**Supplemental Materials**

**QSAR models of human data can enrich or replace LLNA testing for human skin sensitization**

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# Cluster analysis

In Cluster 1, there are four sensitizers and four non-sensitizers. All sensitizers were predicted correctly by QSAR. Similar to outliers in previous sections, benzyl alcohol (sensitizer) has a high DSA05 of 48.67 µg/cm2 (45.06 mol/m2) and a NOEL of 5,906 µg/cm2 (5.47 mol/m2). The very low skin sensitization potency may be the reason for the wrong prediction by our QSAR model. Propylidene phthalate was also mispredicted. The other two sensitizers (2-mercaptobenzothiazole and benzisothiazole) were correctly predicted. Human non-sensitizers benzoic acid, salicylic acid, methyl salicylate, and resorcinol were correctly predicted by QSAR. Of those, the only compound correctly predicted by LLNA was benzoic acid. In a recent study 1, resorcinol is labeled as a human sensitizer, but no NOEL is available. It is used at high levels in hair dyes and skin preparations, but is not considered to be dangerous, since it has a low frequency of human sensitization.

Pyridine, one of the outliers detected in the previous section, is in the cluster of aromatic amines (Cluster 2). Within this cluster, sulfanilamide (human sensitizer) was the only compound mispredicted by the LLNA. The two non-sensitizers (p-aminobenzoic acid and sulfanilic acid) were mispredicted by QSAR model. Sulfanilamide has a similar structure to sulfanilic acid, with Tanimoto coefficient equal to 0.83. The substitution of a sulfo group or a carboxyl in the *para*- position of aniline decreases sensitization potency, while another amine preserves it.

Phenyl benzoate was also identified as an outlier, due to the high DSA05, which shows that this compound is safe at low concentrations in most of the tested population. All of the non-sensitizers in this cluster (Cluster 3) were mispredicted by the LLNA and correctly predicted by QSAR: benzyl cinnamate, benzyl benzoate, benzyl salicylate, and hexyl salicylate. The sensitizers benzoyl peroxide and phenyl benzoate were mispredicted and correctly predicted, respectively. Both compounds were correctly predicted by the LLNA.

In Cluster 8, α-amylcinnamyl alcohol is the only human sensitizer. This compound was predicted as a non-sensitizer by LLNA and QSAR. All other compounds (α-amylcinnamic aldehyde, hexyl cinnamic aldehyde, and hexyl salicylate) were human non-sensitizers and were mispredicted by LLNA correctly predicted by QSAR. In a recent publication 1, hexyl salicylate and α-amylcinnamic aldehyde are labeled as human sensitizers. The first has a NOELs as 35,433 (15.96 mol/m2), which indicates low sensitization rates in the tested populations at relatively high doses, while the second is in fact a strong sensitizer, with DSA05 of 23.622 (µg/cm2) 0.01 (mol/m2).

In the Cluster 11, lilial was the only compound correctly predicted by QSAR models. This compound is labeled as a sensitizer; however, it has a high DSA05, since at the LOEL of 29.53 µg/cm2 it was positive in only one out of 225 people. Cyclamen aldehyde (non-sensitizer) was mispredicted, while bourgeonal (sensitizer) and majantal (non-sensitizer) were predicted as sensitizers. All the compounds in this cluster, except cyclamen aldehyde, were correctly predicted by LLNA.

# Table S1. Number of records, assay outcome, and QSAR prediction for the 62 substances with multiple records present in the human skin sensitization dataset (Dataset A).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **#** | **Compound name** | **CASRN** | **No. of records** | **No. of sensitizers** | **No. of non-sensitizers** | **QSAR** |
| 1 | (Chloro)methylisothia-zolinone (Kathon) | 26172-55-4 | 14 | 4 | 10 | Not predicted |
| 2 | Hydroxycitronellal | 107-75-5 | 12 | 8 | 4 | Sensitizer |
| 3 | Cinnamyl alcohol | 104-54-1 | 10 | 6 | 4 | Sensitizer |
| 4 | Cinnamic aldehyde | 104-55-2 | 8 | 5 | 3 | Sensitizer |
| 5 | Citral | 5392-40-5 | 7 | 5 | 2 | Non-sensitizer |
| 6 | Streptomycin | 3810-74-0 | 6 | 6 | 0 | Sensitizer |
| 7 | Phenylacetaldehyde | 122-78-1 | 6 | 5 | 1 | Non-sensitizer |
| 8 | Geraniol | 106-24-1 | 6 | 2 | 4 | Sensitizer |
| 9 | Benzoyl peroxide | 94-36-0 | 5 | 5 | 0 | Non-sensitizer |
| 10 | Neomycin sulfate | 1405-10-3 | 5 | 5 | 0 | Sensitizer |
| 11 | Penicillin G | 61-33-6 | 5 | 4 | 1 | Sensitizer |
| 12 | Benzocaine | 94-09-7 | 5 | 4 | 1 | Sensitizer |
| 13 | Ethyl acrylate | 140-88-5 | 5 | 3 | 2 | Sensitizer |
| 14 | Methylisothiazolinone | 2682-20-4 | 5 | 2 | 3 | Sensitizer |
| 15 | Coumarin | 91-64-5 | 5 | 2 | 3 | Sensitizer |
| 16 | Eugenol | 97-53-0 | 5 | 1 | 4 | Sensitizer |
| 17 | *dl*-Citronellol | 26489-01-0 | 5 | 1 | 4 | Non-sensitizer |
| 18 | 4-Phenylenediamine | 106-50-3 | 4 | 4 | 0 | Sensitizer |
| 19 | Methylhexanedione | 13706-86-0 | 4 | 4 | 0 | Non-sensitizer |
| 20 | Tetrachlorosalicylanilide | 1154-59-2 | 4 | 4 | 0 | Sensitizer |
| 21 | Isoeugenol | 97-54-1 | 4 | 2 | 2 | Non-sensitizer |
| 22 | Lilial | 80-54-6 | 4 | 1 | 3 | Sensitizer |
| 23 | Cinnamyl nitrile | 4360-47-8 | 4 | 1 | 3 | Sensitizer |
| 24 | Potassium dichromate | 7778-50-9 | 3 | 3 | 0 | Not predicted |
| 25 | Thioglycerol | 96-27-5 | 3 | 3 | 0 | Non-sensitizer |
| 26 | Nickel (II) salts | 7718-54-9; 7786-81-4 | 3 | 3 | 0 | Not predicted |
| 27 | Formaldehyde | 50-00-0 | 3 | 2 | 1 | Sensitizer |
| 28 | Tetramethylthiruam-disulfide | 137-26-8 | 3 | 2 | 1 | Sensitizer |
| 29 | Propylidene phthalate | 17369-59-4 | 3 | 1 | 2 | Non-sensitizer |
| 30 | α-amylcinnamyl alcohol | 101-85-9 | 3 | 1 | 2 | Non-sensitizer |
| 31 | Benzyl alcohol | 100-51-6 | 3 | 1 | 2 | Non-sensitizer |
| 32 | Oakmoss | 68917-10-2 | 3 | 1 | 2 | Not predicted |
| 33 | Treemoss | 68648-41-9 | 3 | 1 | 2 | Not predicted |
| 34 | Linalool | 78-70-6 | 3 | 0 | 3 | Non-sensitizer |
| 35 | Clove oil (bud, leaf, stem) | 8000-34-8 | 3 | 0 | 3 | Not predicted |
| 36 | 2,4-Dinitrochlorobenzene | 97-00-7 | 2 | 2 | 0 | Sensitizer |
| 37 | 2-Mercaptobenzothiazole | 149-30-4 | 2 | 2 | 0 | Sensitizer |
| 38 | Benzylidene acetone | 122-57-6 | 2 | 2 | 0 | Non-sensitizer |
| 39 | Diethylmaleate | 141-05-9 | 2 | 2 | 0 | Sensitizer |
| 40 | Dihydrocoumarin | 119-84-6 | 2 | 2 | 0 | Non-sensitizer |
| 41 | Cobalt (II) salts | 7646-79-9; 10124-43-3 | 2 | 2 | 0 | Not predicted |
| 42 | Mercuric (II) chloride | 7487-94-7 | 2 | 2 | 0 | Not predicted |
| 43 | Benzoisothiazolione | 2634-33-5 | 2 | 1 | 1 | Sensitizer |
| 44 | Farnesol | 4602-84-0 | 2 | 1 | 1 | Sensitizer |
| 45 | Glutaraldehyde | 111-30-8 | 2 | 1 | 1 | Sensitizer |
| 46 | Imidazolidinyl urea | 39236-46-9 | 2 | 1 | 1 | Sensitizer |
| 47 | Isocyclogeraniol | 68527-77-5 | 2 | 1 | 1 | Non-sensitizer |
| 48 | Methyl 2-nonynoate | 111-80-8 | 2 | 1 | 1 | Sensitizer |
| 49 | Methyl 2-octynoate | 111-12-6 | 2 | 1 | 1 | Sensitizer |
| 50 | *p*-methylhydrocinnamic aldehyde | 5406-12-2 | 2 | 1 | 1 | Sensitizer |
| 51 | *t*-2-Hexenal | 6728-26-3 | 2 | 1 | 1 | Sensitizer |
| 52 | Ylang Ylang | 8006-81-3; 68606-83-7; 83863-30-3 | 2 | 1 | 1 | Not predicted |
| 53 | α-methyl cinnamic aldehyde | 101-39-3 | 2 | 0 | 2 | Non-sensitizer |
| 54 | Benzyl cinnamate | 103-41-3 | 2 | 0 | 2 | Non-sensitizer |
| 55 | Benzyl salicylate | 118-58-1 | 2 | 0 | 2 | Non-sensitizer |
| 56 | Benzylbenzoate | 120-51-4 | 2 | 0 | 2 | Non-sensitizer |
| 57 | *d*-Limonene | 5989-27-5 | 2 | 0 | 2 | Sensitizer |
| 58 | Isocyclocitral | 1335-66-6 | 2 | 0 | 2 | Non-sensitizer |
| 59 | Lyral | 31906-04-4 | 2 | 0 | 2 | Sensitizer |
| 60 | Propylene glycol | 57-55-6 | 2 | 0 | 2 | Sensitizer |
| 61 | β-damascone | 23726-91-2 | 2 | 0 | 2 | Non-sensitizer |
| 62 | Benzalkonium chloride | 8001-54-5 | 2 | 0 | 2 | Not predicted |

# Table S2. Number of records and the data outcome for the 19 substances with different annotations between the records present in the murine skin sensitization dataset (Dataset B).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **#** | **Compound name** | **CASRN** | **No. of records** | **No. of sensitizers** | **No. of non-sensitizers** |
| 1 | Hexyl cinnamic aldehyde | 101-86-0 | 44 | 42 | 2 |
| 2 | Eugenol | 97-53-0 | 31 | 30 | 1 |
| 3 | Benzocaine | 94-09-7 | 24 | 7 | 17 |
| 4 | Nickel (II) salts | 7718-54-9; 7786-81-4 | 16 | 5 | 11 |
| 5 | Methyl salicylate | 119-36-8 | 14 | 2 | 12 |
| 6 | Sodium lauryl sulfate | 151-21-3 | 11 | 10 | 1 |
| 7 | Aniline | 62-53-3 | 11 | 6 | 5 |
| 8 | Potassium dichromate | 7778-50-9 | 10 | 9 | 1 |
| 9 | 2-Mercaptobenzothiazole | 149-30-4 | 8 | 6 | 2 |
| 10 | Geraniol | 106-24-1 | 7 | 6 | 1 |
| 11 | Tetramethylthiuram disulfide | 137-26-8 | 7 | 6 | 1 |
| 12 | Streptomycin | 3810-74-0 | 6 | 2 | 4 |
| 13 | Coumarin | 91-64-5 | 4 | 2 | 2 |
| 14 | Ethyl acrylate | 140-88-5 | 3 | 2 | 1 |
| 15 | Zinc sulfate | 7733-02-0 | 3 | 2 | 1 |
| 16 | Resorcinol | 108-46-3 | 3 | 1 | 2 |
| 17 | Benzyl benzoate | 120-51-4 | 2 | 1 | 1 |
| 18 | Ethylenediamine | 107-15-3 | 2 | 1 | 1 |
| 19 | Salicylic acid | 69-72-7 | 2 | 1 | 1 |

# Table S3. List of chemical compounds predicted as sensitizers and confirmed in the literature.

|  |  |  |  |
| --- | --- | --- | --- |
| **Compound name** | **CASRN** | **Function** | **Reference** |
| Styrene | 100-42-5 | Perfuming | 2 |
| Benzonitrile | 100-47-0 | Perfuming | 3 |
| *p*-aminodiphenylamine | 101-54-2 | Hair dyeing | 4 |
| Triethylene glycol dimethacrylate | 109-16-0 | Nail conditioning | 5,6 |
| Ethanolamine | 141-43-5 | Buffering | 7 |
| Diallyl disulfide | 2179-57-9 | Perfuming | 8 |
| Diethylene glycol dimethacrylate | 2358-84-1 | Nail conditioning, film forming | 5 |
| Laureth-9, polidocanol | 3055-99-0 | Emulsifying | 9 |
| Glyceryl monothioglycolate | 30618-84-9 | Hair waivening or straightening | 10 |
| Iodopropynyl butylcarbamate | 55406-53-6 | Preservative | 11 |
| Chlorhexidine | 55-56-1 | Antimicrobial, oral care, preservative | 12 |
| C.I. Solvent Red 3 | 6535-42-8 | Colorant | 13 |
| Ethyl cyanoacrylate | 7085-85-0 | Film forming | 6,14 |
| Trichloroethane | 71-55-6 | Solvent | 15 |
| Chloroacetamide | 79-07-2 | Preservative | 16 |
| Methyl methacrylate | 80-62-6 | Anticaking, opacifying | 6,17 |
| Ethyleneglycol dimethacrylate | 97-90-5 | Nail conditioning | 6,18 |

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