinEthic HCE™ showed 2.74 ± 2.69% and 76.73 ± 40.42%; EpiDerm™ showed 13.48 ± 20.76% and 76.85 ± 34.44%; and LabCyte EPI-MODEL24 showed 22.44 ± 16.18% and 83.19 ± 29.72% for irritants and non-irritants, respectively.

The predictive capacity of VRMs is shown in Table 3. The sensitivity, specificity, and accuracy of EpiSkin™ were 97.1%, 79.3%, and 86.0%; those of SkinEthic™ were 97.1%, 69.4%, and 80.7%; those of LabCyte EPI-MODEL24 were 94.1%, 70%, and 77.2%; and those of KeraSkin™ were 100%, 82.2%, and 87.5%, respectively (Table 3). The sensitivity of OECD TG439 RhE SITs is in the order of KeraSkin™ 100% (20 out of 20 irritants were correctly identified), EpiSkin™ 97.1% (34 out of 35 irritants), SkinEthic™ 97.1% (33 out of 34 irritants), LabCyte EPI-MODEL24 94.1% (16 out of 17 irritants), and EpiDerm™ 89.5% (17 out of 19 irritants). The specificity is in the order of KeraSkin™ 82.2% (37 out of 45 non-irritants...
were correctly identified), EpiDerm™ 81.5% (44 out of 54 non-irritants), EpiSkin™ 79.3% (46 out of 58 non-irritants), LabCyte EPI-MODEL24 70% (28 out of 40 non-irritants), and SkinEthic™ 69.4% (34 out of 49 non-irritants). The RhE STIs mostly satisfied the OECD TG 439 PS criteria (sensitivity ≥ 80%, specificity ≥ 70%, and accuracy ≥ 75%) for the reference chemicals in ChemSkin DB outside of PS reference chemical lists.

Table 3. Predictive capacity of VRM viability.

|                      | KeraSkin™ | EpiSkin™ | SkinEthic™ | EpiDerm™ | LabCyte EPI-MODEL24 | PS |
|----------------------|-----------|----------|-------------|-----------|---------------------|----|
|                      | I         | NI       | I           | NI        | I                   | NI |
| I                    | 20        | 0        | 34          | 1         | 17                  | 2  |
| NI                   | 8         | 37       | 12          | 46        | 15                  | 34 |
| Total (n)            | 65        | 93       | 83          | 73        | 73                  | 57 |
| Sensitivity          | 100%      | 97.1%    | 97.1%       | 89.5%     | 94.1%               | 80%|
| Specificity          | 82.2%     | 79.3%    | 69.4%       | 81.5%     | 70.0%               | 70%|
| Accuracy             | 87.5%     | 86.0%    | 80.7%       | 83.6%     | 77.2%               | 75%|

The viability values for three Cat 3 chemicals in the OECD PS 20 reference substances, were 87.3%, 88.3%, and 2.9% for KeraSkin™; 104.0%, 99.9%, and 7.5% for EpiSkin™; 91.5–102.3%, 93.7–99.2%, and 1.2–1.7% for SkinEthic HCE™; 96.4%, 96.4%, and 4.7% for EpiDerm™; and 108.1%, 106.7%, and 29.1% for LabCyte EPI-MODEL24. These results confirm the inability of TG 439 to distinguish Cat 3 from No Cat or Cat 2.

A correlation analysis of the in vivo score and in vitro tissue viability was performed. The correlation coefficient was in the order of SkinEthic™ (−0.803), KeraSkin™ (−0.773), EpiSkin™ (absolute value of 0.760), LabCyte EPI-MODEL24 (−0.752), and EpiDerm™ (−0.749) (Table 4), suggesting that all VRMs and KeraSkin™ of OECD TG 439 produced tissue viability data highly correlated with in vivo scores.

Table 4. Correlation between in vivo score and VRM viability.

|                      | In Vivo Score | KeraSkin™ | EpiSkin™ | SkinEthic™ RHE | EpiDerm™ | LabCyte EPI-MODEL24 |
|----------------------|---------------|-----------|----------|----------------|-----------|---------------------|
| In vivo score        | 1.000         | 1.000     | 0.899    | 1.000          | 1.000     | 1.000               |
| KeraSkin™            | −0.773 *      | 1.000     | 0.938 *  | 0.957 *        | 1.000     | 1.000               |
| EpiSkin™             | −0.760 *      | 0.940 *   | 0.933 *  | 0.933 *        | 1.000     | 1.000               |
| SkinEthic™ RHE       | −0.803 *      | 0.919 *   | 0.938 *  | 0.978 *        | 1.000     |                     |
| EpiDerm™             | −0.749 *      | 0.960 *   | 0.919 *  | 0.938 *        | 0.978 *   |                     |
| LabCyte EPI-MODEL24  | −0.752 *      |           |          |                |           |                     |

* Correlation is significant at the 0.01 level (2-tailed).

4. Conclusions

In this study, we compiled the ChemSkin DB listing 100 reference chemicals for the development and evaluation of new test methods for skin irritation test through the review of more than 317 reference chemicals. The selection of correct reference chemicals is pivotal in the establishment and optimization of a new test method. Detailed information such as supporting literature, in vivo Draize scores, UN GHS/EU CLP classifications, and commercial sources were included, which could be invaluable for the developers of new skin irritation test methods. In addition, the test results produced by five methods approved in the current OECD Test No. 439 (2021) were included, compared in a table, a scatter plot, and analyzed for the correlation with in vivo Draize scores. Overall, the current RhE methods of TG 439 could not distinguish Category 3 from other categories, but strong correlations between viability and in vivo scores suggest an opportunity for further
improvement. Collectively, we believe that our study will provide important insight into the future development of new in vitro skin irritation testing methods.

Author Contributions: Conceptualization, methodology, M.-J.K. and K.-M.L.; investigation, J.H., G.-Y.L. and M.-J.K.; resources, K.-M.L.; data curation, J.H., G.-Y.L., G.B. and M.-J.K.; writing—original draft preparation, J.H., and G.-Y.L.; writing—review and editing, K.-M.L.; supervision, K.-M.L.; project administration, M.-J.K. and K.-M.L.; funding acquisition, K.-M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the Ministry of Food and Drug Safety in 2021 (21162MFD5015-2), the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (Grant No. HP20C0061), and the RP-Grant 2020 of Ewha Womans University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors have no conflict of interest to declare.

References

1. Whitehead, H.D.; Venier, M.; Wu, Y.; Eastman, E.; Urbanik, S.; Diamond, M.L.; Shalin, A.; Schwartz-Narbonne, H.; Bruton, T.A.; Blum, A.; et al. Fluorinated Compounds in North American Cosmetics. *Environ. Sci. Technol. Lett.* 2021, 8, 538–544. [CrossRef]

2. Adler, S.; Basketter, D.; Creton, S.; Pelkonen, O.; Van Benthem, J.; Zuang, V.; Andersen, K.E.; Angers-Loustau, A.; Aptula, A.; Bal-Price, A. Alternative (non-animal) methods for cosmetics testing: Current status and future prospects—2010. *Arch. Toxicol.* 2011, 85, 367–485. [CrossRef]

3. Akbarsha, M.A.; Mascarenhas, B. Cosmetic Regulation and Alternatives to Animal Experimentation in India. In *Alternatives to Animal Testing—Proceedings of Asian Congress 2016*; Kojima, H., Seidle, T., Spielmann, H., Eds.; Springer: Singapore, 2019; pp. 57–62.

4. Avonto, C.; Wang, M.; Chittiboyina, A.G.; Vukmanovic, S.; Khan, I.A. Chemical stability and in chemico reactivity of 24 fragrance ingredients of concern for skin sensitization risk assessment. *Toxicol. In Vitro* 2018, 46, 237–245. [CrossRef]

5. Kim, J.Y.; Kim, M.K.; Kim, K.B.; Kim, H.S.; Lee, B.M. Quantitative structure-activity and quantitative structure-property relationship approaches as alternative skin sensitization risk assessment methods. *J. Toxicol. Environ. Health Part A* 2019, 82, 447–472. [CrossRef]

6. Kim, M.K.; Kim, K.B.; Kim, H.S.; Lee, B.M. Alternative skin sensitization prediction and risk assessment using proinflammatory biomarkers, interleukin-1 beta (IL-1beta) and inducible nitric oxide synthase (iNOS). *J. Toxicol. Environ. Health Part A* 2019, 82, 361–378. [CrossRef] [PubMed]

7. Organisation for Economic Co-Operation and Development. *Series on Testing and Assessment No. 34: Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*; Organisation for Economic Co-Operation and Development Environment: Paris, France, 2005.

8. Engelke, M.; Zorn-Kruppa, M.; Gabel, D.; Reisinger, K.; Rusche, B.; Mewes, K. A human hemi-cornea model for eye irritation testing: Quality control of production, reliability and predictive capacity. *Toxicol. In Vitro* 2013, 27, 458–468. [CrossRef] [PubMed]

9. Jírová, D.; Basketter, D.; Liebsch, M.; Bendová, H.; Kejlová, K.; Marriott, M.; Kandárová, H. Comparison of human skin irritation patch test data with In vitro skin irritation assays and animal data. *Contact Dermat.* 2010, 62, 109–116. [CrossRef] [PubMed]

10. Gerberick, F.G.; Ryan, C.A.; Kern, P.S.; Schlatter, H.; Dearman, R.J.; Kimber, I.; Patlewicz, G.Y.; Basketter, D.A. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 2005, 16, 157–202.

11. Barroso, J.; Pfannenbecker, U.; Adriaens, E.; Alépée, N.; Cluzel, M.; De Smedt, A.; Hibatallah, J.; Klaric, M.; Mewes, K.R.; Millet, M. Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: The Draize eye test Reference Database (DRD). *Arch. Clin. Toxicol.* 2017, 91, 521–547.

12. Fentem, J.H.; Botham, P.A. ECVAM’s activities in validating alternative tests for skin corrosion and irritation. *Altern. Lab. Anim.* 2002, 30, 61–67. [CrossRef]

13. Organisation for Economic Co-Operation and Development. *Test Guideline No. 404: Acute Dermal Irritation/Corrosion. OECD Guidelines for the Testing of Chemicals*; Organisation for Economic Cooperation and Development: Paris, France, 2015.

14. Draize, J.H.; Woodard, G.; Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Pharmacol. Exp. Ther.* 1944, 82, 377–390.

15. European Commission. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Off. J. Eur. Communities L* 2001, 225, 1–333.
16. European Commission. 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, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006. Off. J. Eur. Union L 2008, 50, 353.

17. United Nations. Globally Harmonised System of Classification and Labelling of Chemicals (GHS); United Nations: New York, NY, USA; Geneva, Switzerland, 2019.

18. Organisation for Economic Co-Operation and Development. Test Guideline No. 439: In Vitro Skin Irritation (Reconstructed Human Epidermis Test Method); Organisation for Economic Cooperation and Development: Paris, France, 2015.

19. Organisation for Economic Co-Operation and Development. Test Guideline No. 439: In Vitro skin Irritation (Reconstructed Human Epidermis Test Method); Organisation for Economic Cooperation and Development: Paris, France, 2021.

20. Pedrosa, T.D.N.; Catarino, C.M.; Pennacchi, P.C.; Assis, S.R.; Gimenes, F.; Consolaro, M.E.L.; Barros, S.B.M.; Maria-Engler, S.S. A new reconstructed human epidermis for In vitro skin irritation testing. Toxicol. In Vitro 2017, 42, 31–37. [CrossRef] [PubMed]

21. Spielmann, H.; Hoffmann, S.; Liebsch, M.; Botham, P.; Fentem, J.H.; Eskes, C.; Roguet, R.; Cotovio, J.; Cole, T.; Worth, A.; et al. The ECVM international validation study on In vitro tests for acute skin irritation: Report on the validity of the EPISKIN and EpiDerm assays and on the Skin Integrity Function Test. Altern. Lab. Anim. 2007, 35, 559–601. [CrossRef]

22. Katoh, M.; Hamajima, F.; Ogasawara, T.; Hata, K. Assessment of human epidermal model LabCyte EPI-MODEL for In vitro skin irritation testing according to European Centre for the Validation of Alternative Methods (ECVAM)-validated protocol. J. Toxicol. Sci. 2009, 34, 327–334. [CrossRef] [PubMed]

23. Katoh, M.; Hata, K. Refinement of LabCyte EPI-MODEL24 Skin Irritation Test Method for Adaptation to the Requirements of OECD Test Guideline 439. Altern. Anim. Test. Exp. 2011, 16, 111–122.

24. Eskes, C.; Cole, T.; Hoffmann, S.; Worth, A.; Cockshott, A.; Gerner, I.; Zuang, V. The ECVM international validation study on In vitro tests for acute skin irritation: Selection of test chemicals. Altern. Lab. Anim. 2007, 35, 603–619. [CrossRef]

25. Shimazaki, T.; Nagai, T.; Kawamoto, Y.; Ozawa, M.; Suzuki, M. SAGAN COAT Photocatalyst Coating Solution TPX for Primary Skin Irritation in Rabbits; Japan Food Research Laboratories: Tokyo, Japan, 2007.

26. Lee Rodgers, J.; Nicewander, W.A. J.T.A. S. Thirteen ways to look at the correlation coefficient. Am. Stat. 1988, 42, 59–66. [CrossRef]

27. Zhou, H.; Deng, Z.; Xia, Y.; Fu, M. A new sampling method in particle filter based on Pearson correlation coefficient. Neurocomputing 2016, 216, 208–215. [CrossRef]

28. Taylor, R. Interpretation of the correlation coefficient: A basic review. J. Diagn. Med. Sonogr. 1990, 6, 35–39. [CrossRef]

29. Basketter, D.; Jirova, D.; Kandárová, H. Review of skin irritation/corrosion Hazards on the basis of human data: A regulatory perspective. Interdiscip. Toxicol. 2012, 5, 98–104. [CrossRef] [PubMed]

30. Kandárová, H.; Hayden, P.; Klausner, M.; Kubilus, J.; Kearney, P.; Sheasgreen, J. In vitro skin irritation testing: Improving the sensitivity of the EpiDerm skin irritation test protocol. Altern. Lab. Anim. 2009, 37, 671–689. [CrossRef]

31. Organisation for Economic Co-Operation and Development. Series on Testing and Assessment No. 137: Explanatory Background Document to the OECD Test Guideline on In Vitro Skin Irritation Testing; Organisation for Economic Co-Operation and Development Environment: Paris, France, 2010.

32. Organisation for Economic Co-Operation and Development. Series on Testing and Assessment No. 220: Performance Standards for the Assessment of Proposed Similar or Modified In Vitro Reconstructed Human Epidermis (RHE) Test Methods for Skin Irritation Testing as Described in TG 439 (Intended for the Developers of New or Modified Similar Test Methods); Organisation for Economic Co-Operation and Development Environment: Paris, France, 2015.

33. Alépée, N.; Granddidier, M.-H.; Tornier, C.; Cotovio, J. An integrated testing strategy for In vitro skin corrosion and irritation assessment using SkinEthic™ Reconstructed Human Epidermis. Toxicol. In Vitro 2015, 29, 1779–1792. [CrossRef]

34. Alépée, N.; Granddidier, M.-H.; Tornier, C. Usefulness of the EpiSkin™ reconstructed human epidermis model within Integrated Approaches on Testing and Assessment (IATA) for skin corrosion and irritation. Toxicol. In Vitro 2015, 29, 147–167. [CrossRef] [PubMed]

35. Tornier, C.; Amsellem, C.; de Fraissinette, A.D.B.; Alépée, N. Assessment of the optimized SkinEthic™ Reconstructed Human Epidermis (RHE) 42 bis skin irritation protocol over 39 test substances. Toxicol. In Vitro 2010, 24, 245–256. [CrossRef]

36. Alépée, N.; Tornier, C.; Robert, C.; Amsellem, C.; Roux, M.-H.; Doucet, O.; Pachot, J.; Méloni, M.; de Fraissinette, A.D.B. A catch-up validation study on reconstructed human epidermis (SkinEthic™ RHE) for full replacement of the Draize skin irritation test. Toxicol. In Vitro 2010, 24, 257–266. [CrossRef]

37. Li, N.; Liu, Y.; Qiu, J.; Zhong, L.; Alépée, N.; Cotovio, J.; Cai, Z. In vitro skin irritation assessment becomes a reality in China using a reconstructed human epidermis test method. Toxicol. In Vitro 2017, 41, 159–167. [CrossRef]

38. Alépée, N.; Granddidier, M.-H.; Cotovio, J. Usefulness of the EpiSkin™ reconstructed human epidermis model within Integrated Approaches on Testing and Assessment (IATA) for skin corrosion and irritation. Toxicol. In Vitro 2019, 54, 147–167. [CrossRef] [PubMed]

39. Tornier, C.; Rosdy, M.; Maibach, H.I. In vitro skin irritation testing on reconstituted human epidermises: Reproducibility for 50 chemicals tested with two protocols. Toxicol. In Vitro 2006, 20, 401–416. [CrossRef]

40. Kandárová, H.; Liebsch, M.; Schmidt, E.; Geschow, E.; Traue, D.; Spielmann, H.; Meyer, K.; Steinhoff, C.; Tornier, C.; De Wever, B. Assessment of the skin irritation potential of chemicals by using the SkinEthic reconstructed human epidermal model and the common skin irritation protocol evaluated in the ECVM skin irritation validation study. Altern. Lab. Anim. 2006, 34, 393–406. [CrossRef]
41. European Chemicals Agency. Disseminated Registration Dossier for Heptanoic Acid (CAS No. 111-14-8): Irritation/Corrosion_Endpoint Summary. Available online: https://echa.europa.eu/registration-dossier/-/registered-dossier/15829/7/4/2?documentUUID=66f6c7f6b-666f-43bf-a71e-40486573130d (accessed on 14 November 2021).

42. European Centre for Ecotoxicology and Toxicology of Chemicals. Skin Irritation and Corrosion: Reference Chemicals Data Bank, Technical Report No 66; European Centre for Ecotoxicology and Toxicology of Chemicals: Brussels, Belgium, 1995; pp. 1–255.

43. Jacobs, J.J.; Lehé, C.; Cammans, K.D.; Das, P.K.; Elliott, G.R. Methyl green-pyronine staining of porcine organotypic skin explant cultures: An alternative model for screening for skin irritants. Altern. Lab. Anim. 2000, 28, 279–292. [CrossRef]

44. Sugiyama, M.; Akita, M.; Alépée, N.; Fujishiro, M.; Hagino, S.; Handa, Y.; Ikeda, H.; Imai, N.; Jitsukawa, S.; Katoh, M. Comparative assessment of 24-h primary skin irritation test and human patch test data with In vitro skin irritation tests according to OECD Test Guideline 439 (for quasi-drugs in Japan). Toxicol. Sci. 2018, 43, 751–768. [CrossRef]

45. Hoffmann, S.; Saliner, A.G.; Patlewicz, G.; Eskes, C.; Zung, V.; Worth, A.P. A feasibility study developing an integrated testing strategy assessing skin irritation potential of chemicals. Toxicol. Lett. 2008, 180, 9–20. [CrossRef] [PubMed]

46. Alépée, N.; Barroso, J.; De Smedt, A.; De Wever, B.; Hibatallah, J.; Klaric, M.; Mewes, K.; Millet, M.; Pfannenbecker, U.; Tailhardat, M. Use of HPLC/UPLC-spectrophotometry for detection of formazan in In vitro reconstructed human tissue (RHT)-based test methods employing the MTT-reduction assay to expand their applicability to strongly coloured test chemicals. Toxicol. In Vitro 2015, 29, 741–761. [CrossRef]

47. European Chemicals Agency. European Chemicals Agency. Disseminated Registration Dossier for Triclocarban (CAS No. 131–145. [CrossRef] [PubMed]

48. Scientific Committee on Consumer Safety. Opinion on Alkyl (C16, C18, C22) Trimethylammonium Chloride: For Other Uses than as a Preservative COLIPA no P72; European Union, Scientific Committee on Consumer Safety: Brussels, Belgium, 2009.

49. Golla, S.; Madhially, S.; Robinson, R.L., Jr.; Gasem, K.A. Quantitative structure–Property relationships modeling of skin irritation. Toxicol. In Vitro 2009, 23, 178–184. [CrossRef]

50. Molinari, J.; Eskes, C.; Andres, E.; Remoué, N.; Sá-Rocha, V.; Hurtado, S.; Barrichello, C. Improved procedures for In vitro skin irritation testing of sticky and greasy natural botanicals. Toxicol. In Vitro 2013, 27, 441–450. [CrossRef]

51. Scientific Committee on Consumer Products. Reports of the Scientific Committee on Cosmetology (Ninth Series); European Commission: Luxembourg, 2000.

52. Alépée, N.; Hibatallah, J.; Klaric, M.; Mewes, K.; Pfannenbecker, U.; McNamee, P. Assessment of cosmetic ingredients in the In vitro reconstructed human epidermis test method EpiSkin™ using HPLC/UPLC-spectrophotometry in the MTT-reduction assay. Toxicol. In Vitro 2016, 33, 105–117. [CrossRef]

53. Scientific Committee on Consumer Safety. Opinion on Tetrabromophenol, 4,4′-(4,5,6,7-Tetrabromo-1,1-Dioxido-3H-2,1-Benzoxathiol-3-Ylidin)bis-2,6-Dibromophenol (C183)-Submission IV; European Union, Scientific Committee on Consumer Safety: Brussels, Belgium, 2019.

54. Scientific Committee on Consumer Safety. Memorandum (Addendum) on the In Vitro Test EPISKIN™ for Skin Irritation Testing; European Union, Scientific Committee on Consumer Safety: Brussels, Belgium, 2010.

55. Scientific Committee on Consumer Safety. Opinion on Alkyl (C16, C18, C22) Trimethylammonium Chloride: For Other Uses than as a Preservative COLIPA no P72; European Union, Scientific Committee on Consumer Safety: Brussels, Belgium, 2009.

56. European Chemicals Agency. Disseminated Registration Dossier for Oxybenzone (CAS No. 131–57–7): Irritation/Corrosion_Endpoint Summary. Available online: https://echa.europa.eu/registration-dossier/-/registered-dossier/15829/7/4/2?documentUUID=66f6c7f6b-666f-43bf-a71e-40486573130d (accessed on 14 November 2021).

57. Fiume, M.M.; Heldreth, B.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, D.C.; Liebler, D.C.; Marks, J.G., Jr.; Shank, R.C.; Slaga, T. Safety assessment of microbial polysaccharide gums as used in cosmetics. Int. J. Toxicol. 2016, 35, 88–495. [CrossRef] [PubMed]

58. Joint FAO/WHO Expert Committee on Food Additives. Toxicological Evaluation of Certain Food Additives and Contaminants: WHO Food Additives Series 21 Xanthan Gum; Cambridge University Press: Cambridge, UK, 1987.

59. Kandlack, O.; Liebsch, M.; Genschow, E.; Gerner, I.; Traue, D.; Slawik, B.; Spielmann, H. Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on In vitro skin irritation tests. Altern. Anim. Exp. 2004, 21, 107–114.

60. Liu, Y.; Li, N.; Chen, L.; Alépée, N.; Cai, Z. A ready-to-use integrated In vitro skin corrosion and irritation testing strategy using EpiSkin™ model in China. Toxicol. In Vitro 2020, 65, 104778. [CrossRef]

61. Fentem, J.; Briggs, D.; Chesné, C.; Elliott, G.; Harbell, J.; Heylings, J.; Portes, P.; Roguet, R.; Van de Sandt, J.; Botham, P. A prevalidation study on In vitro tests for acute skin irritation: Results and evaluation by the Management Team. Toxicol. In Vitro 2001, 15, 57–93. [CrossRef]

62. Alépée, N.; Granddidier, M.; Cotovio, J. Sub-categorisation of skin corrosive chemicals by the EpiSkin™ reconstructed human epidermis skin corrosion test method according to UN GHS: Revision of OECD Test Guideline 431. Toxicol. In Vitro 2014, 28, 131–145. [CrossRef] [PubMed]

63. Organisation for Economic Co-Operation and Development. Series on Testing and Assessment No. 219: Performance Standards for the Assessment of Proposed Similar or Modified In Vitro Reconstructed Human Epidermis (RHE) Test Methods for Skin Corrosion Testing as Described in TG 431 (Intended for the Developers of New or Modified Similar Test Methods); Organisation for Economic Co-Operation and Development Environment: Paris, France, 2015.
64. Ishii, S.; Ishii, K.; Nakadate, M.; Yamasaki, K. Correlation study in skin and eye irritation between rabbits and humans based on published literatures. *Food Chem. Toxicol.* **2013**, *55*, 596–601. [CrossRef] [PubMed]

65. European Chemicals Agency. Disseminated Registration Dossier for Benzylidimethylamine (CAS No. 103-83-3): Irritation/corrosion_Endpoint summary. Available online: [https://echa.europa.eu/registration-dossier/-/registered-dossier/13869](https://echa.europa.eu/registration-dossier/-/registered-dossier/13869) (accessed on 14 November 2021).

66. Lee, D.H.; Kim, S.-H.; Lee, J.H.; Yang, J.-Y.; Seok, J.-H.; Jung, K.; Lee, J.K. Flow cytometric evaluation of the potential of metal oxide nanoparticles for skin sensitization using 5-Bromo-2-deoxyuridine. *Toxicol. Res.* **2021**, *37*, 369–377. [CrossRef]