

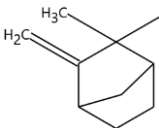
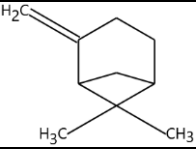
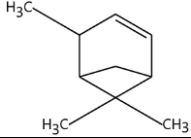
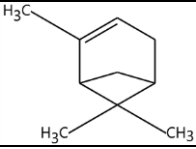
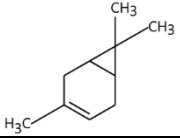
Case study:

Category consistency assessment in Toolbox for a list of *Cyclic unsaturated hydrocarbons* with respect to repeated dose toxicity.

1. Introduction

The aim of this case study is to demonstrate the use of the category consistency module within the Toolbox analyzing the group of six chemicals, which were exemplified in the paper “Category consistency in the OECD QSAR Toolbox: assessment and reporting tool to justify read-across”. The chemicals are represented in Table 1.

Table 1. Six cyclic unsaturated hydrocarbons thought to be analogues

#	CAS RN	Name	2D representation
1	79-92-5	Camphene	
2	127-91-3	beta-Pinene	
3	3387-41-5	Sabinene	
4	1330-16-1	Pinane, didehydro derivative	
5	80-56-8	alpha-Pinene	
6	13466-78-9	delta-3-Carene	

One chemical, camphene (#1 in Table 1) has experimental repeated-dose data which could be used in a read-across analysis based on similarity with the rest of five chemicals and thus, may or may not be used as a source material for reading across to fill the data gaps of the remaining five group members.

2. Repeated dose toxicity of Camphene

Camphene (CAS# 79-92-5) was administered daily by oral gavage to SPF Wistar rats (5/sex/dose) for 28 days at doses of 0, 62.5, 250 and 1000 mg/kg(bw)/day (see <https://echa.europa.eu/registration-dossier/-/registered-dossier/14290>). Tests were conducted according to OECD guideline 407. In all dose groups, behaviour and general health were examined daily. The highest dose group (1000 mg/kg bw/day) rats showed an increased salivation. Behaviour and general health from other treated groups were not significantly different from control group. The body weight and food- and water-consumption were not affected by the administration of camphene.

Based on the results of the experimental study of camphene toxicity, for female rats, the 28-day repeated-dose NOEL is 250 mg/kg(bw)/day. For male rats, the 28-day repeated-dose NOEL could not be determined but it is lower than 62.5 mg/kg(bw)/day.

3. Category consistency functionality in Toolbox

The Category consistency functionality is located at the “Category definition” module of the Toolbox. It is illustrated in Figure A1.

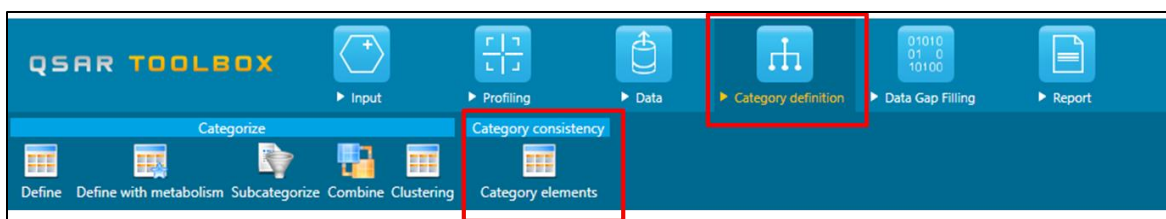


Figure A1. Screen shot of the location of Category consistency functionality in Toolbox

In order to activate the Category consistency a list with structures need to be available for the assessment and the target endpoint needs to be defined. Once activated, a wizard guides the user in the selection of the relevant information for category consistency assessment. There are four main tabs in the wizard: Physicochemical similarity, Structural similarity, Mechanistic similarity, (Eco)tox experimental data. In each tab, some of the elements deemed important for the endpoint (i.e. “suitable” profilers, if any) are shown as first and pre-selected by default. As second, profilers classified as “plausible” for the target endpoint are proposed in the selection list. Nevertheless, the selections can be changed by the user. While the “Physicochemical” and the “Structural” layers could be regarded as endpoint non-specific, the “Mechanistic layer” is strongly connected to the specified endpoint being considered. It is therefore noteworthy that category consistency is endpoint-specific.

A screen shot of the Category consistency wizard is shown in Figure A2.

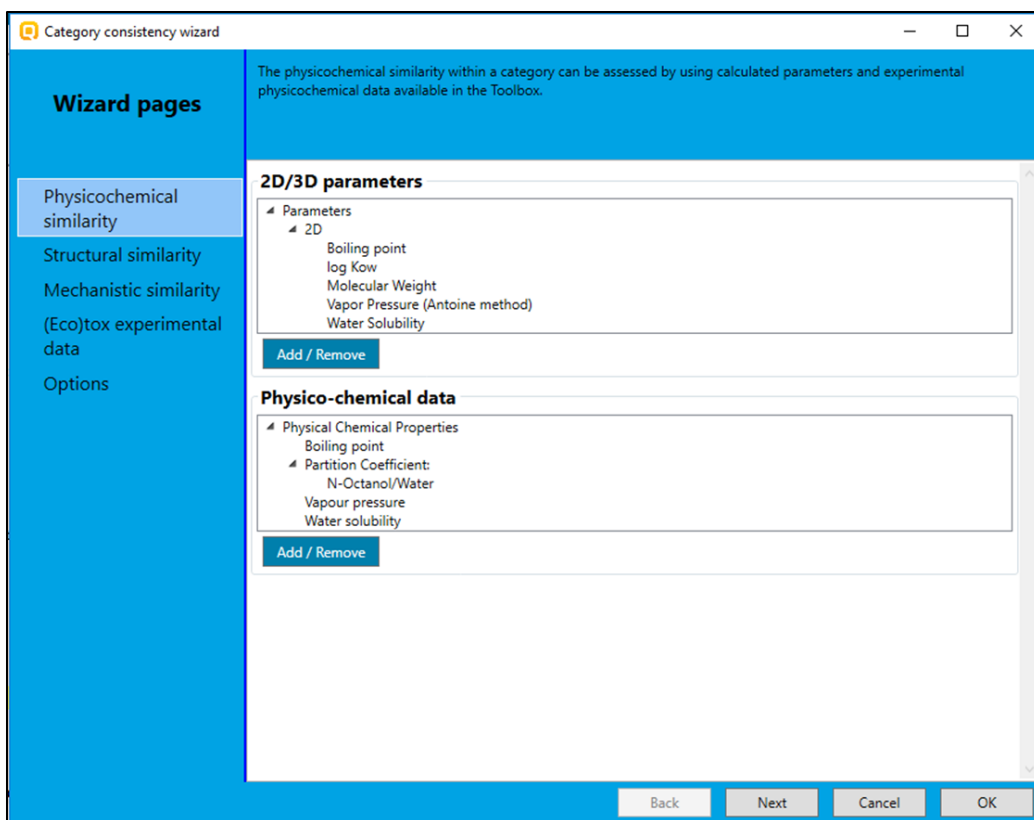


Figure A2. Screen shot of the Category consistency wizard with the contents shown for “Physicochemical similarity” layer.

The information related to these layers and selected by the user is collected automatically and appears in the working data matrix. The user can then use his/her expertise to conclude on whether the category is consistent and can eventually be used for data gap filling. The user is also able to export this information, including the data matrix, in the new “Category report” in the “Report” module.

3.1. Physicochemical similarity

The first layer of the category consistency is associated to the physicochemical properties of the chemicals, which are in turn related to bioavailability. “Physicochemical similarity” provides the

possibility to select calculated and experimentally determined 2D and 3D parameters for physical chemical properties. While five properties (boiling point, log Kow, molecular weight, vapor pressure, water solubility) are pre-selected by default, the entire dropdown list of parameters and properties available in the Toolbox can be open. The user can select additional parameter or remove already selected ones as required.

3.2. Structural similarity

The “Structural similarity” section includes the different elements of empirical knowledge from empirical profilers that can be used to assess similarity. The chemistry-based profilers identify the chemical elements (e.g., O, N), substituents (e.g., OH, NH₂) and/or extended fragments (e.g., O=CC=C) found in the category members. Structure similarity include a variety of indices (Tanimoto, Dice, etc.), molecular features (atom pairs, atom centered fragments, etc.), type of calculation (hologram, fingerprint) and atom characteristics (atom type only or accounts of hybridization, hydrogen atoms attached, etc.). It should be noted that structure similarity is a relative but not an absolute estimation of closeness between chemicals. Again, the user can modify the options and calculation within the empirical knowledge similarity data matrix.

3.3. Mechanistic similarity

The third layer of category consistency is associated with the mechanism(s) of interaction of the chemicals with biological macromolecules calculated by mechanistic and endpoint specific profilers. Explicitly, it is desirable that the category members have the same predicted mechanism. Furthermore, metabolism needs to be taken into account for those cases where it is not the parent compound itself that is responsible for the toxicity but its metabolite(s). The “Mechanistic similarity” section in the category consistency wizard lists the profilers and metabolic simulators that are associated with modes of actions. Depending on the endpoint, some

profilers will be more relevant than others (e.g., DNA binding alerts for reverse bacterial mutagenicity assay, Protein binding alerts for Chromosomal aberration, Protein binding alerts for Skin sensitization). Thus, the selection of the target endpoint prior to running the category consistency module is essential. Mechanistic profilers which are relevant to the defined endpoint are pre-selected by default for defining mechanistic similarity. These profilers are also characterized as “suitable”. Like for the other modules, the user can modify the selection.

3.4. (Eco)tox experimental data

An additional section in the wizard is “(Eco)tox experimental data”, which allows the user to extract additional experimental data. Specifically, data within the Toolbox that the user deems to be toxicologically-related to the apical endpoint under consideration can be included in the consistency evaluation. Also other addition information (e.g., new methods data) can be included here.

4. Category consistency assessment for the list with *cyclic unsaturated hydrocarbons* in Toolbox

4.1. Application of Category consistency functionality in Toolbox

The chemicals from Table 1 were uploaded into the Toolbox. The consistency between the chemicals was assessed with respect to *Repeated dose toxicity, NOEL* which was selected as the target endpoint. Then the Category consistency functionality was activated. No additional selections for the first two layers in the wizard – the “Physicochemical” and the “Structural similarities” were done. There were no “suitable” profilers in the “Mechanistic similarity” layer which means that no default selections of mechanistically relevant to the target endpoint profilers

were available. However, the proposed “plausible” profilers were manually selected as illustrated in Figure A3.

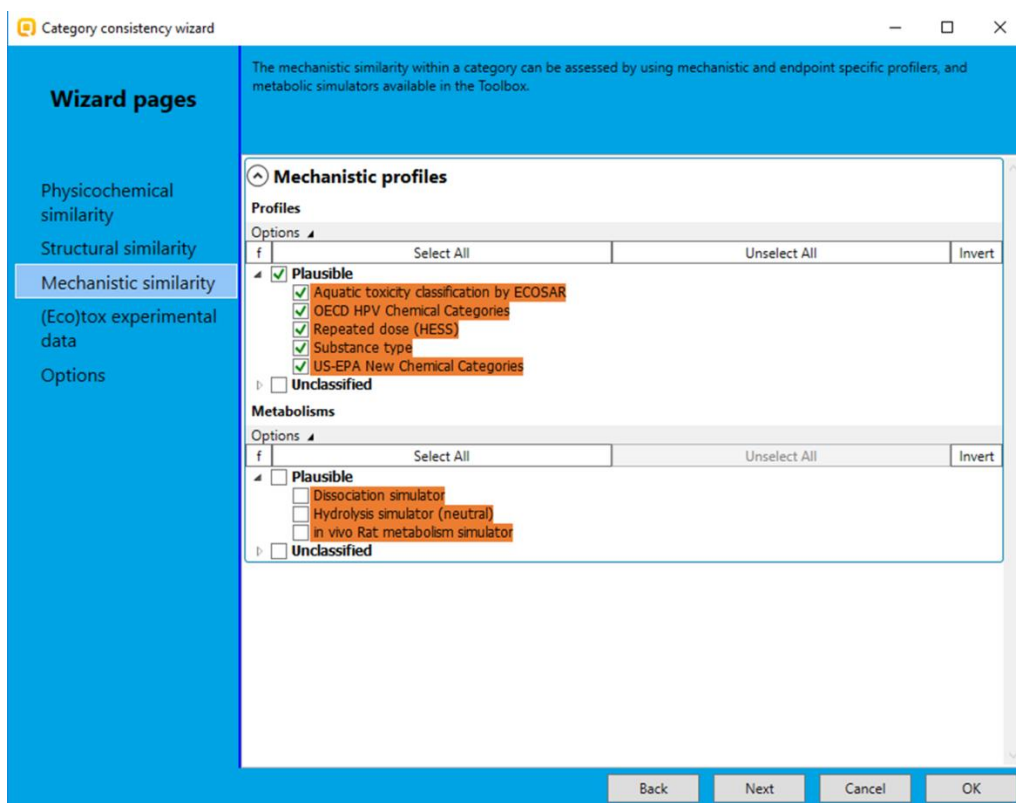


Figure A3. Screen shot with selected all “Plausible” profilers in the “Mechanistic similarity” layer of Category consistency wizard for the current case example

Experimental data for the target endpoint (Repeated dose toxicity, NOEL) was selected by default to be collected in the “(Eco)tox experimental data” layer.

The selections in the wizard were confirmed by clicking on the “OK” button. The information related to the selected elements in the wizard appeared in a data matrix as illustrated in Figure A4.

Filter endpoint tree...	1	2	3	4	5	6
Structure						
Structure info						
CAS Number	79-92-5	127-91-3	3387-41-5	1330-16-1	80-56-8	13466-78-9
Chemical name(s)	2,2-dimethyl-3-methylenebicyclo[3.1.1]heptane	(1)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	4-methylidene-1-(propan-2-yl)bicyclo[3.1.1]heptane	4,6,6-trimethylbicyclo[3.1.1]heptane	2,6,6-trimethylbicyclo[3.1.1]heptane	3,7,7-trimethylbicyclo[4.1.0]heptane
Predefined substance type	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent
SMILES	<chem>CC1(C)C2CCC(C2)C1=C</chem>	<chem>CC1(C)C2CCC(=C)C1C2</chem>	<chem>CC(C)C12CCC(=C)C1C2</chem>	<chem>CC1C=CC2CC1C2(C)C</chem>	<chem>CC1=CCC2CC1C2(C)C</chem>	<chem>CC1CC2C(CC=1)C2(C)C</chem>
Parameters						
2D						
Physical Chemical Properties						
Boiling point	4/12 M: 156+160 °C	M: 166 °C			M: ≥155+156 °C	M: ≥123+124 °C
Partition Coefficient	4/7 M: 4.22	M: 4.16			M: 3.07E+04	M: 4.38
Vapour pressure	4/11 M: 333 Pa	M: 391 Pa			M: 633 Pa	M: 273 Pa
Water solubility	3/6 M: 4.2 mg/L				M: +0.04 mg/L	M: +0.5 mg/L
Human Health Hazards						
Repeated Dose Toxicity						
NOEL	1/2 M: +62.5 mg/kg bw/day (actu... M: 250 mg/kg bw/day (actu...					
Profile						
Predefined						
OECD HPV Chemical Categories	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized
Substance type	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical
US-EPA New Chemical Categories	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized
Endpoint Specific						
Aquatic toxicity classification by ECOSAR	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
Empiric						
Organic functional groups	Alkene	Alkene	Alkene, branched with tertiary...	Alkene	Alkene	Alkene
Structure similarity	10+60 %	20+60 %	0+30 %	10+40 %	0+50 %	0+40 %
Toxicological						
Repeated dose (HESS)	Aliphatic/Alcyclic hydrocar...	Aliphatic/Alcyclic hydrocarbo...	Aliphatic/Alcyclic hydrocarbo...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...

Figure A4. Screen picture of the data matrix for the six chemicals.

All the six chemicals were considered ‘Neutral Organics’ by the “Aquatic toxicity classification by ECOSAR” profiler. No specific functional groups were found for all of the chemicals by the predefined profilers – “OECD HPV Chemical Categories” and “US-EPA New Chemical Categories”. Based on analysis of empiric profiling by organic functional groups, similarities and differences between the category members were observed. In order to analyze the profiling results and obtain overall picture for the entire list with chemicals, statistics for the profiling result could be asked for. The profiling statistic could be obtained for any of the profiling results in the data matrix. For example, Figure A5 shows how to obtain the profiling statistics for the organic functional groups (OFG) profiler.

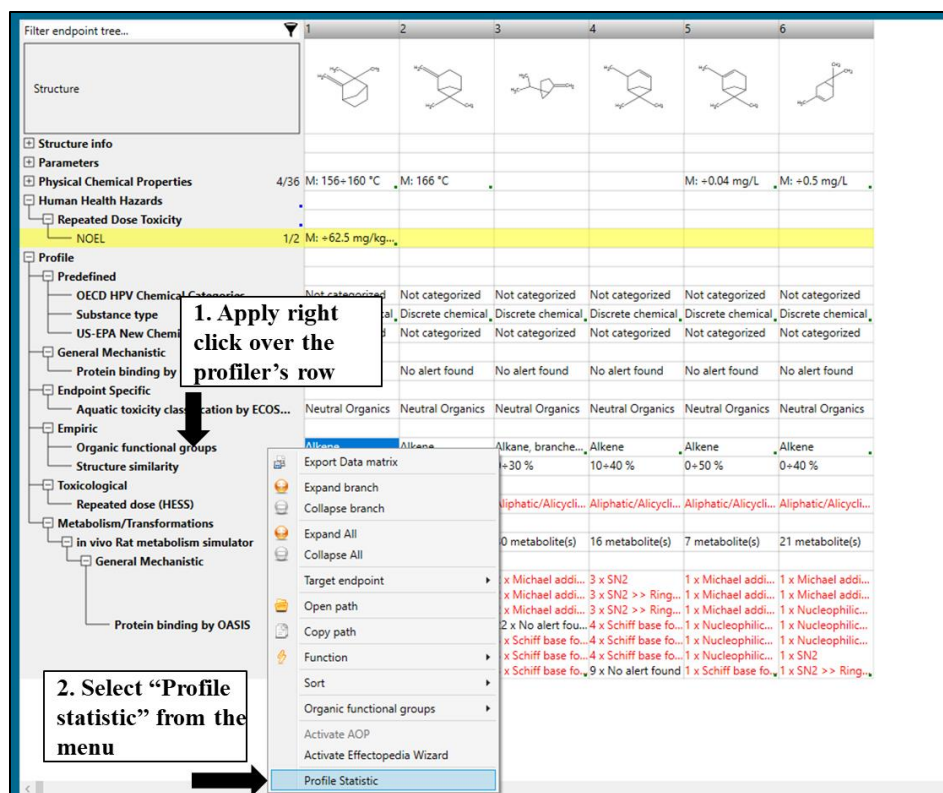


Figure A5. Toolbox screenshot showing how to evoke statistics for the profiling results produced by applied profiler for the list with chemicals in the data matrix

Next Figure A6 shows the profiling statistic of OFG profiler for the six substances. The column “Category” lists the found organic functional category, the column “Count” shows the number of analogues having that specific OFG, and the column “%” give the percent based on the total number of OFG (in this case 36) that a particular organic function contributes.

#	Category	Count	%
1	Alkane, branched with tertiary carbon	1	2.78
2	Alkene	6	16.67
3	Allyl	4	11.11
4	Bicycloheptane	1	2.78
5	Bridged-ring carbocycles	4	11.11
6	Cycloalkane	6	16.67
7	Cycloalkene	3	8.33
8	Fused unsaturated carbocycles	1	2.78
9	Isopropyl	1	2.78
10	Terpenes	6	16.67
11	Unsaturated carbocyclic fragment	3	8.33

Figure 6A. Organic functional group profiling information for the potential category analogues.

The common OFG for all six hydrocarbons are alkene, cycloalkanes, and terpenes.

According to the “Repeated dose toxicity (HESS)” profiler, all six analogues belong to same repeated dose category – “*Aliphatic/Alicyclic hydrocarbons (Alpha 2u-globulin nephropathy) Rank C*”. As found in the mechanistic justification of the repeated dose category, the systemic toxicity of aliphatic and alicyclic hydrocarbons was considered to be a consequence of *alpha-2u-globulin* binding to administrated chemicals or their metabolites [1,2]. In this respect, analysis of the specific mechanism of interaction with proteins of the parent structures and possible metabolites was needed. For that purpose, the general mechanistic “Protein binding by OASIS profiler” was applied on the chemicals and their metabolites obtained by the *in vivo* rat liver metabolism simulator. No protein binding alert was found for any of the analogues as parents but all of them were activated after *in vivo* rat metabolism simulation producing metabolites which have protein binding alerts. The number of predicted metabolites varies from seven to 30. The most common metabolic options for these unsaturated hydrocarbons include: 1) oxidation of the double bond to epoxide with further hydrolysis to glycols, and 2) oxidation of methyl ring substituent groups to the corresponding alcohol, further oxidation to aldehyde and subsequently, carboxylic acid. Mechanistically, all the analogues generate metabolites that are epoxides and aldehydes acting via S_N2 and Schiff base mechanisms, respectively. These simulations are confirmed by the metabolism data found for some saturated and unsaturated alicyclic terpenes such as pinenes, camphene, δ -3-carene, pinane, etc. [3]. The results as appeared in the data matrix are illustrated in Figure A7.

Filter endpoint tree...	1	2	3	4	5	6
Structure						
Human Health Hazards						
Repeated Dose Toxicity						
NOEL	M: +62.5 mg/kg bw/day (ac... 1/2 M: 250 mg/kg bw/day (actu...					
Profile						
Predefined						
OECD HPV Chemical Categories	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized
Substance type	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical	Discrete chemical
US-EPA New Chemical Categories	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized	Not categorized
General Mechanistic						
Protein binding by OASIS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Endpoint Specific						
Aquatic toxicity classification by ECOS...	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
Empiric						
Organic functional groups	Alkene	Alkene	Alkene	Alkene	Alkene	Alkene
Structure similarity	10-60 %	20-60 %	0-30 %	0-30 %	0-30 %	0-30 %
Toxicological						
Repeated dose (HESS)	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...	Aliphatic/Alcyclic hydrocarb...
Metabolism/transformations						
In vivo Rat metabolism simulator	12 metabolite(s)	11 metabolite(s)	30 metabolite(s)	16 metabolite(s)	7 metabolite(s)	21 metabolite(s)
General Mechanistic	1 x SN2 1 x SN2 >> Ring opening S... 1 x SN2 >> Ring opening S... 2 x Schiff base formation >... 2 x Schiff base formation >... 2 x Schiff base formation >... 9 x No alert found	1 x Michael addition 1 x Michael addition >> Mic... 1 x Michael addition >> Mic... 1 x SN2 1 x SN2 >> Ring opening S... 3 x Schiff base formation >... 3 x Schiff base formation >... 3 x Schiff base formation >... 6 x No alert found	2 x Michael addition 2 x Michael addition >> Mic... 2 x Michael addition >> Mic... 22 x No alert found 3 x Schiff base formation >... 3 x Schiff base formation >... 3 x Schiff base formation >... 3 x SN2 3 x SN2 >> Ring opening...	3 x SN2 3 x SN2 >> Ring opening... 3 x SN2 >> Ring opening... 4 x Schiff base formation 4 x Schiff base formation 4 x Schiff base formation 9 x No alert found	1 x Michael addition 1 x Michael addition >> M... 1 x Michael addition >> M... 1 x Nucleophilic addition 1 x Nucleophilic addition 1 x Nucleophilic addition 1 x SN2 1 x Schiff base formation 1 x Schiff base formation 1 x SN2 1 x SN2 1 x SN2 >> Ring opening...	1 x Michael addition >> Mich... 1 x Michael addition >> Mich... 1 x Nucleophilic addition >>... 1 x Nucleophilic addition >>... 1 x Nucleophilic addition >>... 1 x SN2 1 x SN2 >> Ring opening SN... 1 x SN2 >> Ring opening SN... 13 x No alert found 2 x Michael addition >> Mich... 2 x Michael addition >> Mich...

Figure A7. Profiling results for the six **cyclic unsaturated hydrocarbons** by the protein binding profiler. Text box 1 shows the results for the parent chemicals – all with “No alert found” result; Text box 2 shows the results for the package parent and metabolites – protein binding alerts were produced for all of the chemicals after *in vivo* metabolic simulation.

Since the number of predicted metabolites varies between the six parent compounds, the protein binding mechanisms alerted by the structure of the metabolites vary between them. One chemical, didehydropinane (CAS# 1330-16-1; chemical #4 in Figure A7), has the same reactivity pattern (i.e., the same distribution of protein binding alerts) for its metabolites as for the metabolites of the source substance, camphene (CAS# 79-92-5; chemical 1 in Figure A7). Two other chemicals, β -pinene and sabinene, (chemicals 2 and 3 in Figure A7) have **one** additional metabolic-mediated mechanism of interaction with proteins – “Michael addition on conjugated systems with electron withdrawing group”. The two remaining chemicals, α -pinene and δ -3-

carene (chemicals 5 and 6 in Figure A7) have **more than one** additional metabolic-mediated mechanism to interact with proteins.

4.2. Report of the Category assessment results

The information presented in the data matrix related to the category consistency assessment is saved as items for reporting (or report items) in the form of tables and graphs. All information collected and visualized in the data matrix after application of the category consistency assessment is automatically transferred to the report and embedded to a related section there. The calculated or experimental values appear in the report mainly as tables where calculated/experimental values for the chemicals from the assessed list are provided. Each profiler or combination of a profiler and metabolic simulator generates a reporting item in a form of tables and graphics. In case, a metabolic simulator is used in the category consistency assessment, the report item which is automatically generated contains the following components:

- 1) a table with 2D representation of the parent and generated metabolites for each chemical from the assessed list along with the profiling result for each of the structures (parent and metabolites);
- 2) graphical distribution showing the amount of metabolites with specific alerts in the package parent and metabolites;
- 3) summary table with counts of the alerts in each package parent and metabolites for the chemicals from the assessed list.

The appearance of the reporting items could be modified by user, e.g. an automatically added item could be removed and a new one could be generated.

Report for the Category is one of the possible options for reporting in Toolbox. It is located in the “Report” module as illustrated in Figure A8.

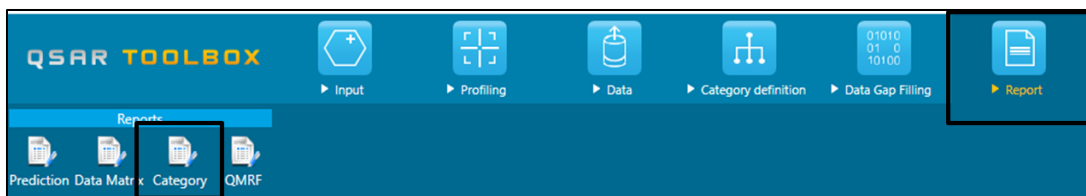


Figure A8. Toolbox screenshot of the Category report location in the Report module

Once selected, the category report is customised by following a wizard dialogue. The sections of this wizard include Customization, Category and Data matrix. The content of the “Customize report” tab is visualized Figure A9.

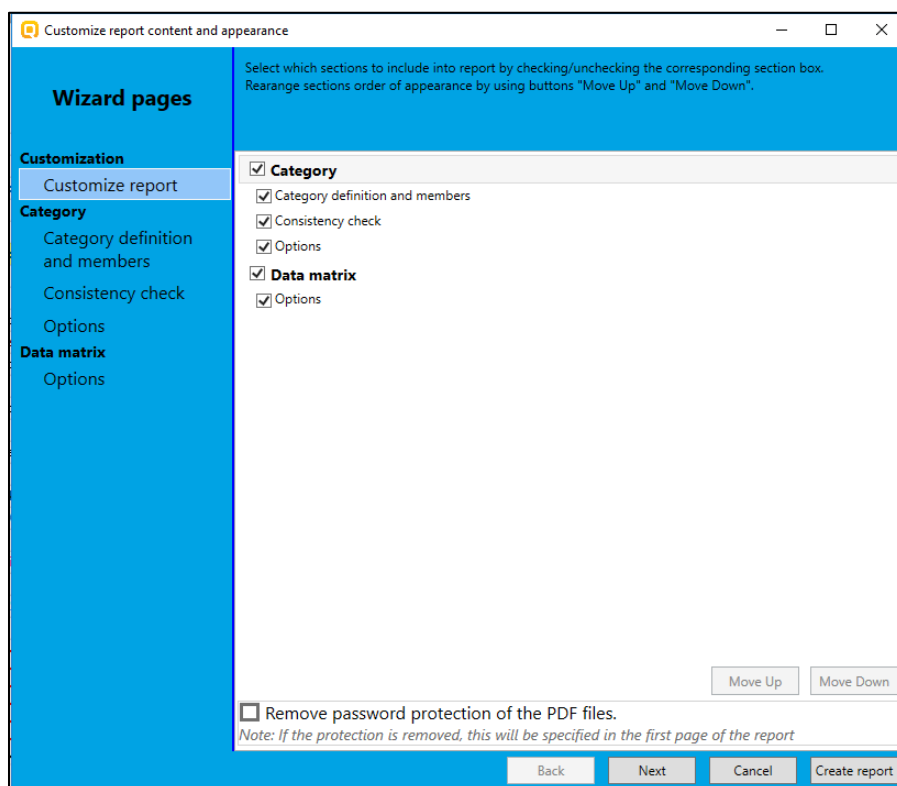


Figure A9. Toolbox screenshot of Category report wizard page with all options checked.

The main information related to the category consistency assessment is reported in the subsection “Consistency check”. The content of “Consistency check” section is illustrated in Figure A10.

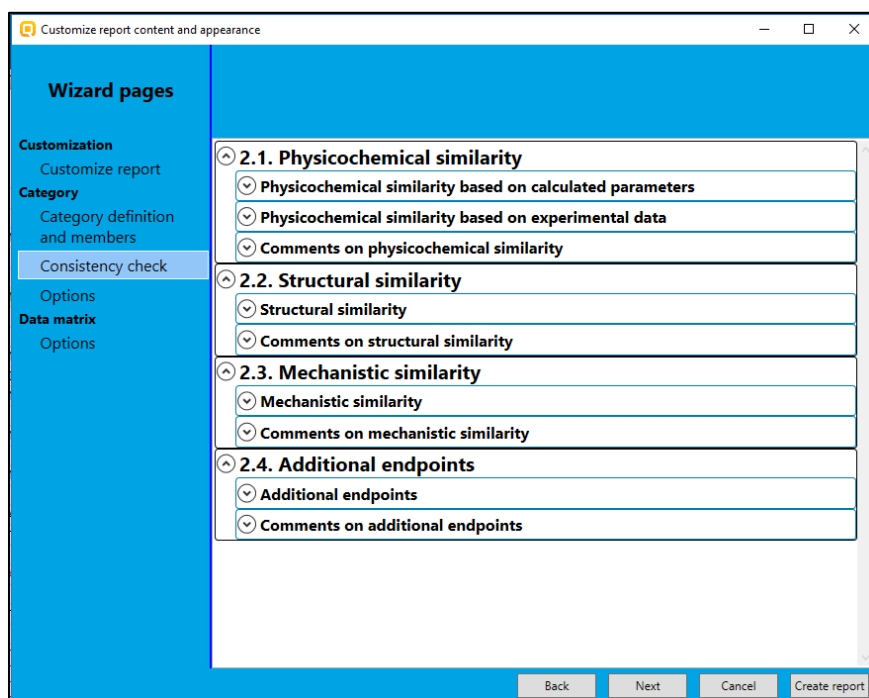


Figure A10. Toolbox screenshot of the Category report wizard with the content of “Consistency check” section

As seen from Figure A10, the three layers of Category consistency (Physicochemical, Structural and Mechanistic similarities) are itemized here as subsections (“2.1”, “2.2” and “2.3” in Figure A10, respectively). Additional experimental data could be collected under subsection 2.4 “Additional endpoints”.

The automatically generated report items for the “Physicochemical similarity” section are tables with calculated and experimental physicochemical data provided for each of the chemicals in the

assessed list. For example, the table with calculated physicochemical properties for the six chemical in the current case example is shown in Figure A11.

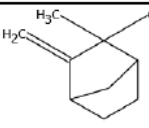
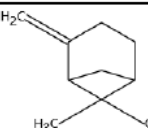
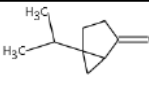
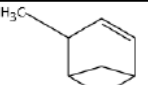
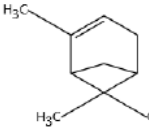
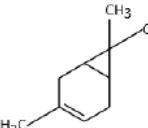
Table with selected 2D/3D parameters for category members		
	1	2
		
Boiling point, °C	151	151
log Kow,	4.35	4.35
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	280	364
Water Solubility, mg/L	6.28	7.06
	3	4
		
Boiling point, °C	142	153
log Kow,	4.69	4.14
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	1.06E+03	655
Water Solubility, mg/L	2.49	7.36
	5	6
		
Boiling point, °C	157	157
log Kow,	4.27	4.61
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	581	303

Figure A11. Illustration of the table with calculated physicochemical properties for each of the chemicals from the assessed list.

Similar tables are automatically generated for the Structural similarity layer. Here, the default category consistency elements are the Organic functional groups and Structure similarity

profilers. The tables in this case contain the profiling results of the applied profilers for the chemicals which were assessed. An illustration of the table with the profiling result by the Organic functional group profiler for the six chemicals is shown in Figure A12.

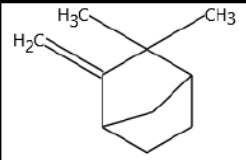
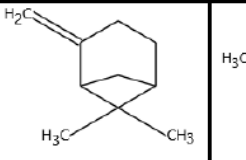
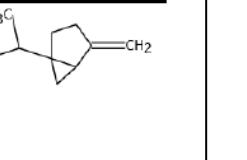
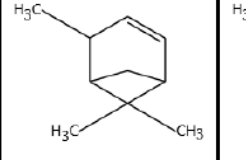
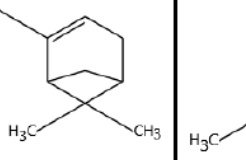
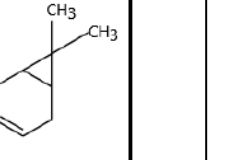
Table with profiling results for "Organic functional groups"		
1	2	3
		
Alkene Bicycloheptane Cycloalkane Bridged-ring carbocycles Terpenes	Alkene Allyl Cycloalkane Bridged-ring carbocycles Terpenes	Alkane, branched with tertiary carbon Alkene Allyl Isopropyl Cycloalkane Terpenes
4	5	6
		
Alkene Cycloalkene Cycloalkane Bridged-ring carbocycles Unsaturated carbocyclic fragment Terpenes	Alkene Allyl Cycloalkene Cycloalkane Bridged-ring carbocycles Unsaturated carbocyclic fragment Terpenes	Alkene Allyl Cycloalkene Cycloalkane Fused unsaturated carbocycles Unsaturated carbocyclic fragment Terpenes

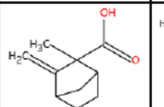
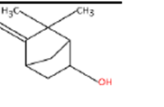
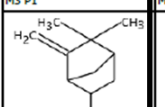
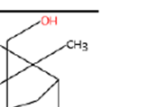
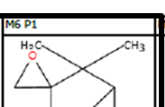
Figure A12. Screen picture of the table with profiling results by the Organic functional group profiler for all the six chemicals from the assessed list

The report items that are automatically generated for the Mechanistic similarity layer again include tables and graphics summarizing the mechanistic behavior of the assessed chemicals. The results which were produced by the "Protein binding by OASIS" profiler and the *in vivo* rat metabolic simulator used in current assessment appeared here as tables and graphics.

For current example, additional selections in the Mechanistic layer were applied. These were the “Protein binding by OASIS” profiler and the “*in vivo* rat metabolism simulator”. This evoked generation of two additional reporting items – one for the protein binding profiler result of the parent chemicals and one for the combination of the protein binding profiler and *in vivo* rat metabolism simulator. The tables and graphics which are automatically generated for the protein binding profiler accounting for *in vivo* metabolism simulation are illustrated in Figures A13-A15. Figure A13 shows the table for chemical with CAS # 79-92-5 with 2D representations of the parent and generated *in vivo* metabolites along with the profiling result by the protein binding profiler. Such tables are produced for each of the chemicals from the assessed list.

Table with profiling similarity accounting for metabolism ("in vivo Rat metabolism simulator" and "Protein binding by OASIS")
Metabolism: in vivo Rat metabolism simulator
Profiler: Protein binding by OASIS

Tables with generated metabolites for each analogue with profiling result

P1 CAS#: 79-92-5  No alert found	M1 P1  No alert found	M2 P1  No alert found
M3 P1  No alert found	M4 P1  Aldehydes	M5 P1  No alert found
M6 P1  Epoxides, Aziridines and Sulfuranes	M7 P1  No alert found	M8 P1  Aldehydes
M9 P1	M10 P1	M11 P1

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Figure A13. Screen picture illustrating table from the report with the parent and simulated *in vivo* rat metabolites of substance with CAS # 79-92-5. Solid lined are the metabolites with protein binding alert.

Next figure in the report summarizes the content of the table with parent and metabolites shown in Figure A13. It is a graphic illustrating the amount of metabolites profiled with a specific alert. Screen picture of the graphic for CAS # 79-92-5 is provided in Figure A14.

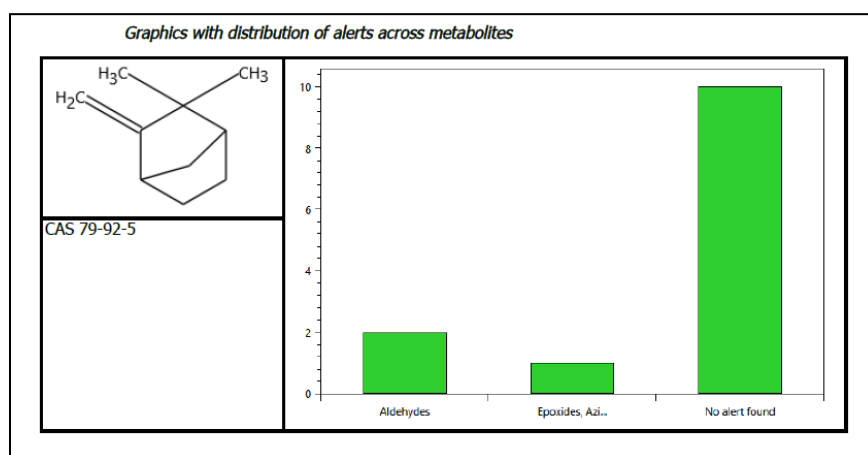


Figure A14. Screen picture of graphic from the category report illustrating the amount of metabolites profiled with specific alert.

After the tables with parent and metabolite and the bar graphics, another one table shows the summary results for all the chemicals from assessed list. This is illustrated in Figure A15 where the summary results for the chemicals from current example are provided.

Table summarizing number of metabolites including parent with specific alerts						
Protein binding by OASIS	P1 79-92-5	P2 127-91-3	P3 3387-41-5	P4 1330-16-1	P5 80-56-8	P6 13466-78-9
Aldehydes	2	3	3	4	1	5
alpha,beta-Aldehydes	0	0	0	0	1	2
alpha,beta-Carbonyl compounds with polarized double bonds	0	1	2	0	0	1
Epoxides, Aziridines and Sulfuranes	1	1	3	3	1	1
Ketones	0	0	0	0	1	1
No alert found	10	7	23	10	5	14

Figure A15. Screen picture of the summarizing table from the category report where the number of alerts (based on the profiling scheme used) for the package parent and metabolites (based on the simulator used) for each chemical from the assessed list are provided.

As seen from the table in Figure A15, the common alerts found for all of the chemicals (parent and metabolites) from the assessed list are “Aldehydes” and “Epoxides, Aziridines and Sulfurans”.

The wizard of the report allows also the information that is prepared for reporting to be previewed. For example, an illustration in Figure A16 shows the preview result of “Physicochemical similarity based on calculated parameters” which produces a table with calculated physicochemical parameters for each of the chemicals in the assessed list.

Click over the button "Preview" in order to see the corresponding illustration which will appear in the final report

	1	2
Boiling point, °C	151	151
log K _{ow}	4.35	4.35
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	280	364
Water Solubility, mg/L	6.28	7.66

	3	4
Boiling point, °C	142	153
log K _{ow}	4.69	4.14
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	1.06E+03	658
Water Solubility, mg/L	2.49	7.36

	5	6
Boiling point, °C	157	157
log K _{ow}	4.27	4.61
Molecular Weight, Da	136	136
Vapor Pressure (Antoine method), Pa	581	303

Figure A16. Preview of the reporting item generated for “Physicochemical similarity based on calculated parameters”

It is also possible the reporting items to be customized by the user, e.g. some of the prepared items to be removed, new ones to be arranged or to reorder the appearance of the items for the final report.

5. Summary and conclusions

All five read-across candidates (# 2-6 in Table 1) are consistent to the source substance camphene (#1 in Table 1), with respect to Predefined, General Mechanistic, Endpoint Specific, Empiric and Toxicological profilers. The “mechanistic hypothesis” for the read-across for this class of chemicals is based on the profiling result from the “Repeated dose toxicity (HESS)”

profiler which is *Alpha 2u-globulin nephropathy (Rank C)* associated with *Aliphatic/Alicyclic hydrocarbons* or their metabolites. It is assumed that this systemic toxicity is driven by the protein binding associated with specific mechanism of interaction of the chemicals or their metabolites to the alpha globulin molecule.

The most common metabolic options for the examined unsaturated hydrocarbons are: 1) oxidation of the double bond to epoxide with further hydrolysis to glycols, and 2) oxidation of methyl ring substituent groups to the corresponding alcohol, aldehyde and subsequently, carboxylic acid. Mechanistically, all the analogues generate metabolites that are epoxides and aldehydes interacting with proteins via S_N2 and Schiff base mechanisms, respectively.

All five read-across candidates are consistent to the source substance with respect to the simulated *in vivo* metabolites producing the epoxide and the aldehyde structural alerts. However, for some of the chemicals in this group, *in vivo* rat liver metabolic simulations introduce additional levels of protein binding complexity.

References:

1. Kanerva R.L., Ridder G.M., Stone L.C., Alden C.L., Characterization of spontaneous and decalin-induced hyaline droplets in kidneys of adult male rats, *Food Chem Toxicol.* 1987; 25(1), pp. 63-82.
2. Bomhard E., Marsmann M., Rühl-Fehlert C., Zywiets A., Relationships between structure and induction of hyaline droplet accumulation in the renal cortex of male rats by aliphatic and alicyclic hydrocarbons, *Arch Toxicol.* 1990; 64(7), pp. 530-8.
3. Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 311. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)2. *EFSA J.*, 2011, 9(6): 2177, pp. 1-126.