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Clustering a Chemical Inventory for Safety Assessment of Fragrance Ingredients: Identifying Read-Across Analogs to Address Data Gaps

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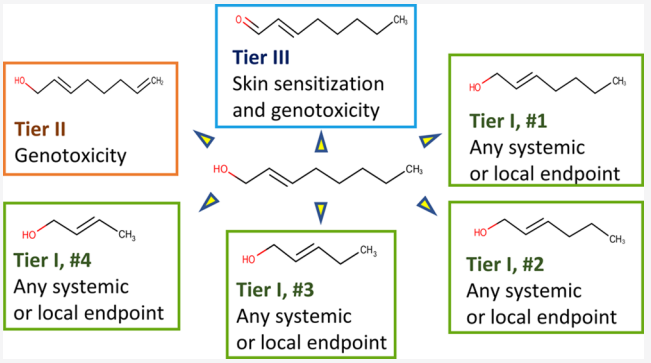
ABSTRACT: A valuable approach to chemical safety assessment is the use of read-across chemicals to provide safety data to support the assessment of structurally similar chemicals. An inventory of over 6000 discrete organic chemicals used as fragrance materials in consumer products has been clustered into chemical class-based groups for efficient search of read-across sources. We developed a robust, tiered system for chemical classification based on (1) organic functional group, (2) structural similarity and reactivity features of the hydrocarbon skeletons, (3) predicted or experimentally verified Phase I and Phase II metabolism, and (4) expert pruning to consider these variables in the context of specific toxicity end points. The systematic combination of these data yielded clusters, which may be visualized as a top-down hierarchical clustering tree. In this tree, chemical classes are formed at the highest level according to organic functional groups. Each subsequent subcluster stemming from classes in this hierarchy of the cluster is a chemical cluster defined by common organic functional groups and close similarity in the hydrocarbon skeleton. By examining the available experimental data for a toxicological endpoint within each cluster, users can better identify potential read-across chemicals to support safety assessments.



INTRODUCTION

Fragrance materials are used in a wide variety of consumer goods, including personal care and household products. The Research Institute for Fragrance Materials (RIFM) evaluates substance safety for fragrance materials used in consumer products. New European Union regulatory framework defined by REACH[1] and the Cosmetics Directive[2] has put an increased emphasis on assessing chemical safety without animal testing. Therefore, RIFM continues to promote efforts to reduce, refine, and replace in vivo toxicity testing.[3]

To support the safe use of fragrance materials, RIFM has undertaken a project to assess the safety of its entire fragrance material inventory. The aim of this project was to evaluate over 3500 materials, including approximately 2600 discrete organic substances. The assessment process entails a preliminary exposure assessment, a complete evaluation of the toxicological profile, and identification of data needs.[4] Reviews of the fragrance and fragrance-like materials reveal that many materials lack individual material toxicity data for all the toxicological endpoints typically examined in a safety assessment. Thus, nontest methodologies are often needed to assist in making a safety assessment.[4] To cover gaps in data, the read-across approach is frequently used to associate toxicological data for structurally similar chemicals. Computational or in silico methods to identify read-across chemicals employ multiple techniques and approaches.[5] Regardless of the read-across method, a key initial step is to assemble the fragrance materials into groups with common characteristics that are toxicologically relevant to a particular endpoint of interest.

The use of chemical grouping approaches is a common practice in industry and within the regulatory community according to OECD guidelines.[6] There are several methods to group or cluster chemicals into categories.[6,7] Read-across is based on the underlying hypothesis that the toxicity of a particular chemical is a function of its molecular structure.[3] Chemicals that share certain common structural elements typically have comparable physicochemical and toxicokinetic properties and may exhibit a common mode of action. Data from one or more tested chemicals thus can be used to predict the toxicity of a structurally similar chemical for the same test or endpoint.

The aim of this work was to cluster the fragrance chemical inventory to robustly support endpoint-specific read-across.

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Table 1. Toxicity Endpoints of Interest and Respective OECD Test Guidelines

	endpoint	test guideline[17]
1	reproductive and developmental toxicity	TG 421, TG 422, TG 414, TG 415, TG 416
2	repeated dose toxicity	TG 408, TG 422, TG 407
3	genotoxicity	TG 471, TG 487
4	skin sensitization	TG 429, TG 442, TG 406
5	phototoxicity	TG 101, TG432
6	respiratory toxicity	TG 412, TG 413, TG 433, TG 403, TG 436

Table 2. Endpoints, Related Computational Profilers, and Software Applications Used for Comparison of Toxicological Properties of the Chemicals

	endpoint	computational profilers	software applications
1	reproductive and developmental toxicity	ER binding	OECD QSAR Toolbox[18]
		developmental toxicity	CAESAR[19,20]
2	repeated dose toxicity	repeated dose HESS categorization	OECD QSAR Toolbox
3	genotoxicity	DNA	OECD QSAR Toolbox
		carcinogenicity	ISS[19,20]
		DNA binding (Ames, MNT, and clastogenecity)	OASIS[21]
		in vivo mutagenicity (micronucleus)	ISS
		in vitro mutagenicity (Ames)	ISS
4	skin sensitization	protein binding	OASIS and OECD QSAR Toolbox
		protein binding potency	OECD QSAR Toolbox
		protein binding alerts for skin sensitization	OASIS
		skin sensitization reactivity domains	ToxTree
5	phototoxicity	phototoxicity (3T NRU, photoinduced toxicity)	OECD QSAR Toolbox
6	respiratory toxicity	respiratory sensitization	OECD QSAR Toolbox

Various methods for clustering inventories and searching read-across analogues have been described previously.[8−11] These approaches employed methods ranging from simple quantitative structure−activity relationships (QSAR) to a complex machine learning techniques on big data.[10] The endpoints of interest include both systemic toxicity, such as in vivo genotoxicity and developmental-reproductive toxicity, and local toxicity, such as skin sensitization or respiratory toxicity.[11] All of these approaches use Tanimoto or other structural similarity scores[12] as a rudimentary basis to identify similar chemicals calculated from either SMILES or fingerprints.[13] The main drawback of clustering an inventory based on such scores is that the substructural features, which can affect toxicodynamic and toxicokinetic properties, are not weighted according to their impact on the toxicity. Consequently, these methods typically produce clusters with divergent substructural features that may confer dramatically different toxicokinetic and toxicodynamic properties. For example, although α,β-unsaturated carbonyl compounds are strong Michael acceptors, a methyl substitution on the β-carbon of the vinylene group renders these molecules unreactive and relatively nontoxic despite minimal effects on the Tanimoto score.[14] Thus, a clustering process based purely on structural similarity will not reliably represent key features that may drive toxicity endpoints.

Here we describe a tier based workflow for clustering or categorizing a chemical inventory and conducting a search for a read-across analogs using (1) classification and grouping of chemicals based on organic functional groups, (2) subclustering within functional group classes based on hydrocarbon skeleton structure similarity and in silico toxicological alerts, (3) further subclustering by incorporating well-documented Phase I and Phase II metabolism, and (4) expert-pruning to optimize the association of physical-chemical properties with toxicokinetic and toxicodynamic properties in the context of specific endpoints. We describe the application of the clustering scheme to the RIFM inventory and demonstrate how this approach improves read-across analogues searches for the safety assessment of fragrance ingredients.

METHOD FOR CLUSTERING A CHEMICAL INVENTORY

Fragrance Material Inventory. The RIFM fragrance materials inventory includes ∼2600 chemicals in active use as fragrance materials, along with another ∼3500 chemicals that are discontinued for fragrance use and share structural similarities with fragrance chemicals. Although no longer used, discontinued materials may serve as a read-across data source.[15] The RIFM inventory of discrete synthetic fragrance chemicals represents the largest chemical inventory in the fragrance industry. The following criteria are evaluated to cluster the RIFM inventory of discrete fragrance materials and form clusters of chemicals with similar toxicodynamic and toxicokinetic properties.

Data Sources. At present RIFM houses data for six human health endpoints and for environmental risk and hazard endpoints. The human health endpoints are skin sensitization, genetic toxicity (mutagenicity and clastogenicity), repeated dose toxicity, reproductive (fertility and developmental) toxicity, phototoxicity, inhalation, or local respiratory toxicity. For environmental toxicity and hazard endpoints, we leverage our data gap filling efforts largely by following the environmental toxicity and hazard assessment scheme published previously.[16] Test data for specific toxicological endpoints were obtained mainly by testing, either by RIFM or by RIFM member companies according to OECD guidelines for testing chemicals.[17] Data from registration dossiers, such as those contained in a REACH registration dossier may also be

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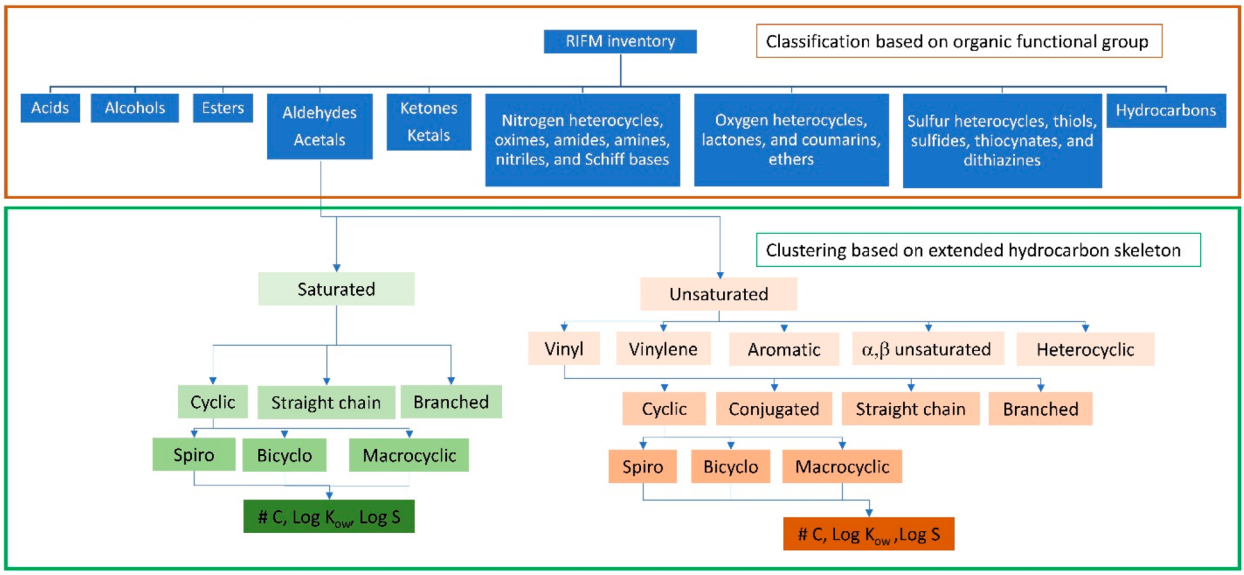

Figure 1. Chemical structure-based clustering of RIFM fragrance chemical inventory. The clustering method represents a top-down dendrogram (clustering tree). The first clustering step generates the main tree branches (blue boxes), which represent clusters driven by functional group classes, and which are further subclustered based on hydrogen saturation and other features of the hydrocarbon skeleton (green and tan boxes). In endpoint specific cases, molecular and physical-chemical properties are used to limit clusters to specific analogs. Every resulting cluster is finally further divided according to predicted bioavailability of the chemicals based on octanol/water partition coefficient (log K_{OW}), aqueous solubility, and number of carbons in the extended fragment attached to the organic functional group.

included. In cases where a REACH registration dossier specifies the usage or a read-across analog, RIFM read-across analog criteria described in this work are applied before accepting or rejecting data from the data source analog. The OECD test guidelines relevant to RIFM safety assessments are given in Table 1.

In Silico Structural Alerts. Computational evaluation of structural alerts was conducted with OECD QSAR Toolbox 4.2[11,18] and VEGA[19,20] as shown in Table 2. These alerts predict properties associated with the toxic effects of chemicals, such as DNA and protein covalent binding and are based on functional groups known to undergo these reactions, either directly or upon biotransformation to a reactive metabolite. All members of a cluster are expected to have similar structural alerts. The structural domain applicability of the QSAR model used to predict the structural alert is used to assess the validity of alert predictions. When experimental data are available for multiple chemicals in the cluster, trend analysis or a category approach is used to confirm the applicability of QSAR prediction by comparison to the data. The models used are listed in Table 2 and are open-source tools with supporting peer-reviewed publications. We note that software applications are rapidly evolving and that those listed are exemplary of our process at the time we performed this work. Limitations are being identified and alternate approaches are available. For example, the Proctor and Gamble rules for DART, which are available in the OECD QSAR Toolbox may supersede the CAESAR model.

Metabolism Studies and Metabolism Predictions. Since fragrance materials include many of the most common chemical classes (e.g., alcohols, esters, etc.) and most fragrance materials contain only one functional group, metabolism data are available for many fragrance-related chemical classes. We considered studies available on the metabolism of representative members of different chemical classes, as well as on specific chemicals.[22] Metabolism predictions were generated using the TIMES[23] platform v2.28.1 with the rat liver S9 metabolism kinetics simulator v01.01.01 for systemic toxicity endpoints. The TIMES platform, in combination with OECD QSAR Toolbox v4.2, gives a detailed explanation on the metabolites generated and the reference used for the prediction. For skin sensitization, the in vivo skin sensitization with autoxidation v22.27 metabolism simulator was used. These platforms broadly represent systemic metabolism applicable to systemic endpoints (genotoxicity, repeated dose toxicity, and developmental and reproductive toxicity) and local metabolism for skin sensitization. Both Phase I and Phase II metabolism were considered for all enzymatic metabolic transformations.

To analyze metabolites as potential read-across analogs, we compared toxicological profiles of phase I metabolites and the target substance. To qualify as read-across analogs, the metabolites should be more reactive and toxic than the target substance, as predicted by structural alerts from toxicological profilers. Except for the biotransformed portion, Phase I metabolites are structurally identical to the target substance. Therefore the structural similarity score (e.g., Tanimoto score) is not considered as a part of the matrix in a tier III read-across search.

Physical−Chemical Properties and Routes of Exposure. Physical−chemical characteristics often play an important role in clustering chemicals and affect their ADME (absorption, distribution, metabolism, and excretion) properties. Quantifiable parameters, such as the aqueous/organic partition coefficient and aqueous solubility are useful properties that affect oral bioavailability and distribution, thereby impacting all systemic endpoints. When these properties are combined with skin absorption coefficient,[24] they facilitate prediction of bioavailability through dermal exposure, which is particularly important for fragrance chemicals. Inhalation is another important route of exposure for fragrance chemicals. Consideration of vapor pressure, Henry's law constant, boiling

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Chem. Res. Toxicol. 2020, 33, 1709−1718

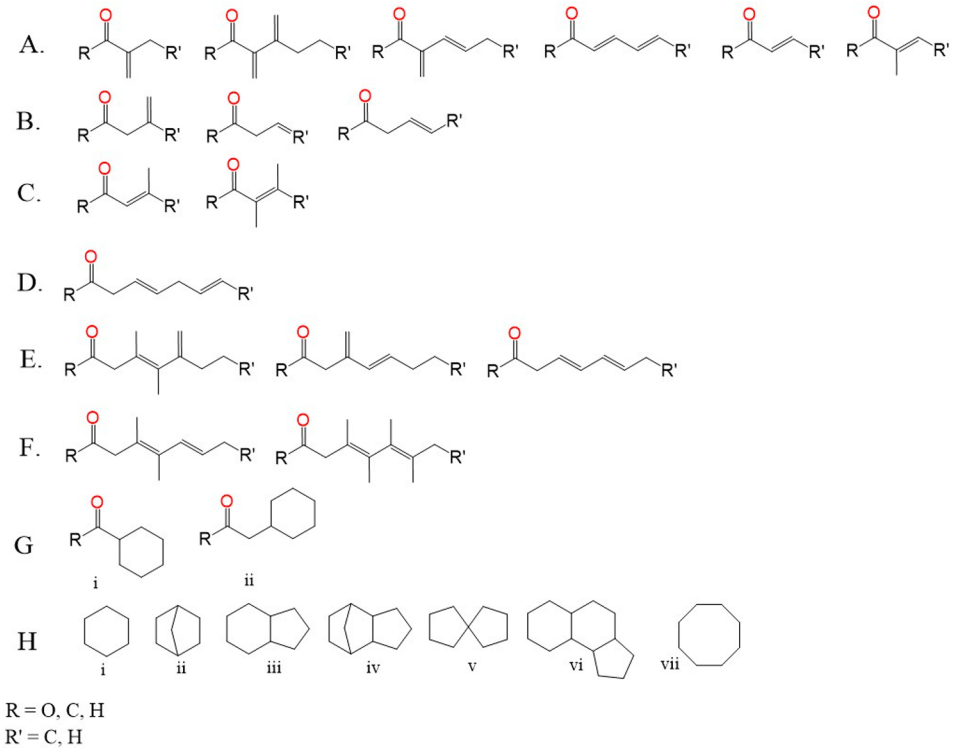

Figure 2. Different extended fragments of hydrocarbon skeletons and substructural features considered in the second clustering step. Examples of subclustering of carbonyl-containing (ketone) chemicals are shown. Rows A to F shows straight-chain and branched unsaturated fragments, in which chemical reactivity is predicted to increase in the left to right direction. (A) Michael acceptors; (B) epoxide formers; (C) Schiff base formers; (D) bis-allylic hydrocarbons; (E) conjugated unsaturated systems; (F) conjugated unsaturated systems with alkyl substitutions; (G) functional group attached i. directly to cyclic fragment, and ii. via alkyl link; and (H) Different cyclic fragment structures considered in G.

point, and melting point can guide estimation of inhalation exposure using appropriate models.[25]

Clustering the Inventory. The overall approach to clustering the RIFM chemical inventory for read across is presented in detail in Figure S1. Within this scheme is the clustering of the inventory based on chemical structural features, which is described in detail inFigure S2. Here we describe first the chemical structural basis of the clustering approach. The first step is classification defined by common organic functional groups (Figure 1). This is accomplished by the organic functional group profiler available within the OECD QSAR Toolbox. This first step is valuable because about 70% of chemicals in the RIFM inventory possess a single organic functional group, whereas another ∼20% have two organic functional groups and remaining ∼10% have three or more functional groups. For chemicals with more than one organic functional group, toxicological profilers such as protein binding, or DNA binding are used to prioritize functional groups, with assignment based on the most reactive functional group in the structure. These classes form the top layer (blue boxes) of the structural cluster tree, as depicted in the top-down dendrogram (classification tree) shown in Figure 1.

Chemicals classified by functional groups then are subclustered based on structural features of the hydrocarbon skeleton, particularly saturated and unsaturated olefinic moieties as they often govern chemical reactivity related to toxicological endpoints[26] (Figure S2). In addition to hydrogen saturation, alkyl groups may be in the form of straight-chain, branched-chain and cyclic structures, which affect metabolism, chemical reactivity and toxicity related to polar functional groups,[27] and binding to receptor targets and transcription factors. Thus, while the hierarchy places polar functional groups first and hydrocarbon features second, overall chemical similarity is dependent on both features. Structural similarity based clustering is facilitated by calculating the Tanimoto score[13,28] using EPFC4 fingerprints[29] from SMILES notation. A Tanimoto score cutoff of 0.7 generally reflects high similarity of core structure.[13] Classes are then clustered using K-medoids.[30] Clustering based on the Tanimoto structural similarity score is performed using Pipeline Pilot.[31] Examples of hydrocarbon skeleton features considered for clustering carbonyl compounds are shown in Figure 2. These steps served as a priliminary steps of clustering a class. A rigorous scrutiny of clusters under each class reveled that clusters needed manual intervension. The clusters required expert pruning and shifting chemicals into appropriate clusters. It showed us that Tanimoto-based clustering is not adequate for safety assessment of chemicals.

Toxicological similarities were compared for each penultimate and ultimate cluster based on common toxicological alerts across human health endpoints. Subclustering of a class of chemicals containing a common organic functional group essentially compares the hydrocarbon skeleton attached to the functional group. Thus, the differences in the toxicity between the adjunct clusters are due to activating and deactivating features of the hydrocarbon skeleton. To finalize the clusters, chemicals constituents within a cluster are refined by combining two or more similar clusters or by further subdividing a cluster with the goal of maintaining a similar predicted toxicological profile based on the structural comparison of hydrocarbon skeleton.

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In step 3 of the clustering, we considered similarities in Phase I metabolic products of the clustered materials for further subclustering. This is particularly important for carboxylic esters and primary and secondary alcohols, but not tertiary alcohols. It is known that hydrocarbon unsaturation may enhance toxicity.[14,32] For example, when the unsaturated moiety is at the 2-position relative to an OH group or an alcohol metabolite of an ester, further oxidation of the alcohol can create reactive electrophiles.[33] For a primary alcohol, the product is an aldehyde, whereas for a secondary alcohol, the product is a ketone.

The last step in clustering an inventory is expert refinement of these clusters based on relevant additional information (e.g., effect of physical−chemical properties and structural-reactivity for the specific endpoint). Each penultimate cluster is scrutinized for consistency of properties that may affect bioavailability through effects on absorption and metabolism, which governs response for certain toxicological endpoints. For example, differences in octanol/water partition coefficient and water solubility, which both are dependent on chain length and substitution patterns may affect subchronic systemic toxicity. Thus, clusters with a series of increasing chain length analogs may be divided to preserve similarity within subclusters. For example, clusters are often divided into ranges of log K_{ow}, such as log K_{ow} < 2.0, 2.0 < log K_{ow} < 5.0, and log K_{ow} > 5.0. These ranges typically distinguish highly water-soluble chemicals, moderately water and lipid soluble chemicals, and highly lipid-soluble chemicals.

Searching a Read-Across Analog from the Clustered Inventory. The organization of the chemical inventory into clusters of structurally and toxicologically similar chemicals together with a corresponding data matrix presents an opportunity for efficient and fast read-across analog search for all human health endpoints. After the inventory is clustered, within each chemical class the component clusters are ranked in increasing order of potential toxicity of the constituent chemicals. As noted, this ranking may differ in the context of different toxicity endpoints based on experimental data whenever available and may be based largely on predicted chemical toxicity as defined by toxicological profilers for data poor endpoints. The data matrix for each endpoint is overlaid on the cluster to identify data gaps. A chemical that lacks certain data for an endpoint is defined as a target chemical and its corresponding cluster is defined as the target cluster. Because of the similarity between its constituent chemicals, the target cluster is the best source of data for filling data gaps.

Read-across between chemicals within a same cluster is defined here as Tier I read-across. When a target cluster does not contain a source chemical with sufficient data for the endpoint according to the requirements given in Table 1, chemicals in adjacent clusters can be searched to find a read-across analog. Data gap filling with chemicals from adjacent clusters is termed a Tier II read-across. Tier-II read-across source analogs should have the same organic functional groups and key structural features as the target but may have dissimilar secondary structural features. For example, an unsaturated straight-chain analog where the unsaturated moiety is not conjugated with the polar group may be used as a source analog for a saturated straight-chain analog.

Prioritization of multiple source chemicals in adjacent clusters should be conducted in terms of similarity in structural features, physical chemical properties, experimentally observed or predicted reactivity and toxicity, bioavailability, and metabolism. Moreover, the predicted reactivity or toxic biotransformation potential of the source or read-across analog should be equal to or greater than for the target chemical. If a satisfactory Tier II read-across analog cannot be identified, then a Phase I metabolite of the target chemical may be used; this is termed Tier III read-across. For a Tier III read-across, the criteria described above for a Tier II read-across should be satisfied and the properties of the metabolite should be appropriate in the context of the toxicity endpoint under consideration.

Application of Clustering to Read-Across Analog Selection for Safety Evaluation of Fragrance Chemicals. The following examples illustrate the clustering of the RIFM fragrance chemical inventory within major chemical classes and provide examples of how the clustering framework guides the selection of read-across source analogs to identify experimental data to support chemicals for which test data are unavailable. The examples represent the roles of clustering, multitier read-across search process, and inferences regarding absorption and metabolism to support the selection of data. As indicated in Table 1, the read-across data are end point specific. As examples, for repeated dose and reproductive toxicity endpoints, read-across provides a no observed adverse effect level (NOAEL), for the skin sensitization endpoint, read-across provides a no expected sensitization induction level (NESIL). For the genotoxicity endpoints, read-across data support the extrapolation of findings from the source analog to the target chemical.

Acids. Carboxylic acids represent approximately 3.5% of chemicals in the RIFM inventory and are direct metabolic products of esters and aldehydes. Both oral and dermal absorption are affected by ionization but decrease with increasing molecular weight. As with alcohols, there are extensive supporting ADME data on carboxylic acids. For example, in cells, straight-chain acids typically are taken up by mitochondria and degraded by β-oxidation, whereas branched carboxylic acids also undergo Phase II metabolism prior to excretion in the urine.[34,35]

While carboxylic acids are generally nontoxic, two structural features enhance toxicity. First, $\alpha-\beta$ unsaturated acids, such as acrylic acid and crotonic acid display enhanced electrophilic reactivity and toxicity. Second, selected saturated acids with branched chains of C2 to C4 in length may display systemic toxicities. For example, the fragrance material 2-ethylhexanoic acid, is a known teratogen.[36] Data gaps for carboxylic acids are most often filled by a Tier I or Tier II read-across, as this class is relatively data rich. For example, the data rich small acids (C2−C5) are often read-across to the comparatively data poor intermediate size (C6−C12) acids. Any chemical that can form such acids via Phase 1 metabolism may be used for Tier III read-across.

Another major subcluster in the carboxylic acid class consists of fatty acids. These undergo metabolism to carbon dioxide and water via beta-oxidation and the citric acid cycle.[37] A subcluster within the long-chain fatty acids contains bis-allylic polyunsaturated fatty acids, such as linoleic, linolenic, and arachidonic acid, which undergo facile autoxidation to form toxic lipid peroxides and related oxidation products.[38]

Alcohols. Alcohols are a chemical class represented in approximately 10% of the chemicals in the RIFM inventory. This class is well-studied with a comparative wealth of toxicokinetic and toxicodynamic data, which help in sub-clustering this class. Around 90% of the inventory chemicals in

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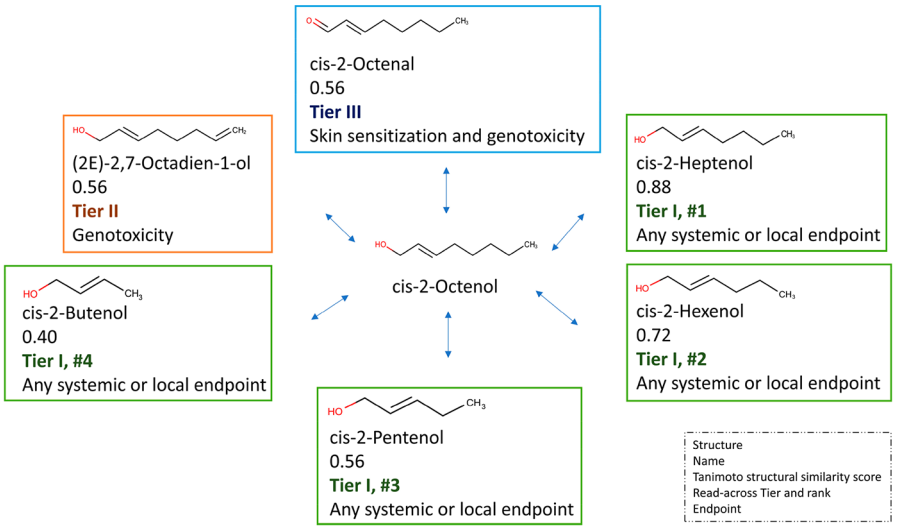

Figure 3. Prioritization of read-across analogs to fill data gaps for the target substance *cis*-2-Octenol. The approach combines a tier-based protocol for prioritizing chemicals in the context of specific human health endpoints. See text for discussion.

the alcohols class are monohydroxyl compounds, which are initially subclassified based on their primary, secondary, or tertiary hydroxyl structures and then further subclustered according to hydrocarbon skeletal features. Read-across case studies have demonstrated that such structural considerations govern toxicokinetic and toxicodynamic similarity (e.g., metabolism and toxic potency respectively).[39−42]

The 90-day oral repeated-dose toxicity data for saturated straight-chain primary alcohols revealed NOAEL values >1000 mg/kg bw/d, based on mild changes, such as decreased body weight accompanied by minor clinical chemical and hematological changes, but without concurrent histopathological effects.[40] In contrast, the 90-day oral repeated dose NOAEL values for saturated branched primary alcohols are typically 10-fold less (<150 mg/kg bw/d) than for otherwise similar straight-chain alcohols. The 90-day oral repeated-dose toxicity data for branched chain primary alcohols also involve mild changes, such as those produced by straight chain alcohols, again without concurrent histopathological effects.

Whereas the metabolism of primary straight-chain saturated monoalcohols occurs by stepwise oxidation to CO_2, saturated branched primary alcohols are metabolized by other pathways such as glucuronidation, oxidation to aldehydes and then to carboxylic acids, and side-chain oxidation yielding polar metabolites, which may be subsequently conjugated.[39] In contrast, secondary alcohols undergo glucuronidation or oxidation to ketones, whereas tertiary alcohols are metabolized by conjugation.[43]

Subclustering of primary, secondary and tertiary alcohols is based on the extended saturated or unsaturated alkyl or aromatic fragments attached to the hydroxyl group. The interplay between alcohol structure and adjacent hydrocarbon features dictates subclusters based on metabolism. For example, metabolism of primary and secondary alcohols having 2,3-unsaturation yields α,β-unsaturated aldehydes and ketones, which are Michael acceptors that may play roles in toxicity, mutagenicity and sensitization reactions.[33,41,44] In searching for appropriate read-across candidates for several endpoints (i.e., genotoxicity, skin sensitization, reproductive and fertility toxicity, and repeat dose toxicity), metabolite structure and properties are essential to assessing similarity of read-across analogs.

Figure 3 illustrates application of our approach to prioritize read-across analogs in the context of multiple endpoints applicable to all chemical classes in the inventory. The target substance *cis*-2-octenol has insufficient data for all human health and environmental toxicity endpoints. We identified six candidate read-across analogs with sufficient data for at least one endpoint. Tanimoto structural similarity scores ranged from 0.40 to 0.88 but provided little guidance for analog selection for different endpoints. A tier-based approach nevertheless guided prioritization of these candidates to fill all data gaps for the target substance. *cis*-2-Heptenol, *cis*-2-hexanol, *cis*-2-pentenol, and *cis*-2-butenol (green boxes) all contain the primary 2-enol functionality of the target and could be considered appropriate for multiple human health end points. These four analogs are ranked in priority based on differences in chain length. All four represent Tier I read-across options. (2*E*)-2,7-octadien-1-ol is a Tier II read-across option, due to the terminal vinyl group. This group can undergo epoxidation, which could make systemic absorption and metabolism substantially different than the target. This analog is thus not appropriate for oral repeat-dose or reproductive toxicity endpoints. However, (2*E*)-2,7-octadien-1-ol would be appropriate for genotoxicity as it would likely be more reactive than the target. *cis*-2-Octenal is a direct phase I metabolite of the target substance and can serve as Tier III read-across analog for skin sensitization or genotoxicity, but not for repeat-dose or reproductive toxicity, again due to substantial differences in bioavailability.

Esters. Esters represent approximately 25% of the RIFM fragrance inventory and show a wide range of substructural diversity. As for the alcohols and carboxylic acids described above, Tier I read-across typically involves analogs with different chain lengths, whereas Tier II read-across involves differences in branching, unsaturation or other hydrocarbon features. A cursory review of the data matrix on the class of esters showed that for systemic endpoints, such as repeated dose toxicity and developmental and reproductive toxicity, majority of members of this class lack safety data from tests performed according to OECD guidelines. Hence, Tier I and II

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Chem. Res. Toxicol. 2020, 33, 1709−1718

read-across searches (i.e., ester to ester) frequently fail to fill the data gaps. Esters are well-known for their ease of metabolic hydrolysis.[45] Upon metabolism, esters produce acid and alcohol metabolites, which may drive observed toxicities. Consequently, esters are subclustered based on both the substructural features of acid and alcohol moiety separately. This enables Tier III read-across from the alcohol or acid metabolites to support safety evaluation of the esters.

A case study for selected simple aryl alcohol alkyl carboxylic acid esters has been reported.[46] This case study reports that in vivo assessment of the toxico-kinetics of 2-phenylethyl propionate has not been reported. However, evidence derived from in vitro studies in both rat liver and intestine tissue samples and artificial gastric juices, demonstrated that the ester readily undergoes hydrolysis to phenethyl alcohol and propionic acid. Results of 90-day repeated-dose study of dermally administered 2-phenylethyl alcohol in male and female Sprague−Dawley rats is further reported.[46] On the basis of body weight abnormalities observed at the higher concentrations, a NOAEL of 0.50 mL/kg/day or 500 mg/kg/day was reported. In the same paper, the 90-day dietary repeated-dosed toxicity of propionic acid in male and female Sprague−Dawley rats revealed a minor decrease in body weight in the higher-dose male group, together with a 12% reduction in kidney size, whereas at the high dose, females displayed increased mass in heart and liver; the NOAEL for males and females were noted as 1250 mg/kg/day and 2500 mg/kg/day, respectively. On the basis of a Tier III ester cluster assessment, the NOAEL value, 500 mg/kg/day, of phenethyl alcohol was used to fill the repeated-dose toxicity data gap for phenethyl propionate.

Aldehydes/Acetals and Ketones/Ketals. The RIFM inventory contains approximately 12% aldehydes and acetals and approximately the same proportion of ketones and ketals. Many saturated aldehydes and ketones show little systemic toxicity. However, short-chain α,β-unsaturated aldehydes and ketones exhibit significant toxicity since they are bifunctional electrophiles and can covalently modify protein and DNA.[47] The aldehyde moiety is associated with Schiff-base formation and is thus linked to skin sensitization. Schiff base reactions of carbonyl compounds can also result in cross-linked DNA adducts.[48] Moreover, the α,β-unsaturated aldehydes and ketones can undergo Michael addition and can be either scavenged by glutathione or undergo adduct formation. For both α,β-unsaturated aldehydes and ketones the bifunctional electrophile can be eliminated by carbonyl reduction. Tier I read-across typically involves analogs with different chain lengths, whereas Tier II read-across may involve differences in branching, unsaturation or other hydrocarbon features, where the analog would likely be more reactive or have greater toxic potency. Tier III read-across usually is not employed because of uncertainty about the ability of alcohol or carboxylic acid metabolites to efficiently undergo biotransformation to the carbonyl target molecule.

Acetals and ketals, which release aldehydes and ketones upon hydrolysis, respectively, are not readily metabolized via human enzymes, although hydrolysis may occur upon oral administration. The acetals and ketals may be considered together with aldehydes and ketones, but still form separate clusters. The subclustering depends upon the extended carbon fragments attached to both the carbonyl and alcohol components of the acetal and ketal, respectively.

Other Oxygen-Containing Compounds. The RIFM inventory contains approximately 9.5% oxygen-containing chemicals other than alcohols, esters, carboxylic acids, aldehydes, and ketones. These are classified under the umbrella term oxygen-containing compounds and include oxygen heterocyclics and heteroaromatics furans, pyrans, coumarins, γ- and δ-lactones, macrocyclic lactones, ethers, and epoxides. Coumarins and epoxides are the most reactive clusters within this class. Members of this group include protein and DNA binders with genotoxic and sensitization properties, and potent repeated-dose hepatotoxicants.[49] Tier I read-across typically involves isomeric structures or modest differences in alkyl substitution. Tier II read-across employs molecules with substantial structural differences, but in which key structural elements (e.g., furan, lactone) are nevertheless represented and are considered to have comparable or greater reactivity than the target molecule.

Epoxides can directly alkylate DNA and proteins.[50,51] Mutagenic and carcinogenic epoxides can also be formed metabolically by enzymatic epoxidation of alkenes. Considerations of structural features, such as the presence and pattern of substituents and other features of the hydrocarbon structure are considered in subclustering and read-across involving epoxides.

The hydrocarbon skeleton attached to the epoxides also affects the bioavailability and is important to consider for systemic end points following oral administration. High molecular weight epoxides show lesser tendency to produce DNA adducts, due to low bioavailability.

Hydrocarbons. The RIFM fragrance inventory contains approximately 3% chemicals that are hydrocarbons, largely comprising different terpenes ranging from monoterpenes to sesquiterpenes. This is by far the least reactive and toxic class of fragrance materials. These hydrocarbons are clustered according to motifs characteristic of terpene chemistry, including single or multiple terpene units and acyclic and cyclic structures. Other hydrocarbons are subclustered as aliphatic straight chain, branched and cyclic, and aromatics included in the fragrance inventory. Aromatic hydrocarbons are given special attention for having more reactivity and distinct routes of metabolism and toxicity. Polycyclic aromatic hydrocarbons (PAHs) are known for mutagenic and/or carcinogenic properties.[52] Both Tier I and Tier II read-across are employed, with the latter involving differences in branching, substitution or unsaturation that yield more reactive structures.

Nitrogen Containing Compounds. The RIFM inventory contains approximately 10.5% nitrogen compounds, which are subclassified into several different clusters of nitrogen-containing functional groups. These clusters include heterocycles (e.g., pyrrolidines, pyrrolines, pyrroles, indoles, piperidines, pyridines, pyrimidines, quinolines, piperazines, pyrazines, and quinoxalines), oximes, amides, amines, nitro, nitriles, nitrite and nitroso, amino acids, and Schiff bases. The nitrogen compounds also most frequently contain two or more functional groups. The additional functional group on a nitrogen heterocycle often is a carbonyl group. Comparisons of the reactivity and toxicity shown by toxicological profilers on nitrogen heterocycles with a carbonyl group suggested that the nitrogen heterocycle was the driver of in vivo reactivity and toxicity.[53] Each cluster in this class is further divided based on characteristics and alerts for additional functional groups. Within the amines cluster, the aromatic amines form a major

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Chem. Res. Toxicol. 2020, 33, 1709−1718

subcluster characterized by high reactivity and potential toxicity and carcinogenicity. The main focus of dividing aromatic amines and heterocyclic amines was to consider N-hydroxylation of aromatic amines leading to their metabolic activation by acetylation or sulfation of N-hydroxy metabolites.[54]

Sulfur Containing Compounds. The RIFM inventory contains approximately 8% sulfur-containing chemicals, which include thiols, sulfides and disulfides, thiophenes, thiocyanates, and dithiazenes. Some sulfur-containing chemicals also contain a furan substructure. These sulfur-containing chemicals may act as skin sensitizers (via protein thiol−disulfide interchange) and cause repeated dose toxicity (hepatotoxicity) according prediction by OECD QSAR Toolbox profilers and references within, including the HESS database. The mechanisms vary depending on the reactive moiety and activating or deactivating factors present in the structure.

In addition to simple nitrogen, oxygen, and sulfur containing cyclic compounds, there are fragrance chemicals that contain two or more different heteroatoms. These are subdivided into nitrogen and oxygen substituted oxazolines, oxazoles, and acetamides, as well as, nitrogen and sulfur substituted tetrahydro and dihydro thiazoles, and thiazoles. Clustering is based not only on the heteroatoms found in the ring but also on associated activating or deactivating factors.

CONCLUSIONS

The ever-expanding need to evaluate the safety of chemicals, together with limited resources and regulatory limits on animal testing have made read-across an indispensable element of chemical safety assessment. Because it blends data with expert judgment, read-across is inevitably subject to uncertainty and bias that can affect the safety assessment process,[55−57]. The purpose of the framework described here is to systematize the selection of read-across analogs based on a hierarchy of chemical similarity, defined as chemical clusters, together with data relevant to toxicological endpoints. Our work is facilitated by the fact that the RIFM inventory represents a small portion of the chemical universe that is relatively uniform in chemical properties-volatile, low molecular weight chemicals. Moreover, the RIFM inventory is relatively rich in data, which enables successful application of read-across in safety assessment. Nevertheless, the hierarchical cluster framework we describe, and the integration of physical chemical properties and metabolism should be broadly applicable to much larger, more diverse chemical inventories. Although data availability is ultimately limiting, the framework we describe could be used to prioritize data generation to systematically fill critical gaps in the cluster hierarchies of broader inventories. The iterative refinement of both data generation and read-across strategies will be critical to improving the quality of future chemical safety assessment.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.9b00518.

Figure S1, Flowchart for structure-based clustering and tier based read-across analog search process; and Figure S2, flowchart for the chemical structural basis of the cluster organization (PDF)

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Author Contributions

M.D., T.W.S., and D.S.T. designed the protocol and generated the data. M.D., D.O.B., D.J.B., and D.S.T. performed data analysis and refinement of the protocol. M.D. and T.W.S. wrote an initial draft. M.D. and D.C.L. wrote the final draft. All authors contributed to the data analysis and draft reviews. All authors have approved the final version of the manuscript.

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Chem. Res. Toxicol. 2020, 33, 1709−1718

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REFERENCES

(1) REGULATION (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Work, E. A. f. S. a. H. a., Ed. 2006; Vol. *1907*/2006.

(2) Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Text with EEA relevance). In *Official Journal of the European Union*, 22–12–2009 ed.; Union, T. E. P. a. o. t. C. o. t. E., Ed. 2009; Vol. *1223*/2009, pp 59–209.

(3) Madden, M. T. D. C. J. C.*In Silico Toxicology: Principles and Applications*; Royal Society of Chemistry: 2011.

(4) Api, A., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M., Dekant, W., Ellis, G., Fryer, A., Fukayama, M., et al. (2015) Criteria for the Research Institute for Fragrance Materials, Inc.(RIFM) safety evaluation process for fragrance ingredients. *Food Chem. Toxicol. 82*, S1–S19.

(5) Cronin, M. An introduction to chemical grouping, categories and read-across to predict toxicity. In *Chemical Toxicity Prediction*, 2013; pp 1–29.

(6) Read-Across Assessment Framework (RAAF). Regulation, R., Ed. European Chemicals Agency: Vol. ECHA-17-R-01-EN.

(7) Enoch, S., and Roberts, D. *Approaches for Grouping Chemicals into Categories*; Royal Society of Chemistry: 2013.

(8) Helman, G. Generalized Read-Across (GenRA): A workflow implemented into the EPA CompTox Chemicals Dashboard*ALTEX* 2019 DOI: 10.14573/altex.1811292.

(9) De Abrew, K. N., Shan, Y. K., Wang, X., Krailler, J. M., Kainkaryam, R. M., Lester, C. C., Settivari, R. S., LeBaron, M. J., Naciff, J. M., and Daston, G. P. (2019) Use of connectivity mapping to support read across: A deeper dive using data from 186 chemicals, 19 cell lines and 2 case studies. *Toxicology 423*, 84–94.

(10) Luechtefeld, T., Marsh, D., Rowlands, C., and Hartung, T. (2018) Machine Learning of Toxicological Big Data Enables Read-Across Structure Activity Relationships (RASAR) Outperforming Animal Test Reproducibility. *Toxicol. Sci. 165* (1), 198–212.

(11) Abe, A., Sezaki, T., and Kinoshita, K. (2019) Development of a read-across workflow for skin irritation and corrosion predictions. *SAR and QSAR in Environmental Research 30* (4), 279–298.

(12) Bajusz, D., Rácz, A., and Héberger, K. (2015) Why is Tanimoto index an appropriate choice for fingerprint-based similarity calculations? *J. Cheminf. 7* (1), 20.

(13) Mellor, C. L., Marchese Robinson, R. L., Benigni, R., Ebbrell, D., Enoch, S. J., Firman, J. W., Madden, J. C., Pawar, G., Yang, C., and Cronin, M. T. D. (2019) Molecular fingerprint-derived similarity measures for toxicological read-across: Recommendations for optimal use. *Regul. Toxicol. Pharmacol. 101*, 121–134.

(14) Schultz, T., and Yarbrough, J. (2004) Trends in structure-toxicity relationships for carbonyl-containing α, β-unsaturated compounds. *SAR and QSAR in Environmental Research 15* (2), 139–146.

(15) RIFM. Fragrance and Flavor Database. https://rifmdatabase.rifm.org/rifmweb/home/home-page (accessed June 10).

(16) Salvito, D., Lapczynski, A., Sachse-Vasquez, C., McIntosh, C., Calow, P., Greim, H., and Escher, B. (2011) Macrocyclic fragrance materials—A screening-level environmental assessment using chemical categorization. *Ecotoxicol. Environ. Saf. 74* (6), 1619–1629.

(17) OECD. Test Guidelines for the Chemicals. https://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm (accessed June 18th, 2019).

(18) Schultz, T. W., Diderich, R., Kuseva, C. D., and Mekenyan, O. G. The OECD QSAR toolbox starts its second decade. In *Computational Toxicology*; Springer: 2018; pp 55–77.

(19) VEGA project website. http://www.vega-qsar.eu/ (accessed June).

(20) Benfenati, E., Manganaro, A., and Gini, G. VEGA-QSAR. In *AI Inside a Platform for Predictive Toxicology*; CEUR-WS: 2013; Vol. *1107*, pp 21–28.

(21) Mekenyan, O., Dimitrov, S., Pavlov, T., Dimitrova, G., Todorov, M., Petkov, P., and Kotov, S. (2012) Simulation of chemical metabolism for fate and hazard assessment. V. Mammalian hazard assessment. *SAR and QSAR in Environmental Research 23* (5–6), 553–606.

(22) Remmer, H. (1970) The role of the liver in drug metabolism. *Am. J. Med. 49* (5), 617–629.

(23) Mekenyan, O., Dimitrov, S., Pavlov, T., and Veith, G. (2004) A Systematic Approach to Simulating Metabolism in Computational Toxicology. I. The TIMES Heuristic Modelling Framework. *Curr. Pharm. Des. 10* (11), 1273–1293.

(24) Guy, R. H. (2010) Predicting the Rate and Extent of Fragrance Chemical Absorption into and through the Skin. *Chem. Res. Toxicol. 23* (5), 864–870.

(25) Angelini, E., Camerini, G., Diop, M., Roche, P., Rodi, T., Schippa, C., and Thomas, T. (2016) Respiratory Health - Exposure Measurements and Modeling in the Fragrance and Flavour Industry. *PLoS One 11* (2), No. e0148769.

(26) Enoch, S., Ellison, C., Schultz, T., and Cronin, M. (2011) A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol. 41* (9), 783–802.

(27) Schwöbel, J. A., Koleva, Y. K., Enoch, S. J., Bajot, F., Hewitt, M., Madden, J. C., Roberts, D. W., Schultz, T. W., and Cronin, M. T. (2011) Measurement and estimation of electrophilic reactivity for predictive toxicology. *Chem. Rev. 111* (4), 2562–2596.

(28) Bajusz, D., Rácz, A., and Héberger, K. (2015) Why is Tanimoto index an appropriate choice for fingerprint-based similarity calculations? *J. Cheminf. 7*, 20–20.

(29) Rogers, D., and Hahn, M. (2010) Extended-Connectivity Fingerprints. *J. Chem. Inf. Model. 50* (5), 742–754.

(30) Park, H.-S., and Jun, C.-H. (2009) A simple and fast algorithm for K-medoids clustering. *Expert Syst. Appl. 36*, 3336–3341.

(31) Pipeline Pilot. http://accelrys.com/products/pipeline-pilot/.

(32) Schultz, T., Netzeva, T., Roberts, D., and Cronin, M. (2005) Structure-Toxicity Relationships for the Effects to Tetrahymena pyriformis of Aliphatic, Carbonyl-Containing, α,β-Unsaturated Chemicals. *Chem. Res. Toxicol. 18*, 330–41.

(33) Enoch, S. J., Cronin, M. T. D., Schultz, T. W., and Madden, J. C. (2008) Quantitative and Mechanistic Read Across for Predicting the Skin Sensitization Potential of Alkenes Acting via Michael Addition. *Chem. Res. Toxicol. 21* (2), 513–520.

(34) Heymann, E. *Carboxylesterases and Amidases, in Enzymatic Basis of Detoxication*; Academic Press: New York, 1980.

(35) Heymann, E. *Hydrolysis of Carboxylic Esters and Amides, in Metabolic Basis of Detoxication*; Academic Press: New York, 1980.

(36) Hanold, K. C. (1986) Teratogenic potential of valproic acid. *Journal of Obstetric, Gynecologic, & Neonatal Nursing 15* (2), 111–116.

(37) Zechner, R., Strauss, J. G., Haemmerle, G., Lass, A., and Zimmermann, R. (2005) Lipolysis: pathway under construction. *Curr. Opin. Lipidol. 16* (3), 333–340.

(38) Oliw, E. H., Brodowsky, I. D., Hornsten, L., and Hamberg, M. (1993) bis-Allylic Hydroxylation of Polyunsaturated Fatty Acids by Hepatic Monooxygenases and Its Relation to the Enzymatic and Nonenzymatic Formation of Conjugated Hydroxy Fatty Acids. *Arch. Biochem. Biophys. 300* (1), 434–439.

(39) Schultz, T. W., Przybylak, K. R., Richarz, A.-N., Mellor, C. L., Bradbury, S. P., and Cronin, M. T. D. (2017) Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected 2-alkyl-1-alkanols. *Computational Toxicology 2*, 28–38.

(40) Schultz, T. W., Przybylak, K. R., Richarz, A.-N., Mellor, C. L., Escher, S. E., Bradbury, S. P., and Cronin, M. T. D. (2017) Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected n-alkanols. *Computational Toxicology 2*, 12–19.

(41) Przybylak, K. R., Schultz, T. W., Richarz, A.-N., Mellor, C. L., Escher, S. E., and Cronin, M. T. D. (2017) Read-across of 90-day rat

https://dx.doi.org/10.1021/acs.chemrestox.9b00518
Chem. Res. Toxicol. 2020, 33, 1709–1718

oral repeated-dose toxicity: A case study for selected β-olefinic alcohols. *Computational Toxicology 1*, 22−32.
(42) Mellor, C. L., Schultz, T. W., Przybylak, K. R., Richarz, A. N., Bradbury, S. P., and Cronin, M. T. D. (2017) Read-across for rat oral gavage repeated-dose toxicity for short-chain mono-alkylphenols: A case study. *Computational Toxicology 2*, 1−11.
(43) Kamil, I. A., Smith, J. N., and Williams, R. T. (1953) Studies in detoxication. XLVI. The metabolism of aliphatic alcohols; the glucuronic acid conjugation of acyclic aliphatic alcohols. *Biochem. J. 53* (1), 129−136.
(44) Mekenyan, O. G., Petkov, P. I., Kotov, S. V., Stoeva, S., Kamenska, V. B., Dimitrov, S. D., Honma, M., Hayashi, M., Benigni, R., Donner, E. M., and Patlewicz, G. (2012) Investigating the Relationship between in Vitro-in Vivo Genotoxicity: Derivation of Mechanistic QSAR Models for in Vivo Liver Genotoxicity and in Vivo Bone Marrow Micronucleus Formation Which Encompass Metabolism. *Chem. Res. Toxicol. 25* (2), 277−296.
(45) Peng, H. M., Raner, G. M., Vaz, A. D., and Coon, M. J. (1995) Oxidative cleavage of esters and amides to carbonyl products by cytochrome P450. *Arch. Biochem. Biophys. 318* (2), 333−9.
(46) Firman, J. W., Patel, A., Date, M., Cronin, M. T. D., and Schultz, T. W. (2018) Read-across of 90-day rodent repeated-dose toxicity: A case study for selected simple aryl alcohol alkyl carboxylic acid esters. *Computational Toxicology 7*, 1−8.
(47) Wang, H.-T., Zhang, S., Hu, Y., and Tang, M.-s. (2009) Mutagenicity and sequence specificity of acrolein-DNA adducts. *Chem. Res. Toxicol. 22* (3), 511−517.
(48) Roberts, D. W., Patlewicz, G., Kern, P. S., Gerberick, F., Kimber, I., Dearman, R. J., Ryan, C. A., Basketter, D. A., and Aptula, A. O. (2007) Mechanistic Applicability Domain Classification of a Local Lymph Node Assay Dataset for Skin Sensitization. *Chem. Res. Toxicol. 20* (7), 1019−1030.
(49) Abraham, K., Wöhrlin, F., Lindtner, O., Heinemeyer, G., and Lampen, A. (2010) Toxicology and risk assessment of coumarin: Focus on human data. *Mol. Nutr. Food Res. 54* (2), 228−239.
(50) Glatt, H., Jung, R., and Oesch, F. (1983) Bacterial mutagenicity investigation of epoxides: drugs, drug metabolites, steroids and pesticides. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 111* (2), 99−118.
(51) Manson, M. M. (1980) Epoxides–is there a human health problem? *Occup. Environ. Med. 37* (4), 317−336.
(52) Murray, J. R., and Penning, T. M. (2018) Carcinogenic Polycyclic Aromatic Hydrocarbons. *Comprehensive Toxicology 7*, 87−153.
(53) Schultz, T. W., Cajina-Quezada, M., and Dumont, J. N. (1980) Structure-toxicity relationships of selected nitrogenous heterocyclic compounds. *Arch. Environ. Contam. Toxicol. 9* (5), 591−598.
(54) Kim, D., and Guengerich, F. *Annu. Rev. Pharmacol. Toxicol.* 2005; Vol. *45*, p 27−49.
(55) Schultz, T. W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D. J., Mahony, C., Schwarz, M., White, A., and Cronin, M. T. D. (2015) A strategy for structuring and reporting a read-across prediction of toxicity. *Regul. Toxicol. Pharmacol. 72* (3), 586−601.
(56) Schultz, T. W., and Cronin, M. T. D. (2017) Lessons learned from read-across case studies for repeated-dose toxicity. *Regul. Toxicol. Pharmacol. 88*, 185−191.
(57) Schultz, T. W., Richarz, A.-N., and Cronin, M. T. D. (2019) Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies. *Computational Toxicology 9*, 1−11.

https://dx.doi.org/10.1021/acs.chemrestox.9b00518
Chem. Res. Toxicol. 2020, 33, 1709−1718