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An End Point-Specific Framework for Read-Across Analog Selection for Human Health Effects

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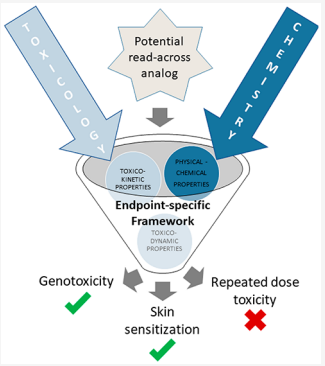
ABSTRACT: Integrating computational chemistry and toxicology can improve the read-across analog approach to fill data gaps in chemical safety assessment. In read-across, structure-related parameters are compared between a target chemical with insufficient test data and one or more materials with sufficient data. Recent advances have focused on enhancing the grouping or clustering of chemicals to facilitate toxicity prediction via read-across. Analog selection ascertains relevant features, such as physical-chemical properties, toxicokinetic-related properties (bioavailability, metabolism, and degradation pathways), and toxicodynamic properties of chemicals with an emphasis on mechanisms or modes of action. However, each human health end point (genotoxicity, skin sensitization, phototoxicity, repeated dose toxicity, reproductive toxicity, and local respiratory toxicity) provides a different critical context for analog selection. Here six end point-specific, rule-based schemes are described. Each scheme creates an end point-specific workflow for filling the target material data gap by read-across. These schemes are intended to create a transparent rationale that supports the selected read-across analog(s) for the specific end point under study. This framework can systematically drive the selection of read-across analogs for each end point, thereby accelerating the safety assessment process.



1. INTRODUCTION

Read-across is a structure−activity analysis[1] encompassing various techniques and approaches[2] to solve gaps in toxicity prediction.[3] The concept of chemical similarity is central to the read-across method.[4] The origins of chemical-based read-across can be traced back several decades.[5] However, the classifying and understanding of toxicological-related properties of organic chemicals has advanced markedly since the beginning of this century. Attempts to standardize modern-day read-across can be traced back to 2007 and the OECD Guidance on Grouping Chemicals,[6] which outlined the need for an all-encompassing structure-similarity rationale, together with a mechanistic basis of chemical categories and metabolic pathways. Regulatory interest in read-across was further driven by the 2009 EU notification under Article 13 on cosmetic products.[7]

While the premise of read-across is straightforward, the practice has become technically elaborate.[8] Today, one of the most challenging applications of read-across is identifying structurally similar materials for use as data sources to fill data gaps in evaluating the safety of a target chemical.[9] The uncertainties of a toxicological read-across prediction are due to the quality of the read-across data, the hypothesis and justification of the toxicokinetic and toxicodynamic bases of the prediction, and the relevant supporting data and information. Confidence in a read-across prediction is increased by identifying uncertainties associated with these factors and reducing them.[10] Read-across frameworks are more likely to be acceptable when undertaken on an end point-by-end point basis, as the mode of actions (MOA) for each end point varies widely.[11] This end point specificity reflects that the extent of test data available from *in vivo* and relevant *in vitro* assays are end point specific.

To aid the safe use of fragrance materials, the Research Institute for Fragrance Materials (RIFM) has undertaken a multiyear project to assess the safety of fragrance materials.[12] Reviews of these published safety assessments of the fragrance materials reveal that over 90% of materials lack toxicity data for at least one of the toxicological end points appraised in safety assessments. Consequently, read-across is often used to assist in safety assessments. Human health end points currently evaluated by RIFM include genotoxicity (mutagenicity and clastogenicity), skin sensitization, phototoxicity (photoirritation and photoallergenicity), repeated dose toxicity, reproductive toxicity (developmental toxicity and fertility), and local

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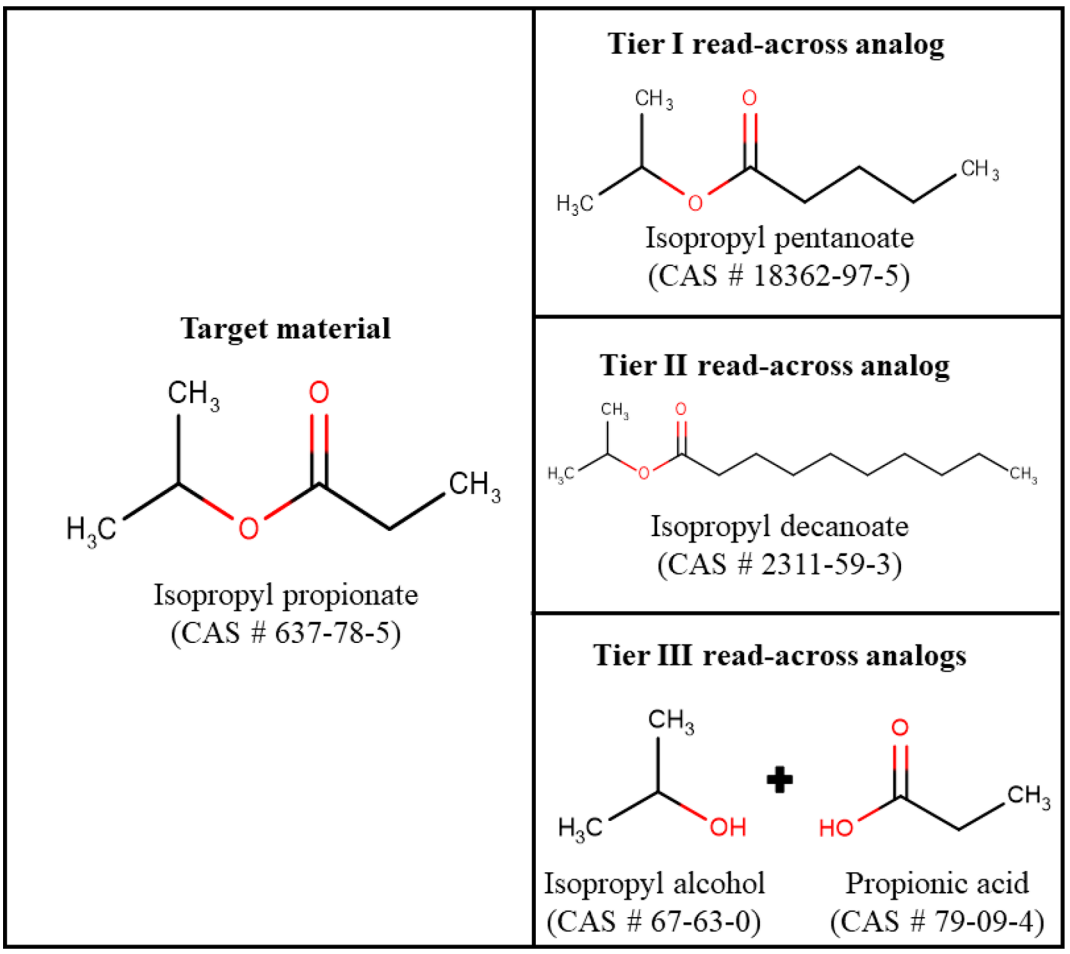

Figure 1. Potential Tier I, II, and III read-across analogs for a typical ester, isopropyl propionate.

respiratory toxicity. The overall aim of read-across within the RIFM safety assessment program is to fill data gaps for the target material with appropriate experimental data from the most suitable of the available read-across analogs.

To support the systematic application of read-across in RIFM safety evaluations, a stepwise workflow for clustering chemicals to support the identification of read-across analogs was developed.[13] First, the chemical inventory is clustered according to the presence of organic functional groups to form a supercluster. Second, these superclusters are further divided into subclusters in which chemicals bearing identical functional group(s) are collected. This grouping is based on the mitigating structural features of the functional group(s) and the structural similarity in the extended hydrocarbon fragment. Third, similarity in the absorption, distribution, metabolism, and excretion (ADME) properties are considered between the chemicals. Chemicals with significantly different ADME properties are further divided into additional subclusters. Finally, expert knowledge-based pruning is applied to optimize the association of physical-chemical properties with toxicokinetic and toxicodynamic properties in the context of a specific end point.

This clustering framework enables a systematic evaluation of read-across analogs for a target chemical. The best data source would be a read-across analog from the same cluster as the target chemical. When a target cluster does not contain a suitable read-across analog with sufficient data for the end point, chemicals in adjacent clusters are investigated for an appropriate read-across analog. Adjacent clusters can be defined as having similar "signatures", which are the functional group-based chemical class and hydrocarbon scaffolding characteristics of the included chemicals. Each cluster described in our earlier work has been defined by a signature.[13] These signatures vary from simple ones (e.g., alcohols/primary/straight chain/saturated/C1 to C5) to more complex ones (e.g., esters/primary/alcohol/straight chain/saturated/C1 to C5/acid/straight chain/unsaturated/vinylene/C14 to C18/ketone). Each cluster typically has four or more adjacent clusters based on its signature. A read-across analog from the same cluster as the target chemical is a Tier I read-across, whereas a read-across analog from an adjacent cluster(s) is a Tier II read-across. It is essential that Tier I and II read-across analogs have the same organic functional groups and critical structural features as the target, but they can differ in secondary structural features in the extended fragment.[13] If a satisfactory Tier I or Tier II read-across analog cannot be identified, Phase I metabolite(s) of the target chemical, chemicals from other clusters, or chemicals from outside the clustering scheme may be considered. This metabolism-based data gap filling is considered a Tier III read-across. For a Tier III read-across, the properties of the metabolite should apply to the end point being considered. The resulting read-across analog from this process must have the same or higher reactivity via an identical MOA as the target chemical. An example of this tiering strategy is shown using isopropyl propionate (CAS # 637-78-5) in Figure 1.

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In the case of isopropyl propionate, an example of a Tier I read-across analog is isopropyl pentanoate. These two chemicals are in the same cluster due to having all the same structural groups and a similar number of carbons. An example of a Tier II read-across for isopropyl propionate is isopropyl decanoate; even though the functional groups are the same, the difference in the number of carbons separates the target and read-across analog into adjacent clusters. In general, clusters are divided based on the number of carbons in the following ways: 1 to 5, 6 to 13, and 14 or more carbons. Finally, an example of Tier III read-across analogs for isopropyl propionate are isopropyl alcohol and propionic acid. These chemicals are the primary metabolites of isopropyl propionate due to ester hydrolysis, which makes them appropriate read-across analogs.

Even considering the process described above, to meet the overall aim of read-across, we have found end point-specific plans of action are required. Here a framework of end point-specific rules applied in identifying acceptable read-across analogs for the six human health end points evaluated by RIFM is reported. The rules described here as end point-specific schemes have been established based on the collective experience developing and evaluating more than 1500 read-across materials. These rules can be used to search databases and select read-across analogs appropriate for particular human health end points.

2. METHODS

From 2017 to 2022, more than 1500 target substances and nearly 3000 read-across analogs have been evaluated for RIFM safety assessments. In an iterative process, each target and read-across analog pair was initially evaluated by the RIFM computational chemistry team and an external panel of experts to create a series of rule-based schemes. The schemes provide a framework to establish end point-specific strategies for data gap filling by read-across.

The end point-specific rules described below build on the previously described rationale for chemical clustering.[13] Chemoinformatics-based similarity measures have been used to develop chemical categories and identify analog pairings for read-across for several end points. The primary example of the chemoinformatic approach is the fingerprint method.[14] Encoding a target chemical and potential read-across chemicals in a database into such fingerprints enables them to be compared using computational measures of similarity, such as a Tanimoto score,[15] which are widely used as an elementary step of a read-across analog assessment. While these structural similarity scores are helpful tools, they can be limited in their considerations. Often, computational measures of similarity can give too much significance to certain parts of a material to increase the similarity score and too little significance to other parts to lower the similarity score. Therefore, it is crucial to use expert judgment based on end point-specific rules along with a structural similarity score to determine the validity of a potential read-across analog.

The primary approach to comparing the reactive potential and other mechanistic and mode of action relevant features of the target material and the read-across analog is through use of structural alerts from well-known *in silico* platforms such as OECD QSAR Toolbox, TIMES, ToxTree, and Vega (CAESAR model). Of particular value is the "category consistency" module in the OECD QSAR Toolbox. However, the final evaluation of the proposed read-across analog is driven primarily by expert knowledge of the chemical mechanisms and biological mode of toxic action. The amount of appropriate experimental data available varies significantly depending on the end point, and the assessment of the reliability of each prediction is end point specific. The experimental data used in the safety assessment process are sourced from a combination of open-source databases, such as ECHA REACH and National Toxicology Program, as well as the RIFM database. The RIFM database is a mix of open literature and proprietary experimental data organized by human health end point.

Building on a previously described strategy[8] augmented with recently described methodologies,[16,17] a flexible workflow was established. This workflow is based on the degree of characterization of the mechanisms or modes of action associated with each toxicity end point, matching target material and read-across analogs based on organic functional groups, heteroatom similarity, and hydrocarbon skeleton configuration (i.e., 2D structure) that vary in stringency.

For genotoxicity and skin sensitization, harmonizing organic functional groups and/or extended fragment similarity between the target material and the read-across analog closely follow the experimental chemistry and structure−activity relationships linked to chemical reactivity.[18] Such information[19,20] is used implicitly as part of the knowledge captured in various software (e.g., genotoxicity and skin sensitization related profilers in the OECD QSAR Toolbox).

It is well-established that some organic chemicals induce phototoxicity due to excitation reactions of a UV-absorbing chromophore.[21] The chromophore may have substituents that modulate its photoabsorbance ability. This modulation is well understood and can be predicted using Woodward-Fieser rules. In addition, *in chemico* testing (i.e., spectrum analysis for shorter wavelength absorbance, both UVB [290−320 nm] and UVA [320−400 nm]) readily excludes nonphototoxicants.

For health end points associated with lesser-understood argumentations, such as repeated dose, reproductive, and local respiratory toxicity, matching the 2D structure of the target material and the read-across analogs is strict. In many cases, matching ADME properties is critical to establishing an acceptable read-across contention.

3. RESULTS

3.1. Genotoxicity. The genotoxicity end point is assessed qualitatively, and the substance in question is classified as either genotoxic or nongenotoxic. Genotoxicity is evaluated by addressing mutagenic and clastogenic potential via a battery of *in vitro* tests (i.e., Ames and the *in vitro* micronucleus test[22]). The genotoxicity *in vitro* assays are well-studied and data-rich, with well understood mechanistic probability.

In read-across to fill a genotoxicity data gap:

- The reactivity of a chemical toward nucleic acids is the most important property for assessing its mutagenic potential.[19] The reactivity of a chemical toward proteins is the most important property for determining its clastogenic potential.[20] The presence of known structural alerts (i.e., reactive functional groups or molecular substructures) associated with genotoxic properties is the seminal factor in establishing mutagenic and clastogenic similarities for the read-across analog.
- The read-across analog must have the same reactivity mechanism as the target. If more than one structural alert is identified in the target, all these alerts must be present in the read-across analog as well. Since reactivity varies within a mechanism, the read-across analog should have the same reactivity as the target substance. For well-studied mechanisms (e.g., Michael addition[23]), the read-across analog must be equi-reactive or more reactive than the target.
- The extended hydrocarbon fragments or molecular skeletons do not play an essential role in assessing genotoxicity. Therefore, the acceptable range of hydrocarbon similarity is wide.
- *In silico* predictions for structure alerts, metabolism, and reactivity must be consistent between the target and read-across analog for relevant models or profilers.

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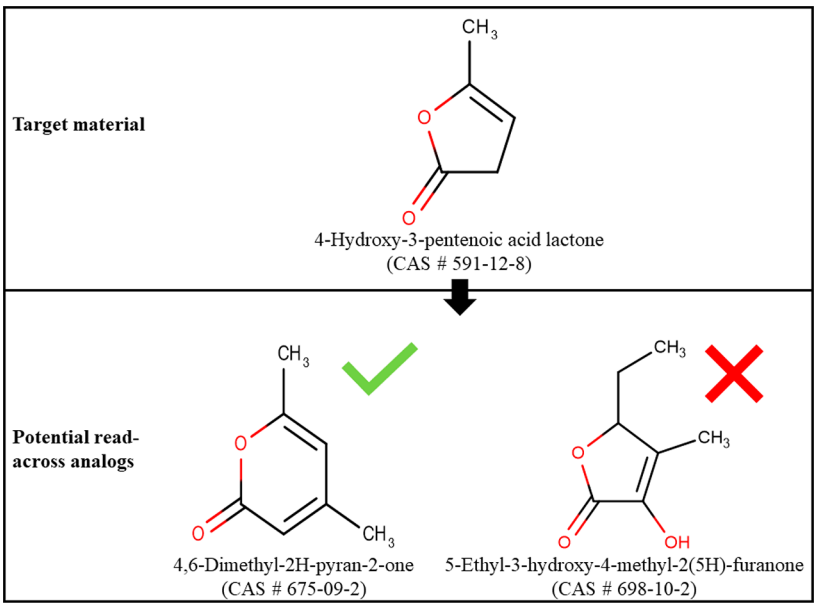

Figure 2. Read-across analog options for the genotoxicity end point. The target material, 4-hydroxy-3-pentenoic acid lactone, is shown at the top and two potential read-across analogs, 4,6-dimethyl-2H-pyran-2-one and 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

- In cases where multiple reactive groups in the target molecule may contribute independently to genotoxicity, an additional molecule with only the lesser reactivity structural feature and accompanying data may serve as "weight of evidence (WoE)" to supplement the read-across analog.
- Liver metabolism is often essential in assessing genotoxicity, especially in transforming a nongenotoxic parent structure into a reactive metabolite. Computational approaches for predicting metabolic transformations associated with the liver (e.g., TIssue MEtabolism Simulator [TIMES] simulations) often provide the primary information used to assess metabolic similarity for genotoxicity end points. Read-across analogs should follow the same metabolic pathway(s) and have similar primary and secondary metabolites.

An example of the above workflow in a published safety assessment is for ethyl 3-methyl-2-oxopentanoate (CAS # 26516-27-8), where a read-across pairing was developed and used to fill the data gap for genotoxicity.[24] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a genotoxicity read-across is shown in Figure 2.

The target material, 4-hydroxy-3-pentenoic acid lactone, is a furan with a ketone at the 2 position and a methyl group at the 5-position. While 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone has structural similarity to the target material, it includes a hydroxy group not present in the target material, which may alter the potential reactivity. Specifically, keto−enol tautomerism allows this hydroxy group to convert to a ketone, which would form a diketone in this potential read-across analog. Diketones have differing reactivity to the target material, making 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone an inappropriate read-across. 4,6-Dimethyl-2H-pyran-2-one is a pyran making it larger than the furan and contains an additional unsaturation, but it contains the same reactive features as the target material. The structural differences between the latter two compounds will not cause different reactivity, making 4,6-dimethyl-2H-pyran-2-one a more appropriate read-across analog.

3.2. Skin Sensitization. Skin sensitization is scientifically well-studied and is driven by reactivity, similar to the clastogenicity end point for genotoxicity. The skin sensitization end point has an established Adverse Outcome Pathway (AOP) that is universally accepted.[25] Thus, the mechanistic probability of reactivity is well-understood. This end point is assessed qualitatively, with the target material classified as either a nonsensitizer or a sensitizer with a No Expected Sensitization Induction Level (NESIL).[26,27] The wide variety of test procedures and data richness for skin sensitization support a clearly defined scheme for assessing chemical pairings for read-across.[28]

Skin sensitization is evaluated by first considering all historical *in vivo* (animal and human) and *in vitro* studies available on the target material. In cases where no *in vivo* data are available or *in vitro* test data are considered insufficient to evaluate the skin sensitization potential, read-across may be employed.

In read-across to fill a skin sensitization data gap:

- The read-across analog must have the same structural features that drive protein reactivity as those in the target. If more than one structural alert is identified in the target material, all these alerts must be present in the read-across analog as well. Since reactivity varies for the same mechanism, the read-across analog should have the same reactivity as the target material. For well-studied mechanisms, the read-across must be equi-reactive or more reactive than the target.
- The extended hydrocarbon fragments or molecular skeletons do not play an essential role in assessing skin sensitization. Therefore, the acceptable range of hydrocarbon similarity is wide.
- Consistency between *in silico* predictions for the target material and the read-across analog for relevant models or profilers add a WoE to the read-across.

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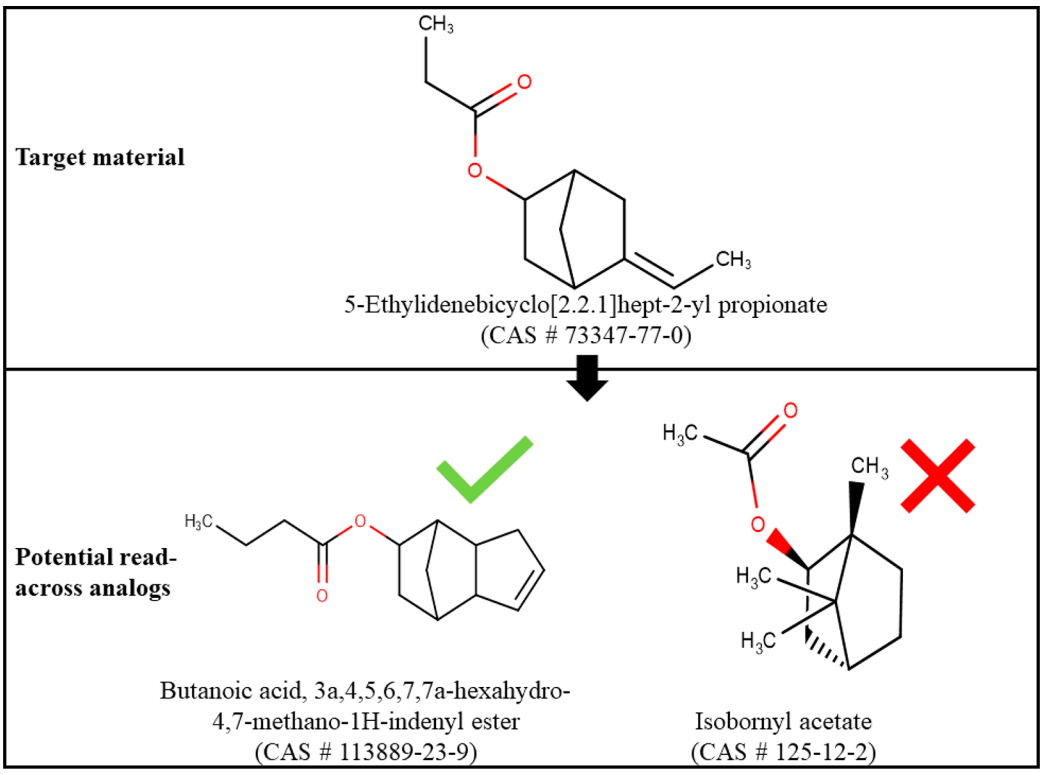

Figure 3. Read-across analog options for the skin sensitization end point. The target material, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate, is shown at the top and two potential read-across analogs, butanoic acid, 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-indenyl ester and isobornyl acetate, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

- Skin absorption is an essential parameter for assessing the sensitization end point. Thus, the read-across analog must be absorbed at least as readily as the target. The absorption parameter is often appraised by comparable target material and read-across analog molecular weights and/or the read-across analog having higher water solubility and lower logK_{ow} (within 1 order of magnitude) of the target material.
- Skin metabolism can be an important consideration in assessing skin sensitization, especially in transforming a nonsensitizing parent structure to a reactive sensitizing metabolite (i.e., an experimentally determined or *in silico* simulated metabolite having a structural alert). The **TI**ssue **ME**tabolism **S**imulator platform for predicting **S**kin **S**ensitization (TIMES-SS), a mechanistically based expert rule system, is the primary source of metabolic information used in assessing metabolic similarity for skin sensitization. Read-across analogs should follow the same metabolic pathways as the target and have similar primary and secondary metabolites.

An example of the above workflow in a published safety assessment is for 6-nonenenitrile, (Z)- (9CI) (CAS # 80639-54-9), where a read-across pairing was developed and used to fill the data gap for skin sensitization.[29] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a skin sensitization read-across is shown in Figure 3.

The target material, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate, is a bridged cyclohexane with a propionate ester as a side chain substituent. Isobornyl acetate possesses the bridged cyclohexane and an acetate ester side chain similar to the target material, but it lacks a vinylene group. Since the vinylene in the target material could be a reactive site, it must be present in the read-across analog. Therefore, isobornyl acetate is an inappropriate read-across analog for the target. Butanoic acid, 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-indenyl ester possesses the bridged cyclohexane and ester side chain found in the target material; it also has a vinylene group in the fused cyclopentane ring. In this case, the hydrocarbon skeleton orientation does not affect the reactivity of the vinylene, so the difference in structure is ignored. Therefore, butanoic acid, 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-indenyl ester is an appropriate read-across analog.

3.3. Phototoxicity. Phototoxicity occurs due to the photoactivation of exogenous chemicals and requires adequate exposures to both the chemical and the activating wavelengths of UV−vis radiation.[21] Phototoxicity includes photoirritation and photoallergenicity. Photoirritation manifests as a chemically induced skin "rash" following exposure to light and is not immune-mediated. Sufficient *in vivo* data to address this form of phototoxicity are rarely available. Organic chemicals that induce phototoxicity possess a UV-absorbing chromophore. Both UVB (290−320 nm) and UVA (320−400 nm) may contribute to photoirritation. Since a material with no significant absorbance in the range of 290−760 nm is considered photochemically inactive, UV−vis absorbance spectrum analysis may be used to eliminate materials from phototoxicity consideration. A threshold of 1000 L × mol^{-1} × cm^{-1} is generally accepted as the cutoff value for phototoxicity potential.[30,31]

Photoallergenicity is far less common than photoirritation and is a Type IV delayed hypersensitivity reaction in the skin

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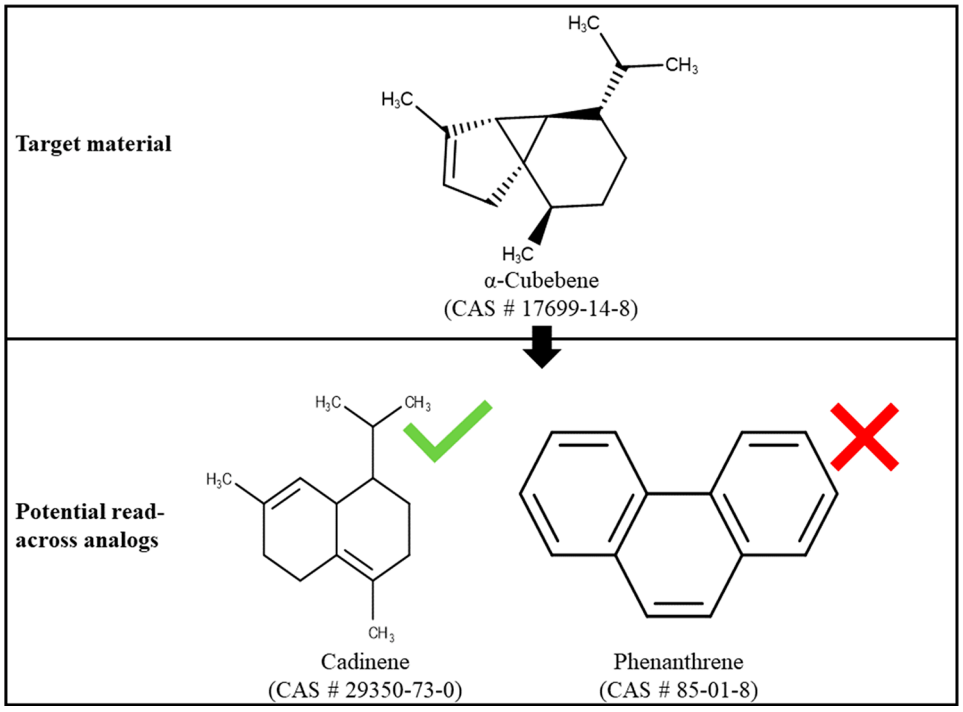

Figure 4. Read-across analog options for the phototoxicity end point. The target material, α-cubebene, is shown at the top and two potential read-across analogs, cadinene and phenanthrene, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

in response to a photoantigen in subjects previously sensitized to the same substance.[32,33] Photoallergic responses are thought to involve two mechanisms. In the first, light converts the photosensitizer to a photoproduct that subsequently binds to tissue proteins producing the hapten-protein complex. In the second mechanism, light absorbed by the photosensitizer results in its conversion to a photoproduct that is a more potent allergen than the parent material. To develop of photoallergenicity, UVA is usually required to create a complete antigen, and the photoallergic substance needs to be in the skin at the time of radiation exposure. For a subject to display photoallergenicity, the skin must contain the photosensitizing chemical at the appropriate concentration and be exposed to sufficient light. Due to the paucity of chemical substances with sufficient data, read-across is seldom used to address data gaps for photoallergenicity.

- For photoallergenicity, the target and read-across analog must belong to the same chemical class (i.e., possess the same number and type of functional group[s]) and have highly similar hydrocarbon moieties (i.e., same extended fragment shape within ±3 carbon atoms of each other).

In read-across to fill a photoirritation data gap:

- The target and read across analog must have the same chromophore(s). Chromophore substituents should be identical or highly similar. If multiple chromophores are present in the target and these chromophores are structurally isolated, two read-across analogs, one for each chromophore, may be used.
- The extended hydrocarbon fragments or molecular skeletons do not play an essential role in assessing skin sensitization. Therefore, the acceptable range of hydrocarbon similarity is wide.

An example of the above workflow in a published safety assessment is for 1-cyclohexene-1-propanal, 4-(1-methylethyl)-, (4R) (CAS # 1378867-81-2), where a read-across pairing was developed and used to fill the data gap for phototoxicity.[34] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a phototoxicity read-across is shown in Figure 4.

The target material, α-cubebene, is a series of three fused rings (cyclopentene, cyclopropane, and cyclohexane) that contains a methyl group substituent in the cyclopentene and cyclohexane ring, and an isopropyl group substituent on the cyclohexane ring. Phenanthrene contains three fused rings; however, it also contains an extended conjugated system, which alters the chromophore and the UV−vis absorbance compared to the target material. This alteration makes it an inappropriate read-across analog for the target material. Cadinene contains only two fused cyclohexane rings, each with a substituted vinylene; both rings have a similar substitution pattern to the target material. Even though cadinene contains an additional substituted vinylene and differing hydrocarbon skeletons, the UV−vis absorbance should not be significantly different since the vinylene groups are sufficiently isolated from each other, and the hydrocarbon skeleton does not play a role in affecting the UV−vis absorbance. In addition, the specific stereochemistry of the material is of no consequence. Therefore, cadinene is an appropriate read-across analog.

3.4. Repeated Dose Toxicity. Repeated dose toxicity evaluates systemic effects remote from the initial exposure site (typically oral or dermal), and after ADME properties are considered.[11] While results based on a 90-day subchronic toxicity protocol are preferred, other data (e.g., results for 28-day and 45 to 54-day repeated dose protocols and chronic studies) are often assessed. In repeated dose tests, various

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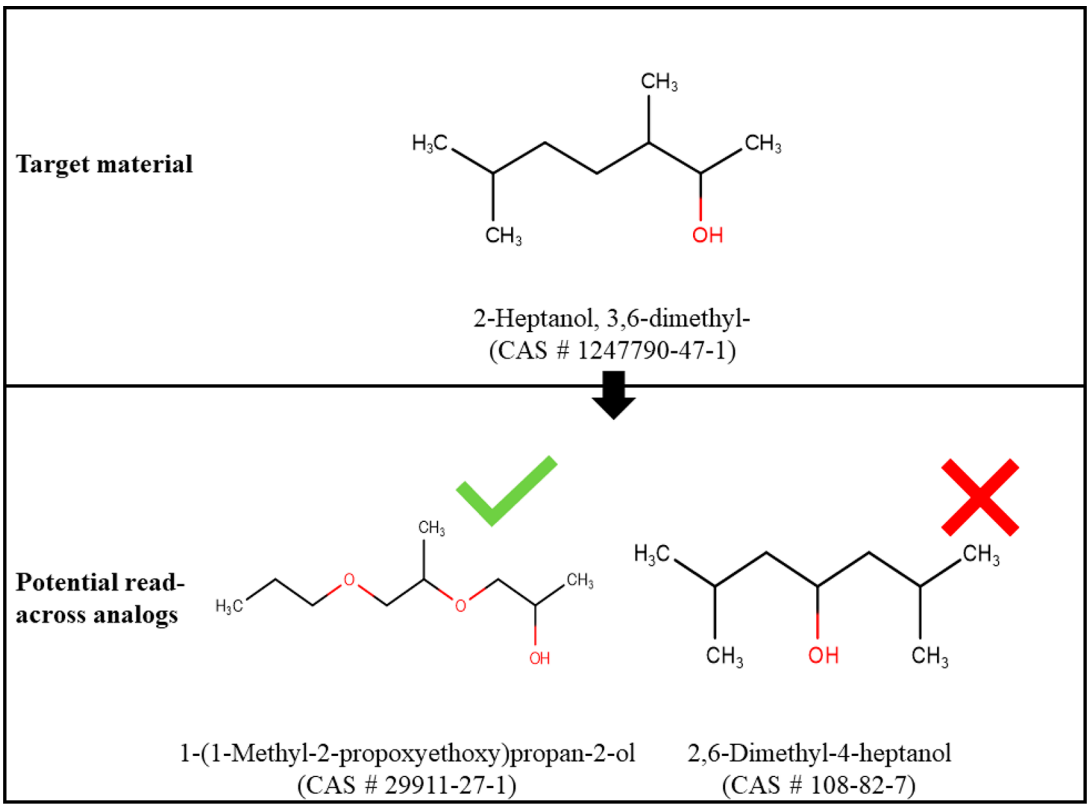

Figure 5. Read-across analog options for the repeated dose toxicity end point. The target material, 2-heptanol, 3,6-dimethyl-, is shown at the top and two potential read-across analogs, 1-(1-methyl-2-propoxyethoxy)propan-2-ol and 2,6-dimethyl-4-heptanol, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

parameters are typically recorded, including nonclinical signs (water and food consumption, behavior, etc.), individual and organ weight-related parameters, hematology, clinical biochemistry and urinalysis, and histopathological evaluation of non-neoplastic effects.[35] Repeated dose findings are often subdivided into adaptive, general whole animal, or organ/tissue-specific adverse effects. Oral and dermal exposure-based assessment of potential impact is based on the No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL), which are compared to the estimated exposure to determine the Margin Of Exposure (MOE). With some exceptions, repeated dose toxicity is not well understood and is data-poor.

In read-across to fill a repeated dose toxicity data gap:

- The mechanisms and modes of action for repeated dose toxicity are not well-known. Repeated dose toxicity is not well-defined. Due to a lack of a test-based end point definition and an incomplete understanding of the toxicodynamics and toxicokinetics related to repeated dose toxicity, a conservative approach is taken for selecting a read-across analog.
- Read-across selection for chemicals that elicit irreversible, organ-specific, histopathological-defined effects (i.e., classic systemic toxicity) follows a strict 2D chemical structure-similarity analysis. The read-across analog should have the same functional group(s) as the target and in structurally similar positions. Moreover, the hierarchy of reactivity-related structural features of the read-across analog should match the hierarchy of the features of the target. High structural similarity between the hydrocarbon skeleton of the target substance and the read-across analog is required to ensure similar ADME properties.
- Read-across analysis for chemicals proposed to elicit reversible or adaptive effects (e.g., nonclinical signs, individual and organ weight-related parameters, etc.) follows an adapted similarity analysis. While the target substance and the read-across analog should have similar ADME properties, greater dissimilarity in the overall 2D structure of the target substance and read-across analog may be acceptable based on expert judgment.
- Consistency between *in silico* predictions for the target and the read-across analog from relevant models or profilers add a WoE to the read-across.

An example of the above workflow in a published safety assessment is for cadinene (CAS # 29350-73-0), where a read-across pairing was developed and used to fill the data gap for repeated dose toxicity.[36] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a repeated dose toxicity read-across is shown in Figure 5.

The target material, 2-heptanol, 3,6-dimethyl-, is a secondary alcohol on a branched hydrocarbon chain. 2,6-Dimethyl-4-heptanol is also a secondary alcohol on a branched hydrocarbon chain. While these chemicals are structurally similar as determined by the Tanimoto score, there is a critical difference in the position of the hydroxy group. In the target material, the hydroxy group can form an α-methyl ketone, which is more reactive than the ketone formed by the hindered hydroxy group in the potential read-across analog, thus making it an inappropriate read-across analog. 1-(1-Methyl-2-propoxyethoxy)propan-2-ol is less structurally similar to the

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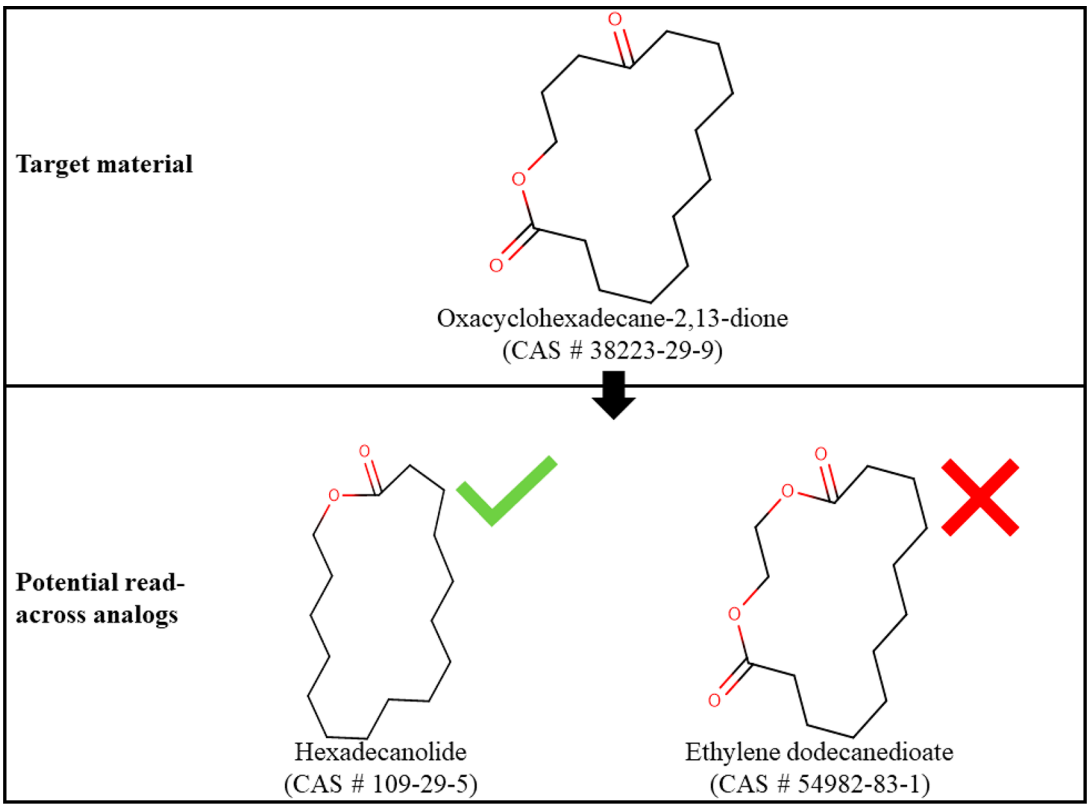

Figure 6. Read-across analog options for the reproductive toxicity end point. The target material, oxacyclohexadecane-2,13-dione, is shown at the top and two potential read-across analogs, hexadecanolide and ethylene dodecanedioate, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

target material, as it has two ether linkages. However, it also has an unhindered secondary alcohol that can form an α-methyl ketone. Since the ether linkages are predicted to be inert, the functionality of this potential read-across analog better matches the target material. Therefore, 1-(1-methyl-2-propoxyethoxy)propan-2-ol is an appropriate read-across analog.

3.5. Reproductive Toxicity. Reproductive toxicity includes fertility-related and developmental effects. Fertility (maternal and/or paternal) effects include reduced fertility, impact on gonads, oogenesis, spermatogenesis, and general disturbances to reproductive cycles. Developmental toxicity affects growth and causes developmental retardation, malformations, and functional deficits in the fetuses, neonates, and maturing offspring.[37] Data on these end points are often difficult to interpret from a structure−activity perspective due to the complexity of ontogenetic development, the interaction of the developing offspring with the pregnant mother, multiple final manifestations or end points recorded, and the variation in susceptibility depending on the developmental stage at the time of exposure.[38] Mammalian maternal−fetal interactions are complex. As such, absorption, distribution, metabolism, and excretion within the mother and the conceptus are ever-changing. With few exceptions, fertility and developmental toxicity are not well-studied and, thus, are data-poor.

In read-across to fill a reproductive toxicity data gap:

- Because fertility centers on maternal and paternal effects, it is assessed for read-across analogs similarly repeated dose toxicity (see above section).
- The mechanisms and modes of action for developmental toxicity are not well-known; there is an incomplete understanding of receptor-mediated responses in fetuses and neonates and a lack of understanding of how maternal toxicodynamics affect fetal toxicokinetics. Thus, a conservative approach is taken for selecting a read-across analog for developmental toxicity. The read-across analog should have the same functional group(s) as the target and in structurally similar positions. High structural similarity between the hydrocarbon skeleton of the target substance and the read-across analog is required to ensure similar ADME properties.
- Consistency between *in silico* predictions for the target material and read-across analog for relevant models or profilers add a WoE to the read-across.

An example of the above workflow in a published safety assessment is for dihydroisocaryophyllene epoxide (CAS # 1209-61-6), where a read-across pairing was developed and used to fill the data gap for reproductive toxicity.[39] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a reproductive toxicity read-across is shown in Figure 6.

The target material, oxacyclohexadecane-2,13-dione, is a macrocyclic molecule with an ester group and a ketone. Ethylene dodecanedioate is also a macrocyclic molecule with two ester groups. This diester configuration allows for ester hydrolysis at two locations and metabolites different from the target material to be formed, so this would be an inappropriate read-across analog. Hexadecanolide is a macrocyclic molecule with a single ester group. Compared to the target material, structurally, it is only missing a ketone. Since the ketone in the target material is isolated, it is not predicted to be of

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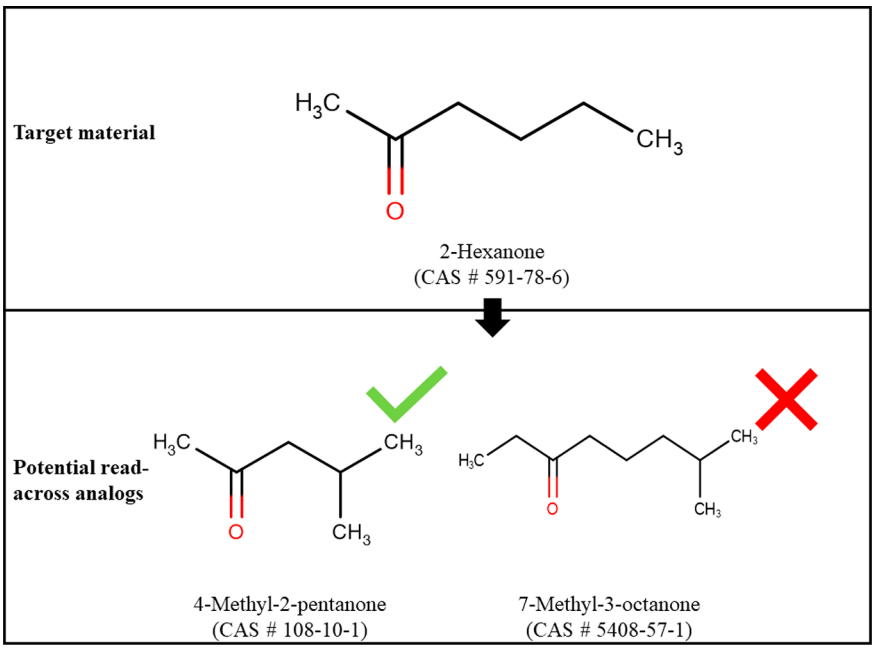

Figure 7. Read-across analog options for the local respiratory toxicity end point. The target material, 2-hexanone, is shown at the top, and two potential read-across analogs, 4-methyl-2-pentanone and 7-methyl-3-octanone, are shown at the bottom. The green checkmark and the red X identify an appropriate and inappropriate read-across analog for the target material, respectively.

toxicological concern. Therefore, the metabolites formed through ester hydrolysis of this analog and the target material are similar, and hexadecanolide is an appropriate read-across analog.

3.6. Local Respiratory Toxicity. Respiratory toxicity is typically caused by exposure to chemical substances via inhalation. It includes adverse local effects at exposure sites (e.g., nose, larynx, etc.) and effects on the proximal airways and distal lung. Local respiratory toxicity is an ill-defined end point with several mechanisms of injury. It includes respiratory irritation and inflammation and effects of immunological origins. If not resolved, the initial effects of inhaled substances often lead to histopathological effects.[40] Inhalation toxicity data-based evaluation of the potential for induction of effects in the respiratory tract is based on the NOAEC (No Observed Adverse Effect Concentration) or the LOAEC (Lowest Observed Adverse Effect Concentration) being compared to the predicted exposure to determine the MOE.

In read-across to fill a local respiratory toxicity data gap:

- Local respiratory toxicity is not well-defined. Standard and validated animal alternative tests are not available for evaluating local irritation effects from inhalation exposure to materials. Due to a lack of an end point definition and poor understanding of the toxicodynamics and toxicokinetics related to respiratory toxicity, a very conservative approach is taken to select an appropriate read-across analog.

- Since vapor pressure and partitioning are fundamental physical-chemical properties for local respiratory toxicity and both properties are 2D structure-dependent, the structural similarity rules for a potential read-across analog are strict. The target material and read-across analog should belong to the same chemical class (i.e., possess the same number and type of functional group[s]) and a highly similar hydrocarbon moiety (i.e., same extended fragment shape within ±3 carbon atoms of each other).

An example of the above workflow in a published safety assessment is for p-mentha-1,4-diene (CAS # 99-85-4), where a read-across pairing was developed and used to fill the data gap for local respiratory toxicity.[41] To further demonstrate the application of the above workflow, an additional example of a suitable and unsuitable chemical pairing for a local respiratory read-across is shown in Figure 7.

The target material, 2-hexanone, is an α-methyl ketone with 6-carbons, a log K_{ow} of 1.38, and vapor pressure of 155 Pa at 25 °C. 7-Methyl-3-octanone is a ketone with 9-carbons, a log K_{ow} of 2.64, and a vapor pressure of 254 Pa at 25 °C. Due to the conservative nature of data gap filling for this end point, the lack of an α-methyl ketone and the carbon and log K_{ow} differences make 7-methyl-3-octanone an inappropriate read-across analog. 4-Methyl-2-pentanone is an α-methyl ketone with 6-carbons, a log K_{ow} of 1.31, and vapor pressure of 265 Pa at 25 °C. The number of carbons and the log K_{ow} make it a better match to the target material, and the α-methyl ketone functionality is preserved. Therefore, 4-methyl-2-pentanone is an appropriate read-across analog.

4. CONCLUSIONS

Read-across is an essential method for deriving a chemical safety assessment.[14] Since read-across typically combines data mining with expert judgment, it is inevitably subject to various uncertainties.[8] Reducing these uncertainties is key to the consistent and reliable application of the read-across approach in chemical safety assessments. In the RIFM Fragrance Material Safety Assessment process, read-across is facilitated through chemical clustering, which enables systematic selection of candidate read-across analogs.[11] This work describes the application of end point-specific schemes for read-across selection. The development of a systematic approach to select read-across analogs in an end point-specific

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context improves the consistency and reliability of the safety assessment process.

The immediate impact of applying end point-specific schemes for read-across selection is the ability to vary the stringency by which read-across analogs are matched to target chemicals. Where mechanisms or modes of action are most uncertain, such as reproductive toxicity, criteria for analog selection allow little flexibility in varying structural features. Where mechanisms are better understood, such as skin sensitization, greater flexibility in analog selection is allowed, focusing mainly on conserving reactive structural features.

An acceptable read-across analog should have a similar mechanism(s) and/or mode(s) of action as the target (i.e., both chemicals are within the same chemical reactivity and/or biological activity domain).[22] Difficulty in establishing commonality in mechanism or mode of action with sufficiently limited uncertainty typically results in rejection of chemical pairings for read-across. Compounding the difficulty in establishing commonality in mechanism/mode of action is the impact of metabolism and, to a lesser extent, other ADME properties on establishing sufficient similarity between the target and read-across analog.

The RIFM fragrance material inventory represents a narrow segment of the chemical universe that is defined by volatility and is relatively uniform in chemical properties. Analysis of critical structural features typically focuses on only one or two of a limited number of well-characterized substituted or functional groups. Since the RIFM inventory is relatively rich in data, many chemical pairings have been assessed in developing the end point-specific schemes. These rules represent the consensus of the RIFM scientists and expert advisors on end point-specific factors that guide the application of read-across in safety assessments. The schemes described here are critical to furthering the acceptance of read-across predictions in assessing chemical safety and, thus, reducing the need for additional testing. Furthermore, the schemes broadly apply to chemical domains associated with flavors and cosmetics.

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Author Contributions

H.M., M.S.D., M.K., and D.T.S. performed the initial computation chemistry assessments. M.S.D., T.W.S., and D.C.L. developed the initial draft. All contributed to revisions of the drafts. H.M., M.S.D., M.K., T.W.S., D.C.L., and T.M.P. performed the expert judgment tasks and T.W.S., D.C.L., and T.M.P. proposed the rules. All authors contributed to the drafting of the rules. All authors have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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