STUDY

# Compilation of Historical Local Lymph Node Data for Evaluation of Skin Sensitization Alternative Methods

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***Background:*** **Within the toxicology community, considerable effort is directed toward the development of alternative methods for skin sensitization testing. The availability of high-quality, relevant, and reliable in vivo data regarding skin sensitization is essential for the effective evaluation of alternative methodologies. Ideally, data derived from humans would be the most appropriate source because the test methods are attempting to predict a toxicologic effect in humans. Unfortunately, insufficient human data of the necessary quality are available, so it is necessary to rely on the best available animal data. In recent years, the local lymph node assay (LLNA) has emerged as a practical option for assessing the skin sensitization potential of chemicals. In addition to accurately identifying skin sensitizers, the LLNA can also provide a reliable measure of relative sensitization potency, information that is pivotal to the successful management of human health risks.**

***Objective:*** **To provide a database of robust in vivo data to calibrate, evaluate, and eventually validate new approaches for skin sensitization testing.**

***Methods:*** **LLNA data derived from previously conducted studies were compiled from the published literature and unpublished sources.**

***Results:*** **We provide a database that comprises LLNA data on 211 individual chemicals. This extensive chemical data set encompasses both the chemical and biologic diversity of known chemical allergens. To cover the range of relative allergenic potencies, the data set includes data on 13 extreme, 21 strong, 69 moderate, and 66 weak contact allergens, classified according to each allergen's mathematically estimated concentration of chemical required to induce a threefold stimulation index. In addition, there are also 42 chemicals that are considered to be nonsensitizers. In terms of chemical diversity, the database contains data pertaining to the chemical classes represented by aldehydes, ketones, aromatic amines, quinones, and acrylates, as well as compounds that have different reactivity mechanisms. In addition to two-dimensional chemical structures, the physicochemical parameters included are log Kp, log $K_{O/W}$, and molecular weight.**

***Conclusions:*** **The list of chemicals contained in the data set represents both the chemical and biologic diversity that is known to exist for chemical allergens and non-allergens. It is anticipated that this database will help accelerate the development, evaluation, and eventual validation of new approaches to skin sensitization assessment.**

THE EVALUATION OF THE POTENTIAL to induce allergic contact dermatitis (ACD) is an important component of the safety assessment process for a new chemical that may be encountered through the skin.[1] For many years, the guinea pig has been the species of choice for the identification of skin-sensitizing hazards. More recently, however, the local lymph node assay (LLNA) has been accepted as a valid alternative approach; its method is based on the characterization of induced proliferative responses in the draining lymph nodes after topical exposure of mice to chemicals.[2–6] The LLNA has been adopted by the Organization for Economic Cooperation and Development (OECD) as a stand-alone test for skin sensitization (OECD Test Guideline 429).[7] This adoption resulted from thorough and independent validations of the LLNA in both the United States[8] and Europe.[9] The LLNA offers clear scientific advantages as well as important animal welfare benefits (in terms of both reduction and refinement) when compared with traditional guinea pig tests. In addition to accurately identifying skin sensitization hazards, the LLNA can provide a reliable measure of relative allergenic potency,

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information that is critical to the risk assessment process. This is achieved by consideration of the vigor with which chemical allergens provoke proliferative responses in draining lymph node cells. The relative potency of a chemical sensitizer is measured by deriving what is known as the EC3 value, which indicates the test chemical concentration necessary to produce a threefold stimulation of proliferation in draining lymph nodes as compared with concurrent vehicle controls.[10] Thus, the intrinsic sensitizing potency of a chemical is defined as a function of the concentration required to elicit a threshold positive response, a stimulation index (SI) equal to 3. For this reason, the lower the EC3 value, the greater the relative skin sensitizing potency of the chemical. This approach has already been applied with some success, and the LLNA is the preferred method for estimating the potency of a contact allergen.[11,12]

Meeting the ultimate challenge of developing nonanimal predictive test methods to replace an in vivo skin sensitization test such as the LLNA requires an understanding of the biologic mechanisms involved in the development of ACD.[13,14] Specifically, the in vitro approach should be designed in such a way that the potential of a chemical to penetrate the skin, react with protein/peptide (with or without the need for biotransformation), and initiate an antigen-specific immune response are incorporated into the developed tests. Approaches could include in vitro methods, based most commonly on chemically induced responses in cell culture systems (eg, single-cell cultures or more complex models such as skin explant and skin-equivalent models), or in silico methods (eg, computer-based expert or quantitative structure activity systems).

Currently, in vitro systems for the identification of skin sensitizers are still in their infancy. One critical requirement for the development of alternative test methods will be the use of robust in vivo data to calibrate, evaluate, and eventually validate new approaches. In this article, we describe the compilation of an extensive chemical data set that embraces a range of chemicals and skin-sensitizing activity. All materials have been evaluated through LLNA; for some chemicals, it has been demonstrated that the LLNA EC3 value correlates closely with what is known of the chemical's relative ability to induce sensitization in humans.[15–17] These data provide a unique and valuable list of chemicals for which the sensitivity, selectivity, and overall accuracy of proposed alternative methods for skin sensitization can be judged. This compilation also provides an invaluable source of data with which to explore other issues, such as the relationship between chemical categorization (on the basis of mechanism of action) and potency. It could also help to define the applicability domain of the LLNA as well as that of existing alternative methodologies and those in development.

## Materials and Methods

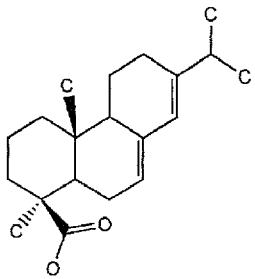
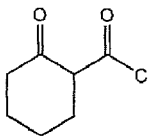
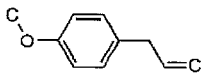
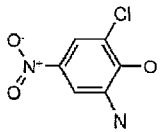
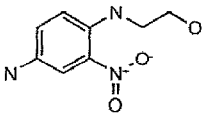
### Chemicals

The chemicals identified in this article have been evaluated for skin sensitization potential with the LLNA. For each chemical listed in Table 1, the chemical abstract service (CAS) number and two-dimensional structure are specified. In addition, the molecular weight, the skin penetration coefficient (log Kp: centimeters × hours − 1, here expressed in its log scale), and the octanol-water partition coefficient (log $K_{O/W}$) values are indicated for each chemical. The structures were graphically drawn with *ChemDraw*, version 6.0 (CambridgeSoft, Cambridge, MA). The molecular weight (MW), log Kp, and log $K_{O/W}$ values were obtained by analysis of the structures with the expert system *DEREK for Windows*, version 7 (Lhasa Limited, Leeds, UK).[18] The log Kp was calculated with the equation of Potts and Guy,[19] and the log $K_{O/W}$ was calculated with Moriguchi's estimation.[20]

### Local Lymph Node Assay Protocol

The LLNA was conducted as described in the literature (Fig 1).[2–6] Briefly, groups of CBA female mice (7–12 weeks of age) were exposed topically on the dorsum of both ears to 25 μL of test material or to an equal volume of the relevant vehicle alone. Treatment was performed daily for 3 consecutive days. Five days after the initiation of exposure, all mice were injected via the tail vein with 250 μL of phosphate-buffered saline containing 20 μCi of tritiated thymidine. The mice were sacrificed 5 hours later, and the draining auricular lymph nodes were excised and pooled for each experimental group or each individual animal. The incorporation of tritiated thymidine measured by beta scintillation counting was reported in disintegrations per minute. An SI was calculated for each chemical-treated group as the ratio of the disintegrations per minute in the treated group (or mean disintegrations per minute when individual animals were assessed) to the disintegrations per minute or mean disintegrations per minute of the concurrent vehicle control group. LLNA methodology assesses skin sensitization, not photosensitization. A substance was classified as a skin sensitizer if, at one or more test concentrations, it induced a threefold or greater increase in local lymph node proliferative activity when compared with concurrent vehicle-treated controls (SI, ≥ 3). The compilation of data from numerous laboratories is reported in

**Table 1.** Chemical Structures, Physicochemical Parameters, LLNA Data, and Potency Categorization

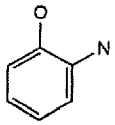
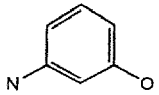
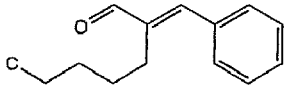
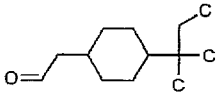
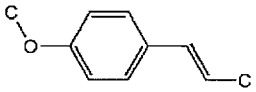
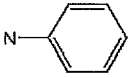
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abietic acid  | 514-10-3 | −1.29 | 4.61 | 302.46 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.5 | 2 | 5.2 | — | — | — | 15 | Weak | Ashby J et al.[21] |
| 2-Acetylcyclohexanone  | 874-23-7 | −2.41 | 1.66 | 143.21 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 0.8 | 0.7 | 0.8 | — | — | — | NC | Nonsensitizer | P&G (unpublished) |
| 4-Allylanisole  | 140-67-0 | −1.82 | 2.54 | 148.21 | AOO | 10.0 | 25.0 | 50.0 | 100.0 | — | — | 1.2 | 4.7 | 4.5 | 8 | — | — | 18 | Weak | Unilever (unpublished) |
| 2-Amino-6-chloro-4-nitrophenol  | 6358-09-4 | −3.69 | 0.26 | 188.57 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 1.7 | 1.4 | 2.1 | 1.5 | 3.4 | — | 2.2 | Moderate | Estrada E et al.[22] |
| 2-(4-Amino-2-nitro-phenylamino)-ethanol (HC Red No. 3)  | 2871-01-41 | −3.84 | 0.12 | 197.19 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 0.5 | 1.2 | 1.9 | 1.8 | 3.3 | — | 2.2 | Moderate | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

(*continued on next page*)

**Table 1.** *Continued*

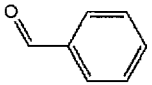
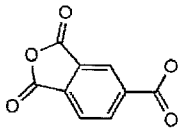
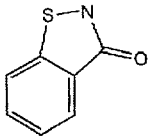
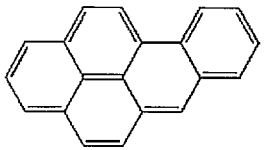
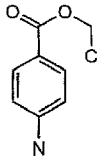
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-Aminophenol  | 95-55-6 | −2.55 | 1.17 | 109.13 | AOO | 0.5 | 1.0 | 2.5 | — | — | — | 3.5 | 5.0 | 7.4 | — | — | — | 0.4[†] | Strong | Ashby J et al.[21] |
| 3-Aminophenol  | 591-27-5 | −2.55 | 1.17 | 109.13 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 2.8 | 3.5 | 5.7 | — | — | — | 3.2 | Moderate | Ashby J et al.[21] |
| α-Amyl cinnamic aldehyde  | 122-40-7 | −1.45 | 3.52 | 202.30 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 1.5 | 1.7 | 2.2 | 2.8 | 8.2 | — | 11 | Weak | Elahi EN et al.[23] |
| 2-(4-tert-Amylcyclohexyl)acetaldehyde (QRM 2113)  | 620159-84-4 | −1.59 | 3.28 | 196.33 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 2.1 | 4 | 9.1 | — | — | — | 37 | Weak | Estrada E et al.[22] |
| *trans*-Anethol  | 104-46-1 | −1.82 | 2.54 | 148.21 | AOO | 4.5 | 9.0 | 22.6 | — | — | — | 13.5 | 24.7 | 37.3 | — | — | — | 2.3[†] | Moderate | Unilever (unpublished) |
| Aniline  | 62-53-3 | −2.18 | 1.56 | 93.13 | AOO | 5.0 | 10.0 | 25.0 | 50.0 | 100.0 | — | 1.1 | 0.9 | 2.0 | 1.9 | 3.3 | — | 89 | Weak | Unilever (unpublished) |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Benzaldehyde  | 100-52-7 | −2.09 | 1.80 | 106.12 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 2.1 | 1.7 | 2.2 | 1.8 | 2 | — | NC | Nonsensitizer | Patlewicz G et al.[24] |
| 1,2,4-Benzenetricarboxylic anhydride (trimellitic anhydride)  | 552-30-7 | −3.36 | 0.75 | 192.13 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 1.1 | 2 | 2.0 | 3.2 | 4.6 | — | 9.2 | Moderate | Estrada E et al.[22] |
| 1,2-Benzisothiazolin-3-one (Proxel active)  | 2634-33-5 | −2.64 | 1.42 | 151.18 | DMF | 10.0 | 30.0 | 50.0 | — | — | — | 3.8 | 4.4 | 4.9 | — | — | — | 2.3† | Moderate | Ashby J et al.[21] |
| Benzo[a]pyrene  | 50-32-8 | −0.44 | 5.39 | 252.32 | AOO | 0.5 | 1.0 | 2.5 | — | — | — | 17.6 | 19.2 | 27.0 | — | — | — | 0.0009† | Extreme | Ashby J et al.[21] |
| Benzocaine  | 94-09-7 | −2.65 | 1.52 | 165.19 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 2.1 | 1.8 | 2.7 | 1.8 | 1.2 | — | NC | Nonsensitizer | Warbrick EV et al.[25] |

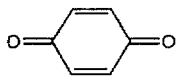
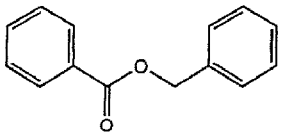
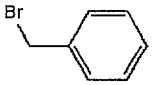
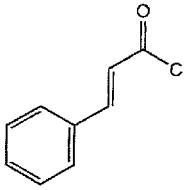
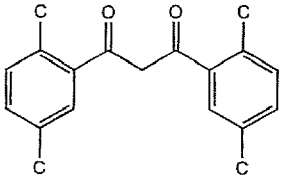
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| p-Benzoquinone  | 106-51-4 | −2.55 | 1.17 | 108.10 | AOO | 0.5 | 1.0 | 2.5 | — | — | — | 36.4 | 42.3 | 52.3 | — | — | — | 0.0099[†] | Extreme | Basketter DA, Scholes EW.[26] |
| Benzyl benzoate  | 120-51-4 | −1.79 | 3.14 | 212.25 | AOO | 5.0 | 25.0 | — | — | — | — | 2.3 | 3.5 | — | — | — | — | 17 | Weak | Smith CK, Hotchkiss SA.[27] |
| Benzyl bromide  | 100-39-0 | −1.95 | 2.56 | 171.04 | AOO | 0.25 | 0.5 | 1.0 | 2.5 | 5.0 | — | 3.5 | 11.5 | 16.1 | 16.4 | 25.1 | — | 0.2[†] | Strong | Unilever (unpublished) |
| Benzylidene acetone (4-phenyl-3-buten-2-one)  | 122-57-6 | −1.81 | 2.54 | 146.19 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 8.5 | 13.6 | 12.8 | — | — | — | 3.7[†] | Moderate | Ryan CA et al.[28] |
| Bis-1,3-(2,5-dimethylphenyl)-propane-1,3-dione  | | −1.34 | 4.37 | 282.38 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 1.8 | 1.6 | 2.1 | — | — | — | NC | Nonsensitizer | P&G (unpublished) |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

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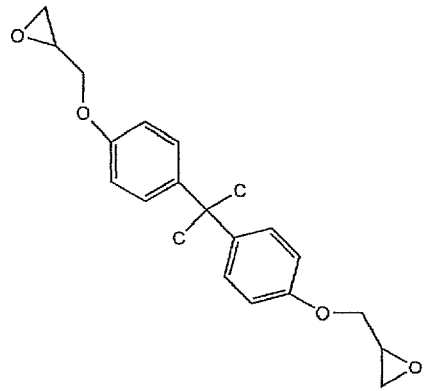
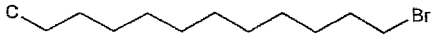
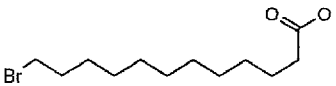
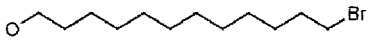
**Table 1.** *Continued*

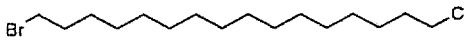
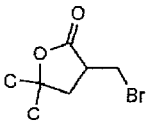
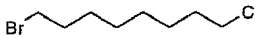
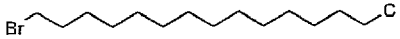
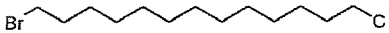
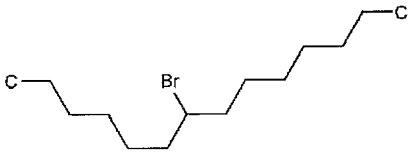
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bisphenol A-diglycidyl ether  | 1675-54-3 | −1.89 | 4.09 | 340.42 | AOO | 1.0 | 3.0 | 10.0 | — | — | — | 2.0 | 6.0 | 17.4 | — | — | — | 1.5 | Moderate | Warbrick EV et al.[29] |
| 1-Bromobutane | 109-65-9 | −2.26 | 1.82 | 137.02 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.1 | 1.2 | 1.0 | — | — | — | NC | Nonsensitizer | Ashby J et al.[21] |
| 1-Bromodocosane | 6938-66-5 | −0.66 | 6.25 | 389.51 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 1.2 | 1.6 | 3.7 | — | — | — | 8.3 | Moderate | Estrada E et al.[22] |
| 1-Bromododecane  | 143-15-7 | −1.55 | 3.79 | 249.24 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.1 | 1.4 | 4.5 | — | — | — | 18 | Weak | Basketter DA et al.[30] |
| 12-Bromododecanoic acid  | 73367-80-3 | −2.28 | 3.02 | 279.22 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.3 | 2 | 3.9 | — | — | — | 18 | Weak | Unilever (unpublished) |
| 12-Bromo-1-dodecanol  | 3344-77-2 | −1.92 | 3.40 | 265.24 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.2 | 4.3 | 9.8 | — | — | — | 6.9 | Moderate | Estrada E et al.[22] |
| 1-Bromoeicosane | 4276-49-7 | −0.84 | 5.76 | 361.45 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.1 | 6.2 | 8.4 | — | — | — | 6.1 | Moderate | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

(*continued on next page*)

**Table 1.** *Continued*

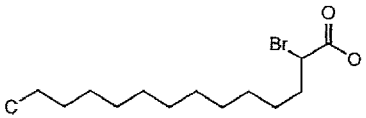
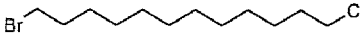
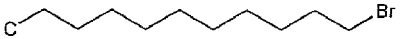
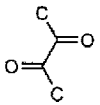
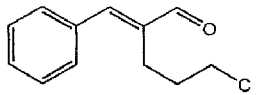
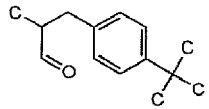
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-Bromoheptadecane  | 3508-00-7 | −1.11 | 5.02 | 319.37 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 3.2 | 6 | 9.6 | — | — | — | 4.8[†] | Moderate | Basketter DA et al.[30] |
| 1-Bromohexadecane | 112-82-3 | −1.20 | 4.77 | 305.34 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | 1.1 | 3.3 | 7.9 | 11.1 | 13.5 | 16.8 | 2.3 | Moderate | Basketter DA et al.[30] |
| 1-Bromohexane | 111-25-1 | −2.09 | 2.31 | 165.07 | AOO | 1.0 | 10.0 | 50.0 | — | — | — | 1.7 | 2.9 | 18.6 | — | — | — | 10 | Weak | Estrada E et al.[22] |
| 3-Bromomethyl-5,5-dimethyl-dihydro-2(3H)-furanone  | 154750-20-6 | −2.72 | 1.79 | 207.07 | AOO | 3.19 | 6.37 | 12.74 | — | — | — | 2.7 | 5.1 | 7.1 | — | — | — | 3.6 | Moderate | Estrada E et al.[22] |
| 1-Bromononane  | 693-58-3 | −1.82 | 3.05 | 207.16 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.2 | 1.4 | 2.8 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[30] |
| 1-Bromooctadecane | 112-89-0 | −1.02 | 5.26 | 333.40 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.8 | 2.2 | 4.5 | — | — | — | 15 | Weak | Basketter DA et al.[30] |
| 1-Bromopentadecane  | 629-72-1 | −1.28 | 4.53 | 291.32 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.9 | 7.8 | 19.6 | — | — | — | 5.1 | Moderate | Ashby J et al.[21] |
| 1-Bromotetradecane  | 112-71-0 | −1.37 | 4.28 | 277.29 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.5 | 3.3 | 11.3 | — | — | — | 9.2 | Moderate | Basketter DA et al.[30] |
| 7-Bromotetradecane  | 74036-97-8 | −1.37 | 4.28 | 277.29 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 0.9 | 1.2 | 3.6 | — | — | — | 21 | Weak | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2- Bromotetradecanoic acid  | 10520-81-7 | −2.10 | 3.51 | 307.27 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 4.7 | 7.7 | 10.1 | — | — | — | 3.4[†] | Moderate | Unilever (unpublished) |
| 1-Bromotridecane  | 765-09-3 | −1.46 | 4.03 | 263.26 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.6 | 2.9 | 10.4 | — | — | — | 10 | Weak | Basketter DA et al.[30] |
| 1-Bromoundecane  | 693-67-4 | −1.64 | 3.54 | 235.21 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.3 | 1.4 | 3.9 | — | — | — | 20 | Weak | Basketter DA et al.[30] |
| 2,3-Butanedione  | 431-03-8 | −2.76 | 0.68 | 86.09 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.4 | 2.8 | 5.2 | — | — | — | 11 | Weak | Roberts DW et al.[31] |
| 1-Butanol | 71-36-3 | −2.42 | 1.06 | 74.12 | dH2O | 5.0 | 10.0 | 20.0 | — | — | — | 1.6 | 1.2 | 1.4 | — | — | — | NC | Nonsensitizer | Ryan CA et al.[28] |
| α-Butyl cinnamic aldehyde  | 7492-44-6 | −1.54 | 3.28 | 188.27 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 1.4 | 1.7 | 1.7 | 2.1 | 13 | — | 11 | Weak | Elahi EN et al.[25] |
| p-tert-Butyl-a-ethyl hydrocinnamal (Lilial)  | 80-54-6 | −1.46 | 3.52 | 204.31 | AOO | 1.0 | 2.5 | 10.0 | 25.0 | 50.0 | — | 1.3 | 2.5 | 2.0 | 3.7 | 9.3 | — | 19 | Weak | Basketter DA et al.[16] |

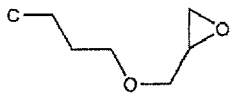
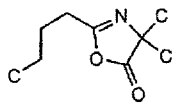
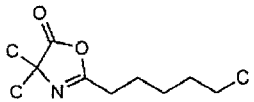
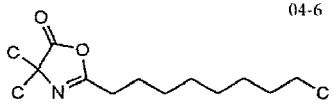
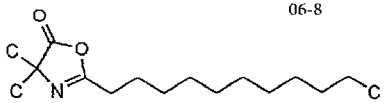
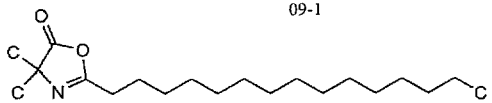
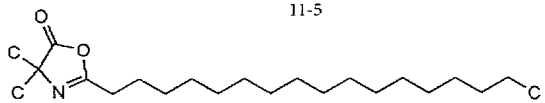
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

(*continued on next page*)

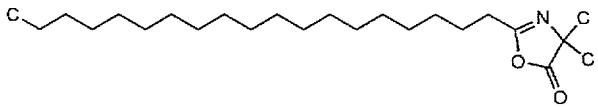
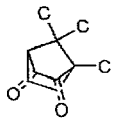
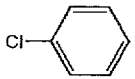
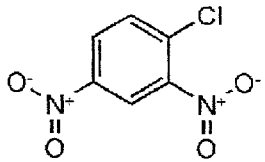
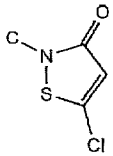
**Table 1.** *Continued*

| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Butyl glycidyl ether  | 2426-08-6 | −2.51 | 1.42 | 130.19 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.4 | 2.2 | 5.6 | — | — | — | 31 | Weak | Basketter DA et al.[32] |
| C4 Azlactone  | 176664-99-6 | −2.67 | 1.52 | 169.22 | AOO | 0.52 | 1.31 | 2.62 | 5.23 | — | — | 1.1 | 2.3 | 4.1 | 11.7 | — | — | 1.8 | Moderate | Estrada E et al.[22] |
| C6 Azlactone  | 176665-02-4 | −2.49 | 2.01 | 197.28 | AOO | 0.61 | 1.52 | 3.05 | — | — | | 1.2 | 3.5 | 7.6 | — | — | — | 1.3 | Moderate | Estrada E et al.[22] |
| C9 Azlactone  | 176665-04-6 | −2.23 | 2.75 | 239.36 | AOO | 1.85 | 3.7 | 7.4 | — | — | — | 1.4 | 4.6 | 10.1 | — | — | — | 2.8 | Moderate | Estrada E et al.[22] |
| C11 Azlactone  | 176665-06-8 | −2.05 | 3.24 | 267.41 | AOO | 8.3 | 20.7 | 41.3 | — | — | — | 1.3 | 4 | 8.5 | — | — | — | 16 | Weak | Estrada E et al.[22] |
| C15 Azlactone  | 176665-09-1 | −1.69 | 4.23 | 323.52 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.8 | 4.1 | 7.5 | — | — | — | 18 | Weak | Estrada E et al.[22] |
| C17 Azlactone  | 176665-11-5 | −1.51 | 4.72 | 351.58 | AOO | 10.87 | 27.17 | 54.33 | — | — | — | 1.7 | 4.3 | 4.6 | — | — | — | 19 | Weak | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C19 Azlactone  | | −1.34 | 5.21 | 379.63 | AOO | 11.73 | 29.33 | 58.67 | — | — | — | 2.5 | 3.1 | 2.5 | — | — | — | 26 | Weak | Estrada E et al.[22] |
| Camphorquinone  | 465-29-2 | −2.20 | 2.15 | 166.22 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.8 | 3.0 | 1.7 | — | — | — | 10 | Weak | Roberts DW et al.[31] |
| Chlorobenzene  | 108-90-7 | −1.85 | 2.19 | 112.56 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.1 | 1.7 | 1.6 | — | — | — | NC | Nonsensitizer | Ashby J et al.[21] |
| 1-Chloro-2,4-dinitrobenzene  | 97-00-7 | −4.05 | −0.13 | 202.55 | AOO | 0.01 | 0.025 | 0.05 | 0.1 | 0.25 | — | 1.5 | 1.8 | 2.4 | 8.9 | 38.0 | — | 0.05 | Extreme | Loveless SE et al.[33] |
| 1-Chlorohexadecane | 4860-03-1 | −1.01 | 4.65 | 260.89 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.6 | 3.3 | 5.7 | — | — | — | 9.1 | Moderate | Unilever (unpublished) |
| 5-Chloro-2-methyl-4-isothiazolin-3-one  | 26172-55-4 | −2.98 | 0.92 | 149.60 | DMF | 0.01 | 0.03 | 0.10 | — | — | — | 3.5 | 12.3 | 27.7 | — | — | — | 0.009[i] | Extreme | Ashby J et al.[21] |

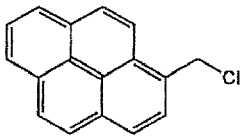
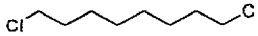
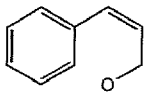
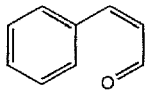
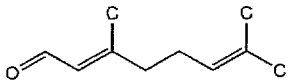
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[i]Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

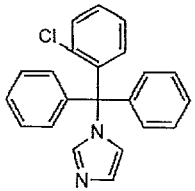
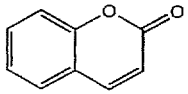
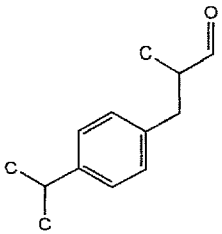
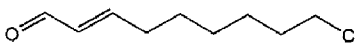
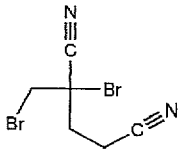
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-Chloromethylpyrene  | 1086-00-6 | −0.77 | 4.89 | 250.73 | AOO | 0.025 | 0.05 | 0.1 | — | — | — | 11.6 | 15.4 | 18.6 | — | — | — | 0.005† | Extreme | Ashby J et al.[21] |
| 1-Chlorononane  | 2473-01-0 | −1.64 | 2.93 | 162.70 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1 | 1.6 | 2.3 | — | — | — | NC | Nonsensitizer | Unilever (unpublished) |
| 1-Chlorooctadecane | 3386-33-2 | −0.83 | 5.14 | 288.95 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.7 | 4.8 | 7.3 | — | — | — | 16 | Weak | Estrada E et al.[22] |
| 1-Chlorotetradecane | 2425-54-9 | −1.19 | 4.16 | 232.84 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.1 | 3.9 | 6.3 | — | — | — | 20 | Weak | Estrada E et al.[22] |
| Cinnamic alcohol  | 104-54-1 | −1.91 | 2.29 | 134.18 | AOO | 10.0 | 25.0 | 50.0 | 90.0 | — | | 1.8 | 3.5 | 3.9 | 5.7 | — | — | 21 | Weak | Estrada E et al.[22] |
| Cinnamic aldehyde  | 104-55-2 | −1.90 | 2.29 | 132.16 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.4 | 0.9 | 1.9 | 7.1 | 15.8 | — | 3.0 | Moderate | Basketter DA et al.[16] |
| Citral  | 5392-40-5 | −1.85 | 2.54 | 152.24 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.2 | 2.1 | 6.3 | — | — | — | 13 | Weak | Patlewicz G et al.[34] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

**Table 1.** *Continued*

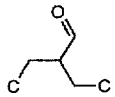
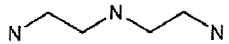
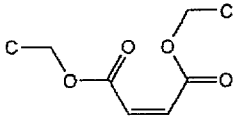
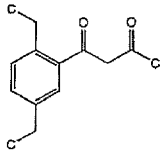
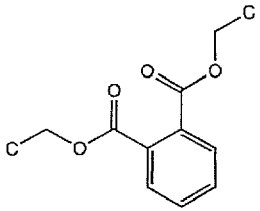
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clotrimazole  | 23593-75-1 | −1.02 | 5.35 | 344.85 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 1.6 | 3.1 | 3.0 | — | — | — | 4.8 | Moderate | Estrada E et al.[22] |
| Coumarin  | 91-64-5 | −2.26 | 1.91 | 146.15 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.7 | 2.9 | 2.3 | — | — | — | NC | Nonsensitizer | RIFM/FEMA database |
| Cyclamen aldehyde  | 103-95-7 | −1.55 | 3.28 | 190.29 | AOO | 1.0 | 2.5 | 10.0 | 25.0 | 50.0 | — | 1.4 | 1.3 | 1.8 | 3.3 | 5.2 | — | 22 | Weak | Basketter DA et al.[16] |
| *trans*-2-Decenal  | 3913-71-1 | −1.86 | 2.54 | 154.25 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.3 | 1.1 | 3 | 6 | 9.5 | — | 2.5 | Moderate | Patlewicz G et al.[34] |
| 1,2-Dibromo-2,4-dicyanobutane  | 35691-65-7 | −2.99 | 1.91 | 265.94 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | — | — | 1.4 | 3.4 | 3.5 | 5.4 | — | — | 0.9 | Strong | Unilever (unpublished) |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

(*continued on next page*)

**Table 1.** *Continued*

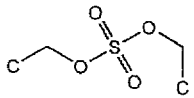
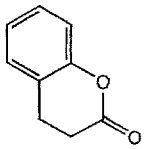
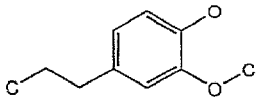
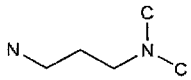
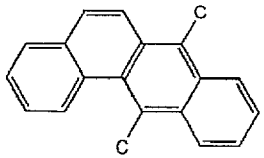
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diethyl acetaldehyde  | 97-96-1 | −2.23 | 1.56 | 100.16 | AOO | 25.0 | 50.0 | 75.0 | 100.0 | — | — | 1.2 | 0.8 | 2.4 | 16.3 | — | — | 76 | Weak | Patlewicz G et al.[34] |
| Diethylenetriamine  | 111-40-0 | −3.14 | 0.29 | 103.17 | AOO | 10.0 | 25.0 | — | — | — | — | 6.4 | 12.1 | — | — | — | — | 5.8 | Moderate | Basketter DA et al.[32] |
| Diethyl maleate  | 141-05-9 | −3.14 | 0.89 | 172.18 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 16.3 | 22.6 | 13.1 | — | — | — | 5.8[†] | Moderate | Ryan CA et al.[28] |
| 1-(2,5-Diethylphenyl)butane-1,3-dione  | 167998-76-7 | −1.84 | 3.14 | 221.32 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 3.9 | 19.2 | 18.7 | — | — | — | 9.6[†] | Moderate | P&G (unpublished) |
| Diethylphthalate  | 84-66-2 | −2.75 | 1.87 | 222.24 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 1.0 | 1.3 | 1.5 | — | — | — | NC | Nonsensitizer | Ryan CA et al.[28] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diethyl sulfate  | 64-67-5 | −3.73 | −0.09 | 154.18 | AOO | 1.0 | 2.5 | 10.0 | — | — | — | 0.8 | 1.9 | 12.0 | — | — | — | 3.3 | Moderate | Ashby J et al.[21] |
| 3,4-Dihydrocoumarin  | 119-84-6 | −2.27 | 1.91 | 148.16 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 1.6 | 2.5 | 6.6 | — | — | — | 5.6 | Moderate | Ashby J et al.[21] |
| Dihydroeugenol  | 2785-87-7 | −2.20 | 2.15 | 166.22 | AOO | 5.1 | 10.1 | 25.3 | — | — | — | 2.7 | 3.6 | 7.8 | — | — | — | 6.8 | Moderate | Smith CK, Hotchkiss SA.[27] |
| 1,4-Dihydroquinone | 123-31-9 | −2.56 | 1.17 | 110.11 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 2.8 | 5.8 | 13.7 | 15.2 | 13.1 | — | 0.11 | Strong | Kimber I et al.[35] |
| 3-Dimethylaminopropylamine  | 109-55-7 | −2.69 | 0.92 | 102.18 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.3 | 1.1 | 3.5 | 7.0 | 13.9 | — | 2.2 | Moderate | Wright ZM et al.[36] |
| 7,12-Dimethylbenz[α]anthracene  | 57-97-6 | −0.46 | 5.39 | 256.35 | DMF | 0.025 | 0.5 | 1.0 | — | — | — | 7.6 | 17.7 | 15.6 | — | — | — | 0.006† | Extreme | Ashby J et al.[21] |

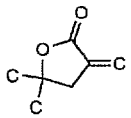
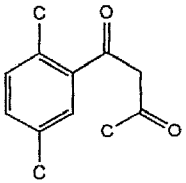
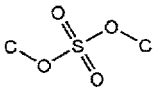
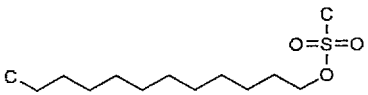
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

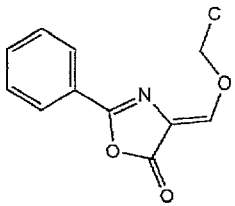
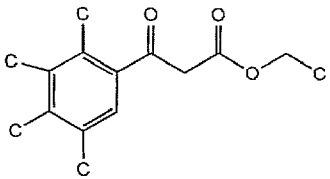
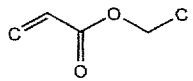
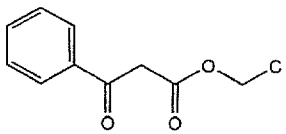
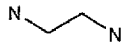
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5,5-Dimethyl-3-methylene-dihydro-2(3H)-furanone  | 29043-97-8 | −2.48 | 1.42 | 126.16 | AOO | 2.0 | 4.0 | 8.0 | — | — | — | 3 | 7.4 | 9.2 | — | — | — | 1.8† | Moderate | Ashby J et al.[21] |
| 1-(2,5-Dimethylphenyl)butane-1,3-dione  | 56290-55-2 | −2.02 | 2.65 | 193.27 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 2.3 | 5.1 | 9.5 | — | — | — | 13 | Weak | P&G (unpublished) |
| Dimethyl sulfate  | 77-78-1 | −3.91 | −0.59 | 126.13 | AOO | 0.25 | 0.5 | 1.0 | — | — | — | 3.8 | 6.0 | 5.7 | — | — | — | 0.19† | Strong | Ashby J et al.[21] |
| Dimethylsulfoxide | 67-68-5 | −2.79 | 0.57 | 78.13 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 2.7 | 2.3 | 3.9 | — | — | — | 72 | Weak | Estrada E et al.[22] |
| Dodecyl methane sulfonate  | 51323-71-8 | −2.55 | 2.51 | 264.42 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.1 | 3.3 | 9 | — | — | — | 8.8 | Moderate | Ashby J et al.[21] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

Table 1. *Continued*

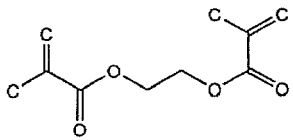
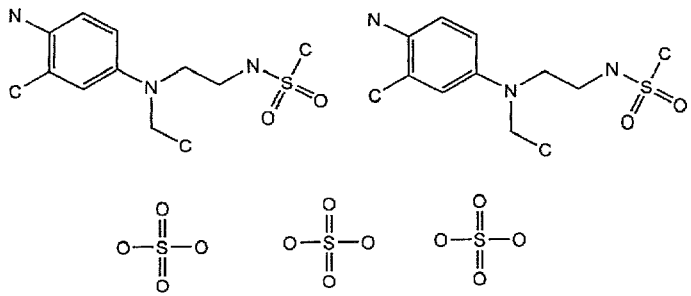
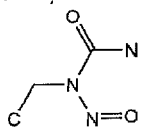
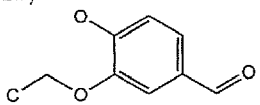
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone)  | 15646-46-5 | −2.71 | 1.87 | 217.22 | AOO | 0.0025 | 0.005 | 0.01 | 0.025 | 0.05 | — | 2.9 | 4.9 | 12.0 | 22.0 | 33.0 | — | 0.003 | Extreme | Loveless SE et al.[33] |
| 3-Ethoxy-1-(2′,3′,4′,5′-tetramethylphenyl)propane-1,3-dione  | 170928-69-5 | −2.11 | 3.00 | 248.32 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 1.1 | 1.7 | 3.7 | — | — | — | 33 | Weak | P&G (unpublished) |
| Ethyl acrylate  | 140-88-5 | −2.67 | 0.92 | 100.12 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.2 | 2.7 | 5 | — | — | — | 28 | Weak | Warbrick EV et al.[29] |
| Ethyl benzoylacetate  | 94-02-0 | −2.46 | 2.01 | 192.21 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 0.9 | 0.9 | 1.2 | — | — | — | NC | Nonsensitizer | P&G (unpublished) |
| Ethylenediamine free base  | 107-15-3 | −2.95 | 0.19 | 60.10 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | 5.0 | 1.1 | 1.2 | 1.6 | 1.9 | 3.3 | 6.1 | 2.2 | Moderate | Kimber I et al.[35] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

(*continued on next page*)

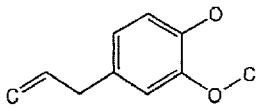
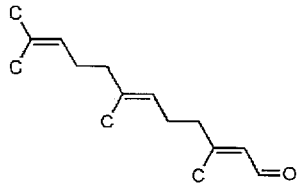
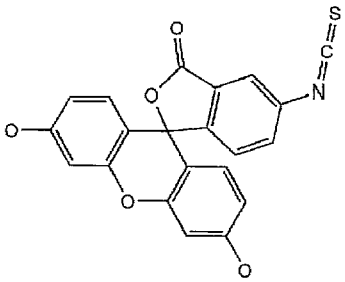
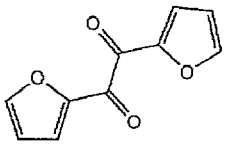
**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ethylene glycol dimethacrylate  | 97-90-5 | −2.95 | 1.38 | 198.22 | MEK | 10.0 | 25.0 | 50.0 | — | — | — | 1.2 | 2.4 | 7 | — | — | — | 28 | Weak | Unilever (unpublished) |
| 4-(N-Ethyl-N-2-methan-sulphonamido-ethyl)-2-methyl-1,4-phenylenediamine (CD3)  | 25646-71-3 | −9.33 | −2.12 | 836.97 | DMSO | 0.1 | 1.0 | 5.0 | 10.0 | — | — | 1.2 | 4.5 | 5.9 | 6.3 | — | — | 0.6 | Strong | Ryan CA et al.[28] |
| *N*-Ethyl-*N*-nitrosourea  | 759-73-9 | −3.95 | −0.73 | 117.11 | AOO | 0.25 | 1.0 | 10.0 | — | — | — | 1.0 | 2.7 | 22.3 | — | — | — | 1.1 | Moderate | Ashby J et al.[21] |
| Ethyl vanillin  | 121-32-4 | −2.65 | 1.52 | 166.18 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 0.65 | 1.05 | 0.74 | 0.36 | 0.29 | — | NC | Nonsensitizer | Basketter DA et al.[16] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eugenol  | 97-53-0 | −2.19 | 2.15 | 164.20 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | — | — | 1.6 | 1.5 | 2.4 | 5.5 | — | — | 13 | Weak | Loveless SE et al.[33] |
| Farnesal  | 502-67-0 | −1.39 | 3.77 | 220.36 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 0.6 | 1.1 | 1.7 | 2.5 | 7 | — | 12 | Weak | Patlewicz G et al.[34] |
| Fluorescein-5-isothiocyanate  | 3326-32-7 | −2.74 | 3.32 | 389.38 | Acetone/ dibutyl phthalate, 50/50 | 0.5 | 1.0 | 2.5 | — | — | — | 8.6 | 11.7 | 16.6 | — | — | — | 0.14† | Strong | Ashby J et al.[21] |
| Formaldehyde‡ C=O | 50-00-0 | −2.67 | 0.33 | 30.03 | Acetone | 0.093 | 0.185 | 0.37 | 0.925 | 1.85 | — | 1.1 | 2.3 | 2.3 | 3.9 | 4.0 | — | 0.61 | Strong | Hilton J et al.[37] |
| Furil  | 492-94-4 | −2.90 | 1.38 | 190.15 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.2 | 1.7 | 2.2 | — | — | — | NC | Nonsensitizer | Roberts DW et al.[31] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

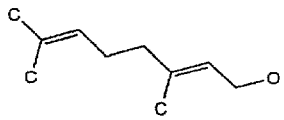
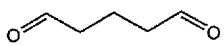
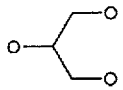
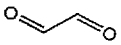
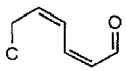
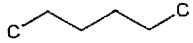
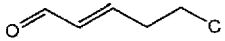
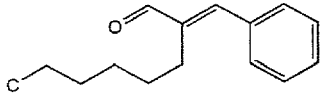
*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

‡Concentration shown as actual concentration of formaldehyde. Material tested was formalin (37% formaldehyde).

(*continued on next page*)

**Table 1.** *Continued*

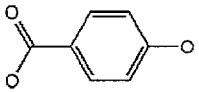
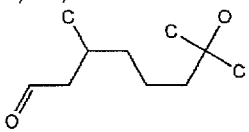
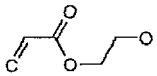
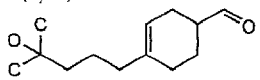
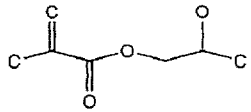
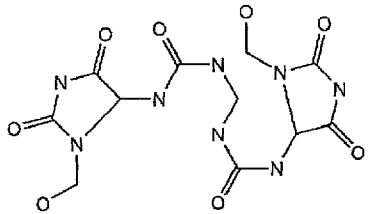
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Geraniol  | 106-24-1 | −1.86 | 2.54 | 154.25 | 75% EtOH/ 25% DEP | 1.0 | 3.0 | 10.0 | 30.0 | 50.0 | — | 1.0 | 1.0 | 1.3 | 3.4 | 3.9 | — | 26 | Weak | Lalko J et al.[38] |
| Glutaraldehyde§  | 111-30-8 | −2.67 | 0.92 | 100.12 | Acetone | 0.05 | 0.125 | 0.25 | 0.5 | 1.25 | 2.5 | 1.3 | 4.3 | 7.6 | 11.6 | 17.7 | 18.0 | 0.1 | Strong | Hilton J et al.[37] |
| Glycerol  | 56-81-5 | −3.25 | 0.05 | 92.09 | DMF | 25.0 | 50.0 | 100.0 | — | — | — | 1.1 | 0.7 | 0.5 | — | — | — | NC | Nonsensitizer | Ryan CA et al.[28] |
| Glyoxal  | 107-22-2 | −2.94 | 0.19 | 58.04 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 2.5 | 4.2 | 5.2 | 10.3 | 15.8 | — | 1.4 | Moderate | Patlewicz G et al.[34] |
| 2,4-Heptadienal  | 5910-85-0 | −2.11 | 1.80 | 110.16 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.1 | 1.4 | 1.9 | 3.7 | 8.1 | — | 4.0 | Moderate | Estrada E et al.[22] |
| Hexane  | 110-54-3 | −1.87 | 1.94 | 86.18 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 0.8 | 0.8 | 2.2 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |
| *trans*-2-Hexenal  | 6728-26-3 | −2.21 | 1.56 | 98.15 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.2 | 1.2 | 2.3 | 2.6 | 6.4 | — | 5.5 | Moderate | Estrada E et al.[22] |
| Hexyl cinnamic aldehyde  | 101-86-0 | −1.36 | 3.77 | 216.32 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 1.3 | 1.1 | 2.5 | 10.0 | 17.0 | — | 11 | Weak | Loveless SE et al.[33] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

§Concentration shown as actual concentration of glutaraldehyde. Material tested was 50% glutaraldehyde solution.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Hydrobenzoic acid  | 99-96-7 | −2.83 | 1.03 | 138.12 | DMSO | 5.0 | 10.0 | 25.0 | — | — | — | 1.4 | 1.5 | 1.3 | — | — | — | NC | Nonsensitizer | Ashby J et al.[21] |
| Hydroxycitronellal  | 107-75-5 | −2.24 | 2.15 | 172.27 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 2.2 | 1 | 0.8 | 1.1 | 7.1 | — | 33 | Weak | Basketter DA et al.[16] |
| 2-Hydroxyethyl acrylate  | 818-61-1 | −3.05 | 0.54 | 116.12 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 10.7 | 14.8 | 18.1 | — | — | — | 1.4† | Moderate | Scholes EW et al.[40] |
| 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (Lyral)  | 31906-04-4 | −1.95 | 2.89 | 210.32 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 0.6 | 0.7 | 0.6 | 1.3 | 4.9 | — | 17 | Weak | Patlewicz G et al.[34] |
| 2-Hydroxypropyl methacrylate  | 923-26-2 | −2.87 | 1.03 | 144.17 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.1 | 1.2 | 1.3 | — | — | — | NC | Nonsensitizer | Basketter DA, Scholes EW.[26] |
| Imidazolidinylurea  | 39236-46-9 | −7.22 | −3.00 | 388.30 | DMF | 10.0 | 25.0 | 50.0 | — | — | — | 1.7 | 3.1 | 5.5 | — | — | — | 24 | Weak | Basketter DA, Scholes EW.[26] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

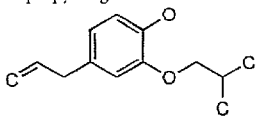
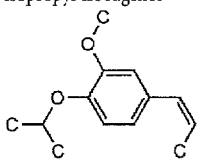
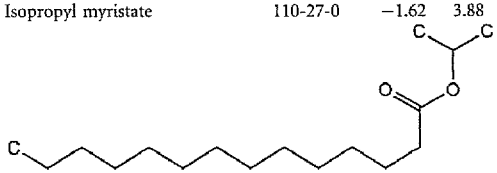
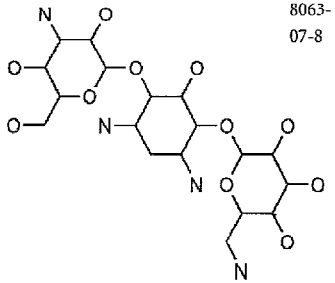
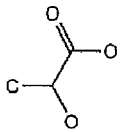
| Chemical Structure | CAS No | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* ($\mu_M$) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-Iodododecane | 4292-19-7 | −1.75 | 3.91 | 296.24 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.7 | 2.3 | 5.7 | — | — | — | 13 | Weak | Estrada E et al.[22] |
| 1-Iodohexadecane | 544-77-4 | −1.39 | 4.89 | 352.35 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.6 | 3.9 | 6.4 | — | — | — | 19 | Weak | Estrada E et al.[22] |
| 1-Iodohexane | 638-45-9 | −2.29 | 2.43 | 212.08 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 0.9 | 1.2 | 2.5 | — | — | — | NC | Nonsensitizer | Unilever (unpublished) |
| 1-Iodononane | 4282-42-2 | −2.02 | 3.17 | 254.16 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.3 | 3.1 | 4.6 | — | — | — | 24 | Weak | Estrada E et al.[22] |
| 1-Iodooctadecane | 629-93-6 | −1.22 | 5.39 | 380.40 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1 | 1.4 | 1.9 | — | — | — | NC | Nonsensitizer | Unilever (unpublished) |
| 1-Iodotetradecane | 19218-94-1 | −1.57 | 4.40 | 324.29 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.7 | 6.9 | 9.7 | — | — | — | 14 | Weak | Ashby J et al.[21] |
| Isoeugenol | 97-54-1 | −2.19 | 2.15 | 164.20 | AOO | 0.50 | 1.0 | 5.0 | — | — | — | 1 | 1.1 | 12.4 | — | — | — | 1.2 | Moderate | Basketter DA, Cadby P.[41] |
| Isononanoyl chloride | 57077-36-8 | −1.99 | 2.54 | 176.69 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 6.6 | 10.6 | 12.6 | — | — | — | 2.7† | Moderate | Unilever (unpublished) |
| Isopropanol | 67-63-0 | −2.51 | 0.82 | 60.10 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.7 | 1.1 | 1.0 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isopropyl eugenol  | 51474-90-9 | −1.93 | 2.89 | 206.29 | AOO | 12.0 | 29.0 | 59.0 | — | — | — | 1.8 | 1.8 | 2.2 | — | — | — | NC | Nonsensitizer | Bertrand F et al.[42] |
| Isopropyl isoeugenol  | 2953-00-7 | −1.93 | 2.89 | 206.29 | AOO | 0.6 | 1.2 | 3.0 | — | — | — | 3 | 5.7 | 10.7 | — | — | — | 0.6† | Strong | Bertrand F et al.[42] |
| Isopropyl myristate  | 110-27-0 | −1.62 | 3.88 | 270.46 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 2.1 | 3.3 | 3.4 | — | — | — | 44 | Weak (false positive) | Ryan CA et al.[28] |
| Kanamycin  | 59-01-8; 8063-07-8 | −6.31 | −0.90 | 484.50 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.2 | 0.8 | 1 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[43] |
| Lactic acid  | 50-21-5 | −3.24 | 0.05 | 90.08 | DMSO | 5.0 | 10.0 | 25.0 | — | — | — | 1.0 | 1.4 | 2.2 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

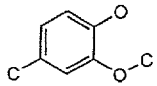
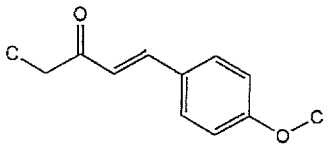
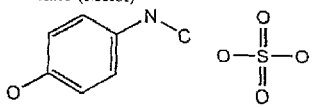
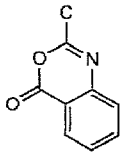
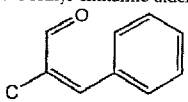
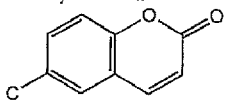
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lauryl gallate (dodecyl gallate) | 1166-52-5 | −2.51 | 3.21 | 338.44 | DMSO | 1.0 | 10.0 | 25.0 | 50.0 | — | — | 12.1 | 29.7 | 29.3 | 36.0 | — | — | 0.3[†] | Strong | P&G (unpublished) |
| R(+)- Limonene | 5989-27-5 | −1.47 | 2.93 | 136.24 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 1.8 | 2.4 | 4.0 | — | — | — | 69 | Weak | Warbrick EV et al.[29] |
| Linalool | 78-70-6 | −1.86 | 2.54 | 154.25 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 2.5 | 4.8 | 8.3 | — | — | — | 30 | Weak | Ryan CA et al.[28] |
| 2-Mercaptobenzothiazole | 149-30-4 | −2.46 | 1.80 | 167.24 | DMF | 1.0 | 3.0 | 10.0 | — | — | — | 2.3 | 4.4 | 8.6 | — | — | — | 1.7 | Moderate | Basketter DA et al.[44] |
| 4′Methoxyacetophenone | 100-06-1 | −2.28 | 1.91 | 150.18 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 1.3 | 1.0 | 1.0 | — | — | — | NC | Nonsensitizer | Ryan CA et al.[28] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-Methoxy-4-methyl-phenol  | 93-51-6 | −2.38 | 1.66 | 138.17 | AOO | 4.2 | 8.4 | 21.0 | — | — | — | 1.8 | 5 | 8.5 | — | — | — | 5.8 | Moderate | Estrada E et al.[22] |
| 1-(p-Methoxyphenyl)-1-penten-3-one  | 104-27-8 | −2.00 | 2.65 | 190.24 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 3.5 | 10.0 | 26.1 | — | — | — | 9.3[†] | Moderate | Ryan CA et al.[28] |
| 4-(Methylamino)phenol sulfate (Metol)  | 55-55-0 | −4.16 | −0.13 | 221.23 | DMF | 0.5 | 1.0 | 2.5 | — | — | — | 2.5 | 3.4 | 6.7 | — | — | — | 0.8 | Strong | Basketter DA, Scholes EW.[26] |
| 2-Methyl-4H,3,1-benzoxazin-4-one (Product 2040)  | 525-76-8 | −2.62 | 1.52 | 161.16 | DMSO | 5.0 | 10.0 | 25.0 | — | — | — | 7.6 | 9.2 | 10.8 | — | — | — | 0.7[†] | Strong | Unilever (unpublished) |
| α-Methyl cinnamic aldehyde  | 101-39-3 | −1.81 | 2.54 | 146.19 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 1.8 | 1.5 | 3.4 | 3.3 | 15.3 | — | 4.5 | Moderate | Elahi EN et al.[23] |
| 6-Methylcoumarin  | 92-48-8 | −2.17 | 2.15 | 160.17 | Acetone | 5.0 | 10.0 | 25.0 | — | — | — | 1.0 | 1.0 | 1.1 | — | — | — | NC | Nonsensitizer | Ashby J et al.[21] |

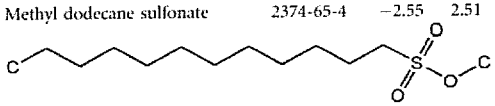
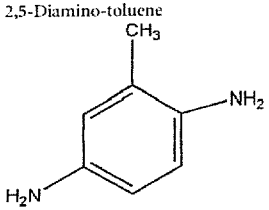
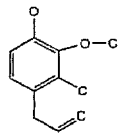
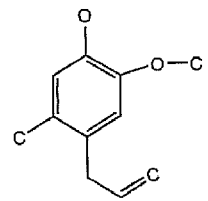
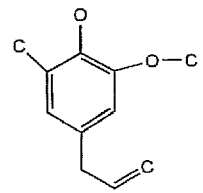
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl dodecane sulfonate  | 2374-65-4 | −2.55 | 2.51 | 264.42 | AOO | 1.0 | 2.5 | 5.0 | — | — | — | 21.6 | 39.9 | 48.6 | — | — | — | 0.39[†] | Strong | Basketter DA, Scholes EW.[26] |
| 2,5-Diamino-toluene  | 95-70-5 | −2.46 | 1.416 | 122.08 | DMSO | 0.125 | 0.25 | 0.5 | 1.0 | — | — | 2.6 | 3.5 | 4.1 | 5.5 | — | — | 0.2 | Strong | P&G (internal) |
| 3-Methyleugenol  | 186743-26-0 | −2.10 | 2.40 | 178.23 | AOO | 11.0 | 27.0 | 54.0 | — | — | — | 1.5 | 2.3 | 6.4 | — | — | — | 32 | Weak | Bertrand F et al.[42] |
| 5-Methyleugenol  | 186743-25-9 | −2.10 | 2.40 | 178.23 | AOO | 11.0 | 27.0 | 54.0 | — | — | — | 2.7 | 4.9 | 4.3 | — | — | — | 13 | Weak | Bertrand F et al.[42] |
| 6-Methyleugenol  | 186743-24-8 | −2.10 | 2.40 | 178.23 | AOO | 11.0 | 27.0 | 54.0 | — | — | — | 1.9 | 4.9 | 8.3 | — | — | — | 17 | Weak | Bertrand F et al.[42] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

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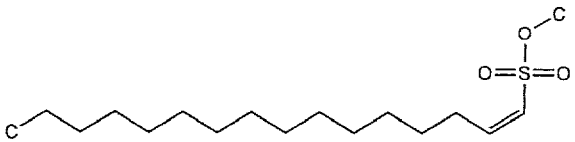
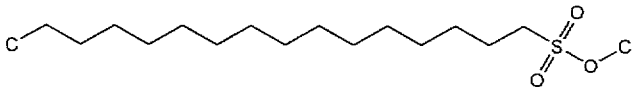
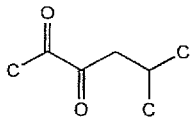
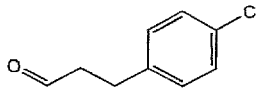
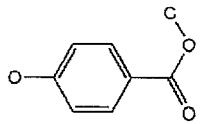
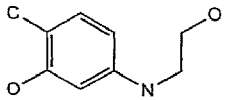
[†]Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl hexadecene sulfonate  | 26452-48-2 | −2.19 | 3.49 | 318.52 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 26.7 | 35.4 | 32.9 | — | — | — | 0.8† | Strong | Ashby J et al.[21] |
| Methyl hexadecyl sulfonate  | 4230-15-3 | −2.20 | 3.49 | 320.53 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1 | 1.3 | 1.5 | — | — | — | NC | Nonsensitizer | Unilever (unpublished) |
| 5-Methyl-2,3-hexanedione  | 13706-86-0 | −2.50 | 1.42 | 128.17 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 2.9 | 6.0 | 14.3 | — | — | — | 26 | Weak | Ryan CA et al.[28] |
| *p*-Methylhydrocinnamic aldehyde  | 5406-12-2 | −1.82 | 2.54 | 148.21 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 1.2 | 1.4 | 2.6 | 4.2 | 10.7 | — | 14 | Weak | Basketter DA et al.[16] |
| Methyl 4-hydroxybenzoate (methylparaben)  | 99-76-3 | −2.74 | 1.28 | 152.15 | DMF | 10.0 | 25.0 | 50.0 | — | — | — | 0.8 | 0.9 | 0.8 | — | — | — | NC | Nonsensitizer | Ryan CA et al.[28] |
| 2-Methyl-5-hydroxyethylaminophenol  | 55302-96-0 | −2.66 | 1.52 | 167.21 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 1.2 | 0.8 | 3.6 | 2.6 | 7.4 | — | 0.4 | Strong | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

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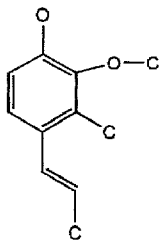
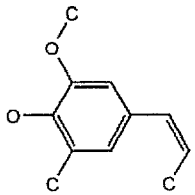
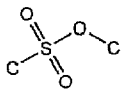
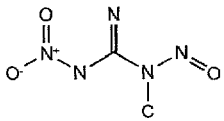
†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

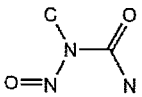
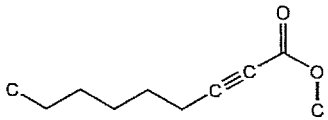
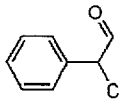
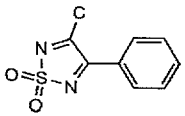
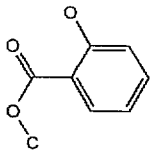
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-Methylisoeugenol  | 186743-29-3 | −2.10 | 2.40 | 178.23 | AOO | 2.5 | 5.5 | 11.0 | — | — | — | 2.2 | 4.3 | 6.0 | — | — | — | 3.6 | Moderate | Bertrand F et al.[42] |
| 6-Methylisoeugenol  | 13041-12-8 | −2.10 | 2.40 | 178.23 | AOO | 2.5 | 5.5 | 11.0 | — | — | — | 5.9 | 11.1 | 15.7 | — | — | — | 1.6[†] | Moderate | Bertrand F et al.[42] |
| 2-Methyl-2H-isothiazol-3-one | 2682-20-4 | −2.94 | 0.68 | 115.15 | AOO | 0.25 | 0.5 | 1.0 | 2.5 | 5.0 | — | 1.5 | 1.5 | 1.8 | 3.8 | 2.5 | — | 1.9 | Moderate | Estrada E et al.[22] |
| Methyl methanesulfonate  | 66-27-3 | −3.53 | −0.20 | 110.13 | AOO | 0.25 | 1.0 | 10.0 | — | — | — | 0.7 | 0.7 | 3.6 | — | — | — | 8.1 | Moderate | Ashby J et al.[21] |
| 1-Methyl-3-nitro-1-nitrosoguanidine  | 70-25-7 | −5.13 | −2.13 | 147.09 | AOO | 0.05 | 0.1 | 0.25 | — | — | — | 27.5 | 60.4 | 78.3 | — | — | — | 0.03[†] | Extreme | Ashby J et al.[21] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

[†]Value estimated.

Table 1. *Continued*

| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *N*-Methyl-*N*-nitrosourea  | 684-93-5 | −4.04 | −0.97 | 103.08 | AOO | 0.05 | 0.1 | 0.25 | — | — | — | 2.7 | 7.1 | 15.4 | — | — | — | 0.05 | Extreme | Ashby J et al.[21] |
| Methyl 2-nonynoate  | 111-80-8 | −2.22 | 2.15 | 168.24 | 80% EtOH | 5.0 | 10.0 | 20.0 | — | — | — | 10.4 | 17.7 | 24.4 | — | — | — | 2.5† | Moderate | Ryan CA et al.[28] |
| α-Methylphenylacetaldehyde  | 93-53-8 | −1.91 | 2.29 | 134.18 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 2 | 2.2 | 1 | 2.2 | 5.2 | — | 6.3 | Moderate | Patlewicz G et al.[34] |
| 3-Methyl-4-phenyl-1,2,5-thiadiazole-1,1-dioxide (MPT)  | 3775-21-1 | −3.18 | 1.14 | 208.24 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 1.3 | 1.1 | 2.1 | 1.9 | 5.6 | — | 1.4 | Moderate | Estrada E et al.[22] |
| Methyl salicylate  | 119-36-8 | −2.74 | 1.28 | 152.15 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 20.0 | — | 1.0 | 1.1 | 1.6 | 1.4 | 0.9 | — | NC | Nonsensitizer | Kimber I et al.[35] |

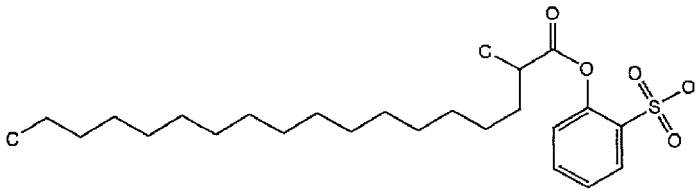
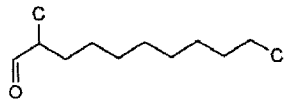
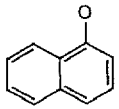
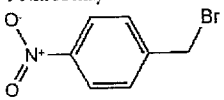
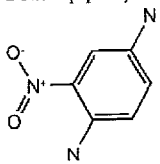
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

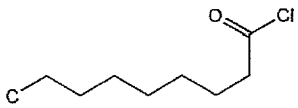
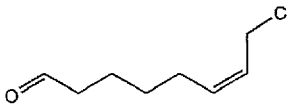
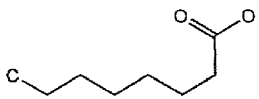
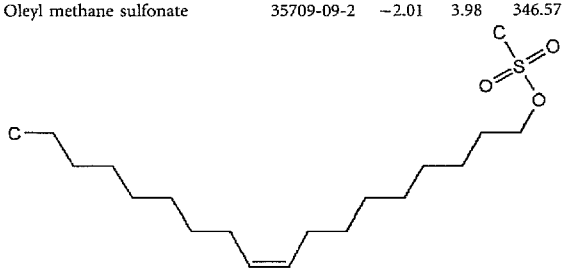
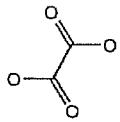
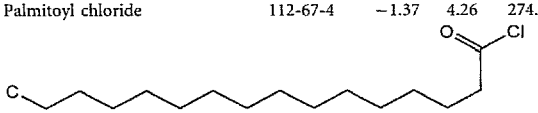
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl 2-sulphophenyl octadecanoate  | — | −2.17 | 4.69 | 454.67 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 5.1 | 11.6 | 25.6 | — | — | — | 2.0† | Moderate | Unilever (unpublished) |
| 2-Methylundecanal  | 110-41-8 | −1.69 | 3.03 | 184.32 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.4 | 1.3 | 1.3 | 2.4 | 3 | — | 10 | Weak | Patlewicz G et al.[34] |
| 1-Naphthol  | 90-15-3 | −1.80 | 2.54 | 144.17 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 1.4 | 1 | 1.2 | 1.5 | 8.5 | — | 1.3 | Moderate | Estrada E et al.[22] |
| 4-Nitrobenzyl bromide  | 100-11-8 | −3.04 | 1.40 | 216.03 | AOO | 0.01 | 0.03 | 0.05 | 0.1 | — | — | 0.9 | 1.3 | 3.5 | 11.5 | — | — | 0.05 | Extreme | Unilever (unpublished) |
| 2-Nitro-p-phenylenediamine  | 5307-14-2 | −3.65 | 0.01 | 153.14 | AOO | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | — | 1.8 | 2.2 | 3.3 | 7.9 | 11.9 | — | 0.4 | Strong | Estrada E et al.[22] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

Table 1. *Continued*

| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nonanoyl chloride  | 764-85-2 | −1.99 | 2.54 | 176.69 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 12.7 | 19.4 | 20.9 | — | — | — | 1.8† | Moderate | Ashby J et al.[21] |
| *cis*-6-Nonenal  | 2277-19-2 | −1.95 | 2.29 | 140.23 | AOO | 10.0 | 25.0 | 50.0 | 100.0 | — | — | 1.6 | 3.2 | 4.5 | 13.7 | — | — | 23 | Weak | Patlewicz G et al.[34] |
| Octanoic acid  | 124-07-2 | −2.42 | 1.66 | 144.21 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 0.7 | 1.0 | 1.6 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |
| Oleyl methane sulfonate  | 35709-09-2 | −2.01 | 3.98 | 346.57 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1 | 1.3 | 3 | — | — | — | 25 | Weak | Roberts DW, Basketter DA.[45] |
| Oxalic acid  | 144-62-7 | −3.69 | −0.59 | 90.03 | DMF | 5.0 | 10.0 | 25.0 | — | — | — | 2.4 | 2.8 | 3.4 | — | — | — | 15 | Weak | Unilever (unpublished) |
| Palmitoyl chloride  | 112-67-4 | −1.37 | 4.26 | 274.88 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.1 | 3.3 | 4.5 | — | — | — | 8.8 | Moderate | Ashby J et al.[21] |

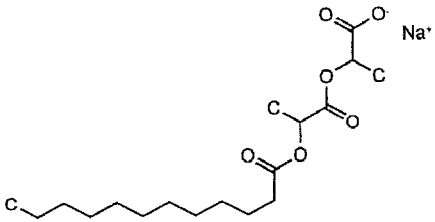
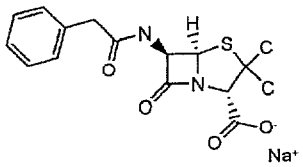
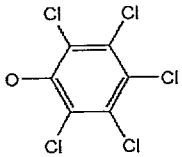
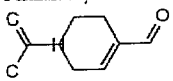
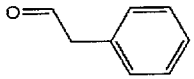
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

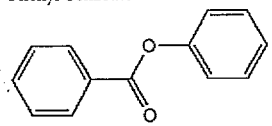
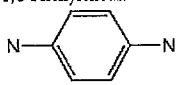
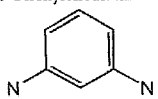
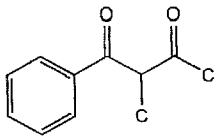
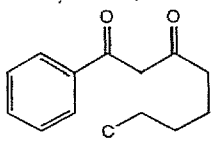
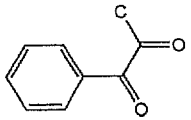
**Table 1.** *Continued*

| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pationic 138C (sodium lauroyl lactylate)  | 13557-75-0 | −3.12 | 2.58 | 366.43 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.4 | 2.5 | 3.9 | — | — | — | 15 | Weak | Unilever (unpublished) |
| Penicillin G  | 61-33-6 | −3.41 | 2.09 | 356.37 | DMSO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 1.0 | 1.0 | 1.4 | 2.1 | 6.6 | — | 30 | Weak | Kimber I et al.[35] |
| Pentachlorophenol  | 87-86-5 | −2.37 | 2.79 | 266.34 | DMSO | 10.0 | 25.0 | 50.0 | — | — | — | 2.1 | 3.5 | 5.4 | — | — | — | 20 | Weak | Basketter DA et al.[43] |
| Perillaldehyde  | 2111-75-3 | −1.83 | 2.54 | 150.22 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 1.2 | 1.1 | 0.9 | 4.3 | — | — | 8.1 | Moderate | Patlewicz G et al.[34] |
| Phenylacetaldehyde  | 122-78-1 | −2.00 | 2.05 | 120.15 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 0.7 | 1.8 | 7.8 | 8.8 | 19.0 | — | 3 | Moderate | Basketter DA et al.[16] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phenyl benzoate  | 93-99-2 | −1.88 | 2.89 | 198.22 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 2.3 | 2.1 | 3.5 | — | — | — | 20 | Weak | Unilever (unpublished) |
| 1,4-Phenylenediamine  | 106-50-3 | −2.55 | 1.17 | 108.14 | AOO | 0.05 | 0.1 | 0.25 | 0.5 | 1.0 | — | 1.9 | 2.3 | 4.0 | 5.7 | 6.6 | — | 0.16 | Strong | Warbrick EV et al.[46] |
| 3-Phenylenediamine  | 108-45-2 | −2.55 | 1.17 | 108.14 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 11.7 | 15.4 | 19.2 | — | — | — | 0.49† | Strong | Ashby J et al.[21] |
| 1-Phenyl-2-methylbutane-1,3-dione  | 6668-24-2 | −2.11 | 2.40 | 179.24 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 1.7 | 2.0 | 4.2 | — | — | — | 29 | Weak | P&G (unpublished) |
| 1-Phenyloctane-1,3-dione  | 55846-68-1 | −1.84 | 3.14 | 221.32 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 2.8 | 6.6 | 8.7 | — | — | — | 11 | Weak | P&G (unpublished) |
| 1-Phenyl-1,2-propanedione  | 579-07-7 | −2.27 | 1.91 | 148.16 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 12.8 | 17.7 | 20.1 | — | — | — | 1.3† | Moderate | Roberts DW et al.[31] |

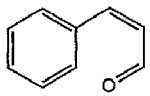
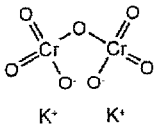
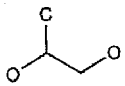
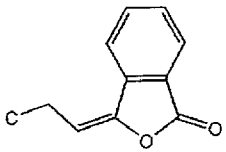
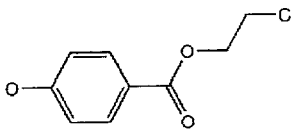
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

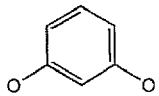
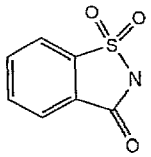
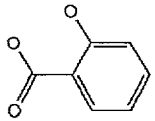
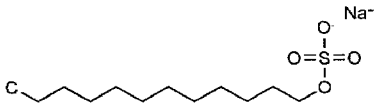
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-Phenyl propenal  | 14371-10-9 | −1.90 | 2.29 | 132.16 | AOO | 1.0 | 2.5 | 5.0 | 10.0 | 25.0 | — | 2.4 | 4.7 | 8.8 | 10.2 | 13.1 | — | 1.4 | Moderate | Patlewicz G et al.[34] |
| Potassium dichromate  | 7778-50-9 | −6.10 | −2.24 | 294.18 | DMSO | 0.025 | 0.05 | 0.1 | 0.25 | 0.5 | — | 1.6 | 1.4 | 3.8 | 5.3 | 16.1 | — | 0.08 | Extreme | Kimber I et al.[47] |
| β-Propriolactone | 57-57-8 | −2.85 | 0.43 | 72.06 | AOO | 0.025 | 1.0 | 2.5 | — | — | — | 1.5 | 13.0 | 19.9 | — | — | — | 0.15 | Strong | Ashby J et al.[21] |
| Propylene glycol  | 57-55-6 | −2.88 | 0.43 | 76.10 | dH2O | 50.0 | 100.0 | — | — | — | — | 1.2 | 1.6 | — | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |
| 3-Propylidenephthalide  | 17369-59-4 | −2.08 | 2.40 | 174.20 | AOO | 5.0 | 10.0 | 20.0 | — | — | — | 4.9 | 9.1 | 15.1 | — | — | — | 3.7† | Moderate | Ryan CA et al.[28] |
| Propylparaben  | 94-13-3 | −2.56 | 1.77 | 180.20 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 1.4 | 1.0 | 1.3 | — | — | — | NC | Nonsensitizer | Ashby J et al.[21] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

**Table 1.** *Continued*

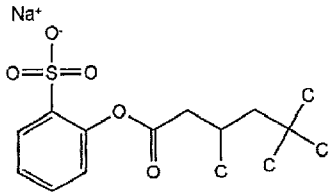
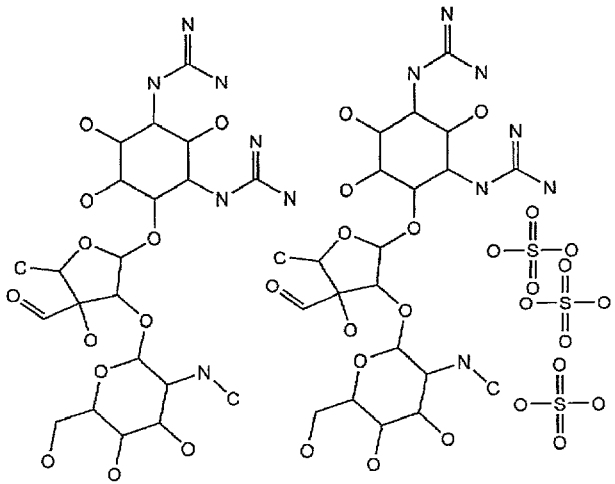
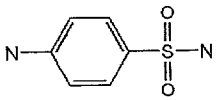
| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pyridine | 110-86-1 | −2.27 | 1.31 | 79.10 | AOO | 25.0 | 50.0 | 100.0 | — | — | — | 1.1 | 2.3 | 3.9 | — | — | — | 72 | Weak | Basketter DA et al.[43] |
| Resorcinol  | 108-46-3 | −2.56 | 1.17 | 110.11 | DMF | 5.0 | 10.0 | 25.0 | — | — | — | 2.2 | 2.2 | 2.7 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[32] |
| Saccharin  | 81-07-2 | −3.38 | 0.64 | 183.18 | DMSO | 25.0 | 50.0 | 75.0 | — | — | — | 1.3 | 1.5 | 1.5 | — | — | — | NC | Nonsensitizer | Warbrick EV et al.[29] |
| Salicylic acid  | 69-72-7 | −2.83 | 1.03 | 138.12 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 0.8 | 1.5 | 2.5 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[39] |
| Sodium lauryl sulfate  | 151-21-3 | −3.15 | 1.87 | 288.38 | DMF | 1.0 | 2.5 | 5.0 | 10.0 | 20.0 | — | 0.9 | 1.1 | 1.7 | 2.6 | 3.5 | — | 14.0 | Weak (false positive) | Loveless SE et al.[33] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

(*continued on next page*)

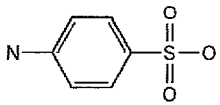
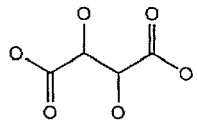
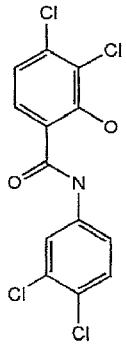
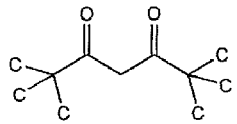
**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sodium 3,5,5-trimethylhexanoyloxy benzenesulfonate  | 94612-91-6 | −3.19 | 2.23 | 336.38 | DMSO | 5.0 | 10.0 | 25.0 | — | — | — | 2.3 | 4.8 | 7.8 | — | — | — | 6.4 | Moderate | Ashby J et al.[21] |
| Streptomycin sulfate  | 3810-74-0 | −17.65 | −8.50 | 1457.38 | DMF | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 1.2 | 1.4 | 1.3 | 2.0 | 1.9 | — | NC | Nonsensitizer | Kimber I et al.[35] |
| Sulfanilamide  | 63-74-1 | −3.49 | 0.40 | 172.20 | DMF | 10.0 | 25.0 | 50.0 | — | — | — | 1 | 1 | 0.9 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[32] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sulfanilic acid  | 121-57-3 | −3.49 | 0.40 | 173.19 | DMF | 5.0 | 10.0 | 25.0 | — | — | — | 1.5 | 1.9 | 2.2 | — | — | — | NC | Nonsensitizer | Basketter DA et al.[48] |
| Tartaric acid  | 87-69-4 | −4.25 | −0.87 | 150.09 | DMF | 5.0 | 10.0 | 25.0 | — | — | — | 1.6 | 3.5 | 6.7 | — | — | — | 8.7 | Moderate | Unilever (unpublished) |
| Tetrachlorosalicylanilide  | 1154-59-2 | −2.38 | 3.49 | 351.02 | Acetone | 0.25 | 0.5 | 1.0 | — | — | — | 11.2 | 14.4 | 18.0 | — | — | — | 0.04† | Extreme | Basketter DA et al.[32] |
| 2,2,6,6-Tetramethyl-heptane-3,5-dione  | 1118-71-4 | −2.15 | 2.40 | 186.30 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 2.1 | 2.8 | 3.4 | — | — | — | 27 | Weak | P&G (unpublished) |

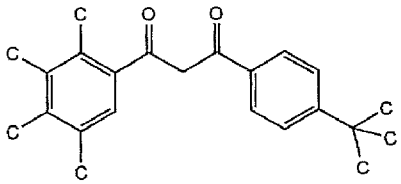
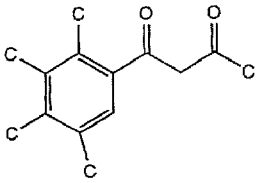
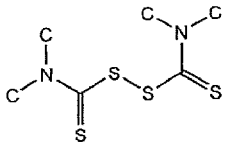
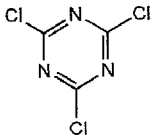
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

Table 1. *Continued*

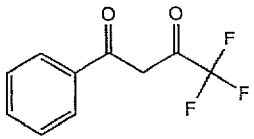
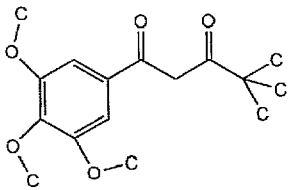
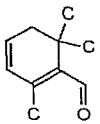
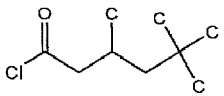
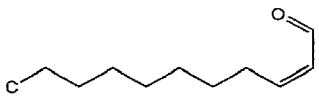
| Chemical Structure | CAS No. | Log Kp | Log $K_{O/W}$ | MW | Vehicle | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA % | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA SI | LLNA EC3* (%) | Potency Category | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-(2',3',4',5'-Tetramethylphenyl)-3-(4'-tertbutylphenyl) propane-1,3-dione  | 55846-68-9 | −0.97 | 5.35 | 336.47 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 1.6 | 1.2 | 1.6 | — | — | — | NC | Nonsensitizer | P&G (unpublished) |
| 1-(2',3'4'5'-Tetramethylphenyl)butane-1,3-dione  | 167998-73-4 | −1.84 | 3.14 | 221.32 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 7.0 | 22.1 | 22.4 | — | — | — | 8.3† | Moderate | P&G (unpublished) |
| Tetramethylthiuram disulfide  | 137-26-8 | −3.36 | 1.17 | 240.42 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 2.4 | 2.9 | 5.1 | — | — | — | 5.2 | Moderate | Basketter DA et al.[43] |
| 2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride)  | 108-77-0 | −3.29 | 0.78 | 184.41 | AOO | 1.0 | 2.5 | 5.0 | — | — | — | 21.8 | 28.9 | 34.0 | — | — | — | 0.09† | Extreme | Ashby J et al.[21] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4,4,4-Trifluro-1-phenylbutane-1,3-dione  | 326-06-7 | −2.27 | 2.52 | 219.18 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 2.1 | 3.0 | 4.6 | — | — | — | 20 | Weak | P&G (unpublished) |
| 1-(3′,4′,5′-Trimethoxyphenyl)-4-dimethylpentane-1,3,-dione  | 135099-98-8 | −2.78 | 2.47 | 297.37 | Acetone | 10.0 | 20.0 | 40.0 | — | — | — | 2.8 | 1.1 | 0.7 | — | — | — | NC | Nonsensitizer | P&G (unpublished) |
| 1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)  | 116-26-7 | −1.83 | 2.54 | 150.22 | AOO | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 | — | 0.7 | 1.1 | 1.1 | 2.7 | 3.3 | — | 7.5 | Moderate | Patlewicz G et al.[34] |
| 3,5,5-Trimethylhexanoyl chloride  | 36727-29-4 | −1.99 | 2.54 | 176.69 | AOO | 5.0 | 10.0 | 25.0 | — | — | — | 7.2 | 12.0 | 19.0 | — | — | — | 2.7† | Moderate | Ashby J et al.[21] |
| Undec-10-enal  | 112-45-8 | −1.77 | 2.79 | 168.28 | AOO | 5.0 | 10.0 | 25.0 | 50.0 | 75.0 | — | 1.7 | 5.3 | 7.5 | 8.7 | 8.8 | — | 6.8 | Moderate | Patlewicz G et al.[34] |

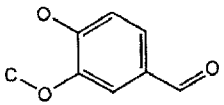
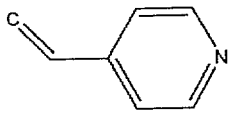
AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

(*continued on next page*)

**Table 1.** *Continued*

| *Chemical Structure* | *CAS No.* | *Log Kp* | *Log $K_{O/W}$* | *MW* | *Vehicle* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA %* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LLNA SI* | *LINA EC3* (%)* | *Potency Category* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vanillin  | 121-33-5 | −2.74 | 1.28 | 152.15 | AOO | 2.5 | 5.0 | 10.0 | 25.0 | 50.0 | — | 0.9 | 1.4 | 1.5 | 1.2 | 1.4 | — | NC | Nonsensitizer | Basketter DA et al.[16] |
| Vinylidene dichloride | 75-35-4 | −2.28 | 1.45 | 96.94 | AOO | 10.0 | 25.0 | 50.0 | — | — | — | 0.8 | 0.8 | 0.9 | — | — | — | NC | Nonsensitizer | Warbrick EV et al.[29] |
| Vinyl pyridine  | 1337-81-1 | −2.08 | 1.80 | 105.14 | AOO | 2.5 | 5.0 | 10.0 | — | — | — | 7.4 | 14.2 | 14.8 | — | — | — | 1.6† | Moderate | Ashby J et al.[21] |

AOO = acetone and olive oil (4:1); CAS = Chemical Abstract Service number; DEP = diethyl phthalate; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH = ethanol; FEMA = Flavor and Extract Manufacturers' Association; $K_{O/W}$ = octanol-water partition coefficient (log scale); Kp = skin penetration coefficient (log scale); LLNA = local lymph node assay; MEK = methyl ethyl ketone; MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; RIFM = Research Institute for Fragrance Materials.

*Mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index (SI).

†Value estimated.

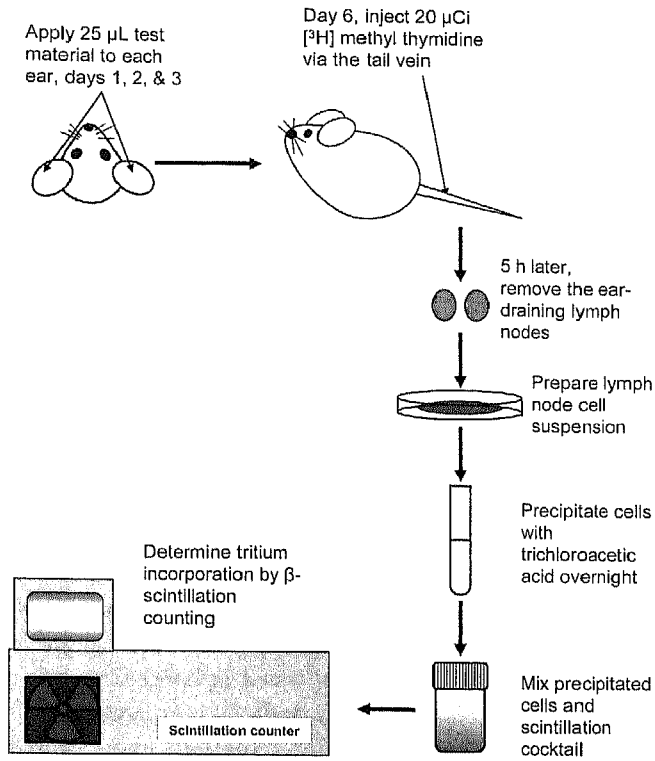



**Figure 1.** Schematic of local lymph node assay.

this article and in Table 1. The data are derived from previous studies that used LLNA methodology as described in the OECD guideline.[7] References for the sources of LLNA data for each of the chemicals are listed in Table 1.[16,21–48]

### Potency Estimation in the Local Lymph Node Assay

The approach to the estimation of the relative skin sensitization potency of chemicals in the LLNA has been previously described in detail.[10] It is based on the mathematical estimation of the concentration of chemical necessary to obtain a threshold positive response (SI = 3); this is termed the EC3 value. When the LLNA dose-response curve included concentrations that induced at least one SI greater than 3 and one SI less than 3, EC3 values were calculated by linear interpolation. For chemicals that induced an SI greater than or equal to 3 at all concentrations tested, an EC3 value was extrapolated from the two lowest doses used.[49] For conducting this extrapolation method, a dose response should be evident. The relative sensitizing potencies of the chemical allergens were categorized with a recently proposed arbitrary classification scheme.[11] The system, shown in Table 2, is composed of four sensitization potency categories based on EC3 values. Compounds that did not induce a threefold increase at any concentration tested (generally > 20%) are categorized as nonsensitizing.

## Results

### Skin Sensitization Data Set: Chemical Information

Table 1 lists 211 chemical compounds along with their respective CAS numbers and two-dimensional chemical structures. It is clear from review of the structures themselves that the data set embraces the wide chemical diversity known to exist among skin allergens. For example, aldehydes, ketones, aromatic amines, quinones, and acrylates are represented in the data set. It is also known that the chemical data set lists compounds that demonstrate different protein reactivity mechanisms (eg, Schiff base, Michael addition). The physicochemical diversity of the allergens is reflected also by the log $K_{O/W}$ values, which range from less than −3 to greater than 5 (Table 3 and Fig 2). The majority of the allergens (92.3%), however, have log $K_{O/W}$ values in the ranges of −1 to 3 and 3 to 5. This is not surprising because chemicals with these ranges are known to be very good and good skin permeants, respectively.[19,50–53] The potency of an allergen does not seem to be associated with any one log $K_{O/W}$ range (eg, extreme allergens are represented in four of the five log $K_{O/W}$ categories). The nonallergens represented in the database have a log $K_{O/W}$ distribution similar to that of the allergens. Similar ranges of log Kp values are evident for both the allergens and nonallergens (data not shown).

Not surprisingly, all of the allergens listed in Table 1 have an MW of less than 500 D, which is consistent with what has been published previously.[54] The MW distribution of the allergens shows that the majority are in the ranges of 100 to 200 D (52.7%) and 200 to 300 D (26.6%) (Table 4 and Fig 3). It is generally believed that chemical allergens have low MWs (< 500 D) and log $K_{O/W}$ values of greater than 1, which are thought to favor the penetration of the chemical across the lipid-rich stratum corneum.[55] For the most part, all potency categories are associated with the MW cutoff ranges depicted in Table 4. The distribution of the nonallergens in the database is similar to that of the allergens.

**Table 2.** Classification of Relative Skin Sensitization Potency by Local Lymph Node Assay EC3 Values

| *EC3 Value (%)* | *Potency Classification* |
|---|---|
| ≥ 10 to ≤ 100 | Weak |
| ≥ 1 to < 10 | Moderate |
| ≥ 0.1 to < 1 | Strong |
| < 0.1 | Extreme |

Table 3. Log $K_{O/W}$ (Log P) Distribution of Allergens by Potency Classification

| *Log $K_{O/W}$ Range* | *Skin Penetration* | *Allergen Potency* Distribution* |
|---|---|---|
| < −3 | Very poor skin permeant, very hydrophobic | 0 |
| ≥ −3 to −1 | Poor skin permeant, hydrophilic | 4 (2E, 1S, 0M, 1W) |
| ≥ −1 to 3 | Very good skin permeant, amphiphilic | 113 (7E, 17S, 54M, 35W) |
| ≥ 3 to 5 | Good skin permeant, slightly lipophilic | 43 (2E, 3S, 11M, 27W) |
| ≥ 5 | Moderate skin permeant but decreasing with increasing log $K_{O/W}$ | 9 (2E, 0S, 4M, 3W) |

*Designated as E (extreme), S (strong), M (moderate), and W (weak).

### Skin Sensitization Data Set: Biologic Data

The LLNA data for each of the 211 chemicals are displayed in Table 1. The data set includes weak, moderate, strong, and extreme skin sensitizers, as well as nonsensitizers. The LLNA data in this database were from studies that used the standard LLNA protocol as described in OECD Test Guideline 429.[7] For some chemicals (such as those that formed the basis of the various interlaboratory trials), several different EC3 values were available albeit within a narrow range. In each of these cases, the data shown in Table 1 derive from one representative experiment that we feel reflects accurately the results obtained with the chemical. For all of the studies represented in Table 1, a common vehicle as described in OECD Test Guideline 429 was used (eg, acetone and olive oil 4:1 [AOO], and dimethyl sulfoxide). In instances in which the chemical was tested in more than one vehicle, the data set for AOO was selected.

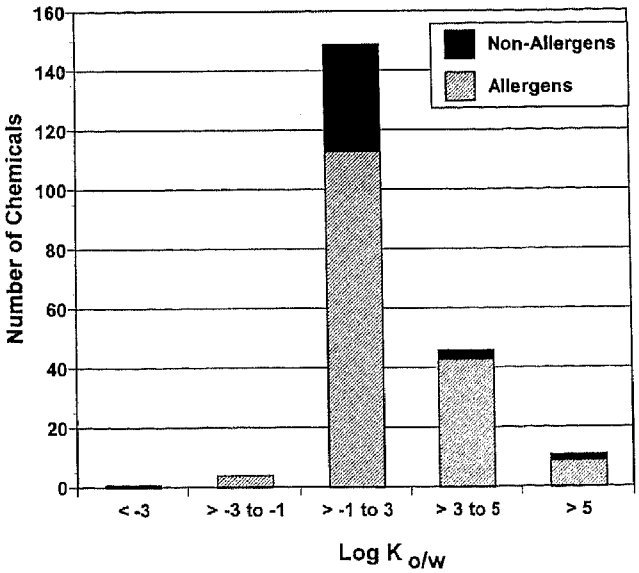



Figure 2. Distribution of log $K_{O/W}$ values of allergens and nonallergens.

The specific reference for the source of the LLNA data for each chemical is indicated in Table 1. The data set includes 42 nonsensitizers, 66 weak sensitizers, 69 moderate sensitizers, 21 strong sensitizers, and 13 extreme sensitizers, for a total of 211 compounds (Fig 4). For the nonsensitizers, materials that did not give a positive response in the LLNA up to the highest dose tested were included (data for nonsensitizers were most often tested at concentrations of > 20%). For positive chemicals in the LLNA database, EC3 values (calculated from linear interpolation) range from as low as 0.003% (for the sensitizer oxazolone) to as high as 89% (for aniline). In Table 1, we have also included EC3 values estimated by the extrapolation method; these are indicated by a footnote marker. Of course, caution should be used in interpreting and using extrapolated EC3 values that have been estimated from less-than-perfect dose-response data. However, it is our opinion that these data can still provide important benchmark information to investigators who are developing alternative skin sensitization testing methods.

## Discussion

One major focus area for scientists from academia and industry over the past decade has been to develop alternative nonanimal test methods for skin sensitization testing.

Table 4. Molecular Weight Distribution of Allergen Potency

| *MW Range* | *Allergen Potency* Distribution* |
|---|---|
| < 100 | 10 (0E, 2S, 3M, 5W) |
| ≥ 100 to 200 | 89 (5E, 11S, 45M, 28W) |
| ≥ 200 to 300 | 45 (7E, 4S, 12M, 22W) |
| ≥ 300 to 400 | 23 (1E, 3S, 8M, 11W) |
| ≥ 400 | 2 (0E, 1S, 1M, 0W) |

MW = molecular weight.
*Designated as E (extreme), S (strong), M (moderate), and W (weak).

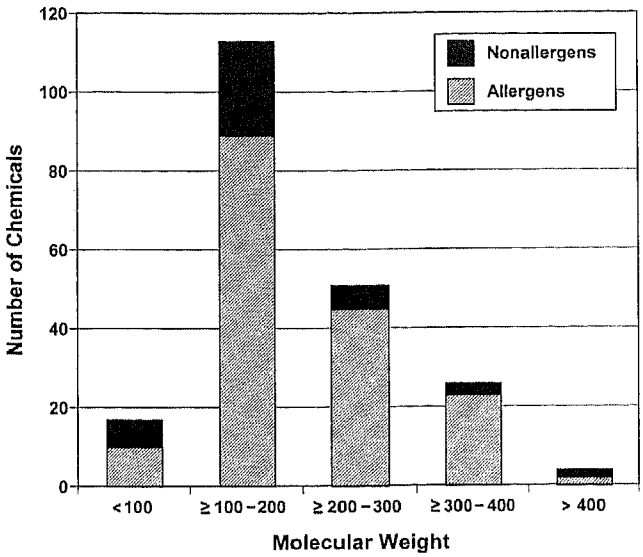



**Figure 3.** Distribution of molecular weights of allergens and nonallergens.

It is an enormous challenge to reproduce accurately in vitro the complex immunobiologic mechanisms that act in concert to permit skin sensitization. For example, numerous cell types (eg, T lymphocytes, Langerhans cells, keratinocytes) and a plethora of immune and inflammatory mediators (including cytokines and chemokines) are involved in the initiation and expression of an ACD response. The key to success will be the development of a test method or methods that incorporate all aspects of our understanding of the chemistry and biology of contact allergy.

Whatever new methods are developed, whether they are in silico quantitative structure activity system models or in vitro cell-based methods, they will be required to have their performance characteristics assessed, particularly their sensitivity, selectivity, and overall accuracy. This in turn requires the selection of a robust chemical data set to interrogate and calibrate the method.[49,56] One essential criterion is that the activity of each of the chemicals used must be supported by relevant and reliable in vivo data of high quality. In addition, it is important to select chemicals that display a wide range of potencies and that represent the relevant classes of chemicals and physical properties of the materials known to cause the specific endpoint. To this end, we compiled a data set of chemicals to be used for evaluating alternative approaches to skin sensitization testing that meet these criteria and that encompass the chemical and biologic diversity of chemicals known to cause skin sensitization in animals and/or in humans.

The chemicals listed in Table 1 have clearly different protein reaction mechanisms (eg, Schiff base formation, Michael addition) and include representatives from various chemical classes of materials, including aldehydes, ketones, diketones, acrylates, and aromatic amines. The two-dimensional structure and CAS number of each compound is provided to aid investigators in obtaining the correct materials. It is well known that skin allergens must have a relatively low MW ($\leq$ 500 D)[54] and appropriate physicochemical properties (eg, lipophilicity).[27,57] The chemicals compiled in this data set demonstrate a distribution of log $K_{O/W}$ values and MWs that are consistent with chemicals known to be good to very good skin penetrants (see Tables 3 and 4 and Figs 2 and 3). It is known also that chemicals of equal molecular weight can have very different skin penetration potentials, depending on their lipophilicity. For example, most skin permeants are amphiphilic (or amphipathic) chemicals with a trend toward slight lipophilicity. It is also interesting that the allergenic potency of the chemicals compiled in the database are not associated with any one particular log $K_{O/W}$ or MW category (see Tables 3 and 4). The nonallergens in the database show similar distributions for the log $K_{O/W}$ and MW categories.

In addition to chemical diversity, it is particularly important that the enormous range of potencies known for skin allergens be encompassed. It is believed that differences between contact allergens with respect to their relative skin sensitizing potencies can span four or more orders of magnitude.[11,15,58] The chemicals compiled in this LLNA database display a range of potencies, which is referred to as the EC3 value.[10] The preferred method for deriving EC3 values is linear interpolation using the data points (concentration and SI) immediately above and below the SI value of 3 on the LLNA dose-response curve. In addition, we employed a method for estimating EC3 values that can be applied in instances in which none of the

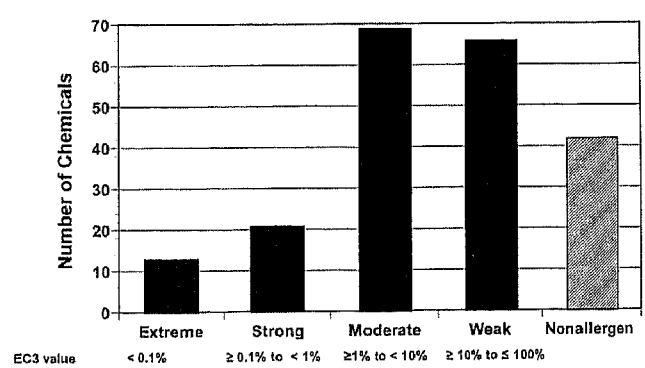



**Figure 4.** European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) potency category distribution for allergens.

tested concentrations results in an SI of less than 3 and in which there is clear evidence of a dose response.[49] Although caution needs to be taken when employing this extrapolation method to obtain an EC3 value estimate, the method can provide information regarding likely threshold values and may obviate the need for repeat animal testing. It is also important to note that the data sets provided in this article are representative of the types of dose responses observed in the assay. For some chemicals (eg, camphorquinone), a decrease in SI values at the highest concentration tested may be indicative of local or systemic toxicity. Additional information such as body weight data can provide guidance in interpreting these types of responses.

The LLNA EC3 values listed in Table 1 show an enormous range of potency, covering four orders of magnitude, from weak allergens to extreme allergens. Each of the ECETOC potency categories established by the European Centre for Ecotoxicology and Toxicology of Chemicals[11,12] is well represented among the 211 chemicals in the data set (ie, 42 nonsensitizers, 66 weak sensitizers, 69 moderate sensitizers, 21 strong sensitizers, and 13 extreme sensitizers) (see Fig 4). Our experience is that the allergenic potency for mice as determined with the LLNA is similar to that known for humans.[16,17,59]

In terms of hazard identification (to the standards required globally for regulatory classification), the LLNA demonstrates the same accuracy as the Buehler and guinea pig maximization tests for predicting the human response.[8] Thus, there are a few compounds that demonstrate discordance between the LLNA results and human experience. For completeness, we chose to include those chemicals as well in this data set. For example, benzocaine, methyl 4-hydroxybenzoate (methylparaben), streptomycin sulfate, and vanillin are chemicals that are not detected as skin sensitizers in the LLNA but that have been reported as human allergens.[16,17,25,28,35] It is important to note that none of these chemicals that escaped detection in the LLNA are considered significant human contact allergens, nor do they typically give positive results in guinea pig tests. In addition, there are chemicals that give positive responses in the LLNA but are not considered skin allergens in humans. For example, isopropyl myristate and sodium lauryl sulfate yielded positive LLNA responses (albeit weak) whereas human experience suggests that these compounds are nonallergens.[28,33]

It is well known that some chemical allergens require biotransformation to be capable of initiating a skin sensitization response in vivo.[27] Thus, chemicals that are known to undergo activation or metabolism in the skin to acquire reactivity are represented in this data set. (Such chemicals are called prohaptens; for example, eugenol and isoeugenol are considered to be prohaptens.)[27,42] Because it is known that some chemical allergens must undergo biotransformation prior to reacting with proteins/peptides, it will be critical to incorporate a metabolism component into alternative assays in order to address these types of molecules.

An important measure of any new in vitro test method will be the extent of its usefulness in the skin sensitization risk assessment process. Although it would be of value to have in vitro methods available to assess the skin sensitization hazard of novel chemicals, it would be more valuable to have available methods that can extrapolate that hazard as risk to humans.[1] In addition to determining skin sensitization potential (hazard), the LLNA yields important information for assessing sensitization risk, the relative allergenic potency of a chemical.[15,17,28] Therefore, it will be useful to compare any new in vitro skin sensitization test method to the murine LLNA for its ability to determine allergenic potency. On the other hand, in vitro methods that can be used only for hazard identification would still be valuable because they can be used for screening purposes and will help to reduce the need for animal testing.

The list of chemicals contained in the data set represents both the chemical and biologic diversity known to exist for chemical allergens and nonallergens. It is our hope that publishing this LLNA data set will provide investigators with key information needed to accelerate the development and subsequent evaluation of alternative test methods and ultimately reduce the reliance on animals for assessing the skin sensitization potential of new chemicals.

## References

1. Gerberick GF, Robinson MK. A skin sensitization risk assessment process for evaluation of new ingredients and products. Am J Contact Dermat 2000;11:65–73.
2. Dearman RJ, Basketter DA, Kimber I. Local lymph node assay—use in hazard and risk assessment. J Appl Toxicol 1999;19:299–306.
3. Gerberick GF, Ryan CA, Kimber I, et al. Local lymph node assay validation assessment for regulatory purposes. Am J Contact Dermat 2000;11:3–18.
4. Basketter DA, Evans P, Fielder RJ, et al. Local lymph node assay—validation and use in practice. Food Chem Toxicol 2002;40:593–8.
5. Kimber I, Dearman RJ, Scholes EW, Basketter DA. The local lymph node assay: developments and applications. Toxicology 1994;93:13–31.
6. Kimber I, Dearman RJ, Basketter DA, et al. The local lymph node assay: past, present and future. Contact Dermatitis 2002;47:315–28.
7. Organization for Economic Cooperation and Development. Guidelines for testing of chemicals. Guideline no. 429. Skin sensitization:

the local lymph node assay. Paris: Organization for Economic Co-operation and Development; 2002.

8. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. Report. 1999. NIH Publication No.: 99-4494. National Institutes of Health, Bethesda, MD.
9. Balls M, Hellsten E. Statement on the validity of the local lymph node assay for skin sensitization testing. ECVAM Joint Research Centre, European Commission, Ispra, Italy, 21 March. ATLA 2000;28:366–7.
10. Basketter DA, Lea LJ, Cooper K, et al. A comparison of statistical approaches to derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 1999;19:261–6.
11. Kimber I, Basketter DA, Butler M, et al. Classification of contact allergens according to potency: proposals. Food Chem Toxicol 2003;41:1799–809.
12. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Contact sensitization: classification according to potency. Brussels: ECETOC; 2003. Technical Report 87.
13. Ryan CA, Hulette BC, Gerberick GF. Approaches for the development of cell based in vitro methods for contact sensitization. Toxicol In Vitro 2001;15:43–55.
14. Casati S, Aeby P, Basketter DA, et al. Dendritic cells as a tool for the predictive identification of skin sensitisation hazard: the report and recommendation of ECVAM workshop 51. ATLA 2005;33: 47–62.
15. Basketter DA, Blaikie L, Dearman RJ, et al. Use of the local lymph node assay for the estimation of relative contact allergenic potency. Contact Dermatitis 2000;42:344–8.
16. Basketter DA, Wright ZM, Warbrick EV, et al. Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis 2001;45:89–94.
17. Gerberick GF, Robinson MK, Ryan CA, et al. Contact allergenic potency: correlation of human and local lymph node assay data. Am J Contact Dermat 2001;12:156–61.
18. Barratt MD, Basketter DA, Chamberlain M, et al. An expert system rulebase for identifying contact allergens. Toxicol In Vitro 1994; 8:1053–60.
19. Potts RO, Guy RH. Predicting skin permeability. Pharmacol Res 1992;9:663–9.
20. Moriguchi I, Hirono S, Nakagome I, Hirano H. Comparison of reliability of log P values for drugs calculated by several methods. Chem Pharm Bull 1994;42:976–8.
21. Ashby J, Basketter DA, Patton D, Kimber I. Structure activity relationships in skin sensitization using the murine local lymph node assay. Toxicology 1995;103:177–94.
22. Estrada E, Patlewicz G, Chamberlain M, et al. Computer-aided knowledge generation for understanding skin sensitization mechanisms: the TOPS-MODE approach. Chem Res Toxicol 2003;16: 1226–35.
23. Elahi EN, Wright Z, Hinselwood D, et al. Protein binding and metabolism influence on the relative skin sensitization potential of cinnamic compounds. Chem Res Toxicol 2004;17:301–10.
24. Patlewicz G, Basketter DA, Smith CK, et al. Skin-sensitization structure-activity relationships for aldehydes. Contact Dermatitis 2001;44:331–6.
25. Warbrick EV, Dearman RJ, Basketter DA, Kimber I. Failure of vehicle to influence local lymph node assay response to benzocaine. Contact Dermatitis 2000;42:164–5.
26. Basketter DA, Scholes EW. Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 1992;30:65–9.
27. Smith CK, Hotchkiss SA. Allergic contact dermatitis: chemical and metabolic mechanisms. London: Taylor & Francis; 2001; Appendix I.
28. Ryan CA, Gerberick GF, Cruse LW, et al. Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 2000;43:95–102.
29. Warbrick EV, Dearman RJ, Ashby J, et al. Preliminary assessment of the skin sensitizing activity of selected rodent carcinogens using the local lymph node assay. Toxicology 2001;163:63–9.
30. Basketter DA, Roberts DW, Cronin M, Scholes EW. The value of the local lymph node assay in quantitative structure-activity investigations. Contact Dermatitis 1992;27:137–42.
31. Roberts DW, York M, Basketter DA. Structure-activity relationships in the murine local lymph node assay for skin sensitization: a,b-diketones. Contact Dermatitis 1999;41:14–7.
32. Basketter DA, Scholes EW, Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol 1994;32: 543–7.
33. Loveless SE, Ladics GS, Gerberick GF, et al. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology 1996;108:141–52.
34. Patlewicz G, Wright ZM, Basketter DA, et al. Structure-activity relationships for selected fragrance allergens. Contact Dermatitis 2002;47:219–26.
35. Kimber I, Hilton J, Dearman RJ, et al. Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: an inter-laboratory evaluation. J Toxicol Environ Health 1998;53:563–79.
36. Wright ZM, Basketter DA, Blaikie L, et al. Vehicle effects on skin sensitizing potential of four chemicals: assessment using the local lymph node assay. Int J Cosmet Sci 2001;23:75–83.
37. Hilton J, Dearman RJ, Harvey P, et al. Estimation of relative skin sensitization potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. Am J Contact Dermat 1998;9:29–33.
38. Lalko J, Isola D, Api AM. Ethanol and diethyl phthalate: vehicle effects in the local lymph node assay. Int J Toxicol 2004;23:171–7.
39. Basketter DA, Gerberick GF, Kimber I. Strategies for identifying false positive responses in predictive skin sensitization tests. Food Chem Toxicol 1998;36:327–33.
40. Scholes EW, Basketter DA, Sarll AE, et al. The local lymph node assay: results of a final inter-laboratory validation under field conditions. J Appl Toxicol 1992;12:217–22.
41. Basketter DA, Cadby P. Reproducible prediction of contact allergenic potency using the local lymph node assay. Contact Dermatitis 2004;50:15–7.
42. Bertrand F, Basketter DA, Roberts DW, Lepoittevin J-P. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. Chem Res Toxicol 1997;10:335–43.
43. Basketter DA, Gerberick GF, Kimber I, Loveless SE. The local

lymph node assay: a viable alternative to currently accepted skin sensitization tests. Food Chem Toxicol 1996;34:985–97.

44. Basketter DA, Selbie E, Scholes EW, et al. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. Food Chem Toxicol 1993; 31:63–7.
45. Roberts DW, Basketter DA. Quantitative structure-activity relationships: sulfonate esters in the local lymph node assay. Contact Dermatitis 2000;42:154–61.
46. Warbrick EV, Dearman RJ, Lea LJ, et al. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. J Appl Toxicol 1999;19:255–60.
47. Kimber I, Hilton J, Dearman RJ, et al. An international evaluation of the murine local lymph node assay and comparison of modified procedures. Toxicology 1995;103:63–73.
48. Basketter DA, Scholes EW, Cumberbatch M, et al. Sulphanilic acid: divergent results in the guinea pig maximization test and the local lymph node assay. Contact Dermatitis 1992;27:209–13.
49. Gerberick GF, Ryan CA, Kern PS, et al. A chemical dataset for evaluation of alternative approaches to skin sensitization testing. Contact Dermatitis 2004;50:274–88.
50. European Commission 2002. Guidance document on dermal absorption. Sanco/222/2000 rev. 6, 27. p. 6. European Commission, Health & Consumer Protection Directorate-General, Brussels.
51. EPA (United States Environmental Protection Agency) 1992. Dermal exposure assessment: principles and applications. Interim report EPA/600/8-91/011B p. 2–31f. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington DC.
52. De Heer C, Wilschut A, Stevenson H, Hackkert BC. 1999. Guidance document on the estimation of dermal absorption according to a tiered approach: an update. Zeist, The Netherlands: TNO Voeding. TNO Report No.: V98 1237, 27p.
53. Blank IH, Scheuplein RJ, MacFarlane DJ. Mechanism of percutaneous absorption. III. The effect of temperature on the transport of non-electrolytes across the skin. J Invest Dermatol 1967;49: 582–9.
54. Bos JD, Meinardi MM. The 500 dalton rule for skin penetration of chemical compounds and drugs. Exp Dermatol 2000;9:165–9.
55. Smith-Pease CK, Basketter DA, Patlewicz GY. Contact allergy: the role of skin chemistry and metabolism. Clin Exp Dermatol 2003; 2:177–83.
56. Balls M, Blaauboer BJ, Fentem JH, et al. Practical aspects of the validation of toxicity test procedures. Altern Lab Anim 1995;23: 129–47.
57. Lepoittevin J-P, Basketter DA, Goossens A, Karlberg A-T. Allergic contact dermatitis: the molecular basis. Berlin: Springer; 1998. p. 143–6.
58. Robinson MK, Gerberick GF, Ryan CA, et al. The importance of exposure estimation in the assessment of skin sensitization risk. Contact Dermatitis 2000;42:251–9.
59. Basketter DA, Clapp C, Jefferies D, et al. Predictive identification of human skin sensitization thresholds. Contact Dermatis [In press]