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Improving Read-Across for GHS Hazard Classification Using ECHA's Read-Across Assessment Framework (RAAF): Alkylbenzenes and Skin Sensitization

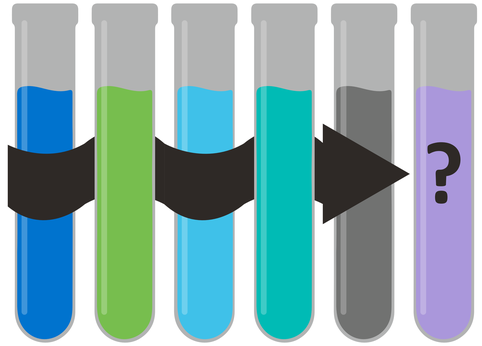
Charlotte M. Marsh, M.S.
Chase H. Butler, M.S.
Joel M. Cohen, Sc.D.

OBJECTIVES

- Apply the European Chemicals Agency's (ECHA) Read-Across Assessment Framework (RAAF) to demonstrate that alkylbenzenes comprising a chemical category is scientifically justified.
- Predict the skin sensitization hazards of data-poor alkylbenzenes based on the data-rich members of the chemical category.
- Compare the results of our chemical category classification approach against structure-activity relationship (SAR)-based hazard predictions.

BACKGROUND

Read-Across and Chemical Categories



- Known hazard information can be "read across" to similar, data-poor chemicals to aid hazard classification of those chemicals.
- Read-across may be applied *via* a single chemical analogue or a category approach.
- Read-across is encouraged by the European Union's (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, under which, no new animal testing can be done without approval.

Skin Sensitization and GHS Hazard Classification

- Under GHS (United Nations, 2015), a substance is classified as a skin sensitizer:
  - A. if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or
  - B. if there are positive results from an appropriate animal test.
- Appropriate animal tests include the local lymph node assay (LLNA) (Organisation for Economic Co-operation and Development Test Guideline [OECD TG] 429), guinea pig maximization test (GPMT) (OECD TG 406), and Buehler test (OECD TG 406).
- Skin sensitization is an important endpoint in the context of consumer products, cosmetics, and occupational exposures.

METHODS

Chemical Category Classification

According to the ECHA RAAF, "[s]ubstances that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances" (ECHA, 2017).

We established a test set of 41 qualitatively similar alkylbenzene compounds. We hypothesized that the alkylbenzenes' lack of electrophilic functional groups, which are necessary for most haptenization reactions (and subsequent immune response), would translate to a lack of skin sensitization potential across the category. The "boundaries" of the category were established using chemicals with available skin sensitization data. Additionally, we ensured that interpolating to any given data-poor category member (from the data-available members "closest" to it with respect to physicochemical properties) would be justifiable. We then evaluated the chemicals (*i.e.*, the category members) according to the RAAF's Seven Assessment Elements (AEs) for chemical categories:

- **AE C.1: Substance characterization**
- **AE C.2: Structural similarity and differences within the category**
- **AE C.3: Link of structural similarities and structural differences with the proposed regular pattern.** We focused on structural and physicochemical properties that are relevant to skin sensitization **(Table 1)**.

Table 1 Structural and Physicochemical Properties

Property	Relevance to Skin Sensitization
Molecular weight	Related to a chemical's ability to penetrate the skin and interact with immune cells
Water solubility	Related to hydrophilicity and the chemical's ability to penetrate the stratum corneum
Vapor pressure	Indication of whether a chemical is likely to volatilize before it can penetrate the skin
Log K_{ow}	A high Log Kow may indicate enhanced skin penetration
Ionizable groups*	Ionized chemicals are less likely to penetrate the skin
Reactive functional groups/structural alerts	Certain functional groups (for example, isocyanates and epoxides) are associated with skin sensitization reactions

*Notes: K_{ow} = Octanol-Water Partition Coefficient; * = Ionizable groups at a neutral pH.*

- **AE C.4: Impact of impurities on the prediction**
- **AE C.5: Consistency of effects in the data matrix**
- **AE C.6: Reliability and adequacy of the source study(ies).** We identified key studies based on:
  - Data from LLNA, GPMT, or Buehler test with a data quality Klimisch score of 1 or 2. Enough study details to confirm the correct interpretation of results and compose a study summary
- **AE C.7: Bias that influences the prediction**

SAR Analysis

Finally, we evaluated all chemicals within the category for skin sensitization reactivity domains using the free SAR program Toxtree. These results were then compared with the results of our chemical category hazard classification approach.

RESULTS

AE C.1 Substance Characterization

Category members were identified by name and Chemical Abstract Services (CAS) registry number or European Community (EC) number.

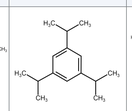
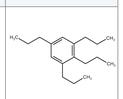
AE C.2 Structural Similarity and Differences Within the Category

The class was limited to hydrocarbons composed of a single aromatic ring on which the only substituents were saturated alkyl groups (methyl and larger). The presence of any other functional group (hydroxyl, carbonyl, amino, sulfate, *etc.*) precluded a chemical from being assessed *via* this category approach. **Table 2** provides an overview of the group's structural similarities, as well as the approximate upper and lower bounds for the category members' physicochemical properties.

AE C.3 Link of Structural Similarities and Structural Differences with the Proposed Regular Pattern

Despite predictable differences in some properties (*e.g.*, the larger compounds are less volatile), the compounds are structurally very similar. None of the category members contain functional groups capable of reactivities associated with skin sensitization. Thus, despite the physicochemical differences, we expected members of the alkylbenzene category to exhibit a similar lack of potential for skin sensitization.

Table 2 Physicochemical Properties of Selected Alkylbenzenes

Parameter	Bookends and Representative Category Members			
CAS Number	108-88-3	105-05-5	717-74-8	25265-78-5
Chemical Name	Toluene	1,4-Diethylbenzene	1,3,5-Triisopropylbenzene	Tetrapropylenebenzene
Structure				
Molecular Weight (g/mol)[1]	92.1	134.2	204.4	246.4
Vapor Pressure (mmHg)[1]	28.4 (e) 23.7 (p)	1.06 (e) 0.92 (p)	5.1 E-2 (p)	1.6 E-4 (p)
Log K_{ow} (unitless)[1]	2.73 (e) 2.54 (p)	4.58 (e) 4.07 (p)	6.36 (p)	8.11 (p)
Water Solubility (mg/L)[1]	526 (e) 471 (p)	24.8 (e) 17.3 (p)	0.26 (p)	1.7E-3 (p)
Ionizable groups*	No	No	No	No
Reactive functional groups?	No	No	No	No

*Notes: e = Experimental; Hg = Mercury; p = Predicted. Values measured at 25°C when possible; * = Ionizable groups at a neutral pH.(1) Estimated using EPISuite.*

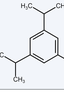
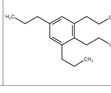
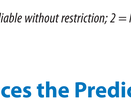
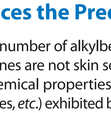
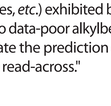
AE C.4 Impact of Impurities on the Prediction

Substances that might contain significant impurities outside the category description were excluded. For example, "C10 aromatic hydrocarbons containing >1% naphthalene" was excluded, due to the known presence of naphthalene as an impurity.

AE C.5 Consistency of Effects in the Data Matrix
AE C.6 Reliability and Adequacy of the Source Studies

Table 3 summarizes the skin sensitization data, including study type, study method, and reliability score, for seven chemicals that are representative of the chemical category.

Table 3 Skin Sensitization Data Matrix for Representative Alkylbenzenes

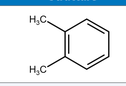
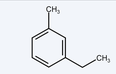
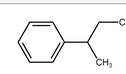
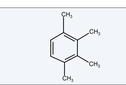
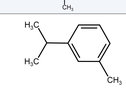
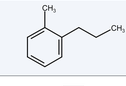
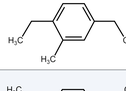
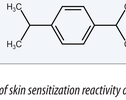
CAS Number	Chemical Name	Structure	Animal Data
108-88-3	Toluene[1]		Not sensitizing. GPMT EU Method B.6 (K = 1)
98-82-8	Cumene		Not sensitizing. GPMT similar to OECD TG 406 (K = 2)
105-05-5	1,4-Diethylbenzene[1]		Not sensitizing. Modified LLNA (K = 1)
98-51-1	4-tert-Butyltoluene		Not sensitizing. GPMT (K = 2)
99-62-7	1,3-Diisopropylbenzene		Not sensitizing. Buehler tests (2) (K = 1 for both)
717-74-8	1,3,5-Triisopropylbenzene[1]		Not sensitizing. Buehler test similar to OECD TG 406 (K = 1)
25265-78-5	Tetrapropylenebenzene (mixture of isomers)[1]		Not sensitizing. LLNA, OECD TG 429 (K = 1)

Notes: K = Klimisch score for study reliability (1 = Reliable without restriction; 2 = Reliable with restriction). (1) Chemical also included in Table 2.

AE C.7 Bias that Influences the Prediction

We obtained sensitization data for a number of alkylbenzenes. These data consistently indicate that alkylbenzenes are not skin sensitizers. Given the variety of structural and physicochemical properties (molecular weight, substitution pattern, physical properties, *etc.*) exhibited by these compounds, interpolating these sensitization data to data-poor alkylbenzenes was deemed appropriate. These results demonstrate the prediction of the absence of an effect, otherwise known as "negative read-across."

Table 4 Representative Data-Poor Category Members

CAS Number	Chemical Name	Structure	Toxtree Alert[1]
95-47-6	o-Xylene		None
620-14-4	Benzene, 1-ethyl-3-methyl-		None
135-98-8	sec-Butylbenzene		None
488-23-3	Benzene, 1,2,3,4-tetramethyl-		None
535-77-3	m-Cymene		None
1074-17-5	Benzene, 1-methyl-2-propyl-		None
13632-94-5	Benzene, 1,4-diethyl-2-methyl-		None
100-18-5	1,4-Diisopropylbenzene		None

Notes: (1) The Toxtree SAR program predicts the presence of skin sensitization reactivity domains.

SAR Analysis

The presence of one of more Toxtree "structural alerts" for a chemical suggests that the chemical has skin sensitization potential. Toxtree identified no skin sensitization reactivity domains for any category members. The lack of structural alerts for chemicals across the category provides supporting evidence that the members of this well-defined chemical category do not cause skin sensitization effects.

CONCLUSIONS

- Based on the lack of skin sensitization effects observed in high-quality animal studies for chemicals throughout the category's physicochemical range, which is supported by the absence of SAR-predicted effects for data-poor chemicals, we can infer that all chemicals within the alkylbenzene category are likely to be non-sensitizers and would therefore not warrant hazard classification under GHS.
- Based on our analysis, our category classification for the skin sensitization hazard of alkylbenzenes falls under Scenario 6 of the ECHA RAAF (2017), defined as "different compounds have qualitatively similar properties… [n]o relevant variations in properties observed among source substances and the same strength predicted for the target substance."