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COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

Use of QSAR models to predict genotoxicity: a scoping paper

Introduction

1. A range of Quantitative Structure-Activity Relationship (QSAR) models have been developed to predict genotoxicity. The COM has previously agreed that where no genotoxicity data are available, initial assessment of potential genotoxicity can be based on the publically available (Q)SAR models. This scoping paper seeks to provide a brief summary of these models prior to determine if the committee would wish to update or amend the COM (2011) "*Guidance On A Strategy For Genotoxicity Testing Of Chemical Substances*" (COM, 2011).

2. For each model detailed within this scoping study, key information has been collated on the endpoints covered and the size of the data set, details on any training and validation sets that have been applied to test the robustness of the model, the adherence of the model to OECD principles (Annex A) and the respective strengths and limitations of the model. The definitions of terms used by these models are described in Annex B.

3. This scoping review considers knowledge-based and statistical-based QSARs as well as hybrid models. Knowledge-based QSARs provide reasoning for predictions, such as a mechanism of action of a functional group, often supported with literature references and expert knowledge. However, the domain of applicability may not be clear and negative results may reflect insufficient knowledge of a mechanism of action within the database, rather than a lack of genotoxic activity for a chemical. Statistical-based QSARs use statistical analyses of data to produce quantitative outputs. As such, they tend to have a higher accuracy of prediction than knowledge-based approaches. However, interpretation of the results is more difficult and there may not be a mechanistic rationale behind the predictions. Hybrid approaches combine the knowledge-based and statistical-based QSARs.

Literature search strategy

4. An initial list of known QSARs was collated. This list was supplemented with information from the JRC review by Serafimova *et al.* (2010). A brief search was also conducted using Scopus (searching title, keywords and abstract) with the following search terms to identify any further models:

QSAR	Predict*	Chrom*
In silico	Ames	Micronucleus
Structural activity	Genotox*	Salmonella
Structure activity	Mutagen*	
SAR	Aberration	

5. Due to the large number of results produced by these broad terms, and the frequency with which QSAR models have been updated, the Scopus search was restricted to papers published after 2007. This year was selected to ensure an 'overlap' with the review conducted by JRC to maximise the probability of identifying all available models. The Scopus search did not identify any models that had not already been listed in the JRC review; however, it did provide supplementary information that assisted in the assessment of commercial models.Several models were identified but not considered within this document. These models and the rationale for their exclusion are provided in Annex C.**Knowledge-Based QSARs**

Toxtree

Name of model, developer, latest version number and release date

7. Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) has been developed by Ideaconsult Ltd. with support from the Computational Toxicology Group at the EC Joint Research Centre (Ideaconsult Ltd, 2015). The current version, 2.6.13, was published in 2015 (Ideaconsult Ltd, 2017).

8. Toxtree primarily uses structural alerts to identify specific functional groups that are anticipated to exhibit toxicological characteristics. Toxtree should be considered a Structure-Activity Relationships (SAR) tool. However, it also contains several QSAR models.

Endpoint(s) covered by the model and the size of the data set

9. Toxtree is a collection of several modules that provide qualitative estimates for different endpoints. The current version, 2.6.13, contains 16 modules; the following are of relevance when assessing potential genotoxicity (Ideaconsult Ltd, 2015):

- *In vitro* mutagenicity (Ames test) alerts by ISS - Benigni / Bossa rulebase.
- Carcinogenicity (genotoxicity and non-genotoxicity) and mutagenicity rulebase - ISS Benigni / Bossa rulebase.
- Structural alerts for the *in vivo* micronucleus assay in rodents – ToxMic module.

- Structural alerts associated with covalent DNA binding - DNA binding alerts. This module does not provide a result output for the assessment of genotoxicity. Therefore, it is not discussed further within this scoping paper.

Benigni / Bossa rulebase

10. The Benigni / Bossa rulebase is applied to two of the modules within Toxtree. This approach compares the functional groups of the chemical under investigation to functional groups within the database that are identified as having a genotoxic mode of action or a non-genotoxic carcinogenic mode of action. In addition, the rulebase contains a number of QSAR models (Benigni *et al.*, 2008). Benigni *et al.* (2008) states the QSARs provide a "more refined" assessment than the structural alert approach. Therefore, the outputs from the QSAR results should be considered of greater weight in evaluating the data. However, the level of refinement provided by this approach is not stated by the authors. Depending on the module in use, different results will be returned.

11. Outputs for *in vitro* mutagenicity (Ames test) alerts module include:
- Structural alert for *S. typhimurium* mutagenicity.
- No alerts for *S. typhimurium* mutagenicity.
- Potential *S. typhimurium* TA100 mutagen based on QSAR.
- Unlikely to be a *S. typhimurium* mutagen based on QSAR.
- For better assessment a QSAR calculation could be applied.
- Error when applying the decision tree.

12. Results for the carcinogenicity (genotoxicity and non-genotoxicity) module include:
- No alerts for carcinogenic activity.
- Structural alert for genotoxic carcinogenicity.
- Structural alert for non-genotoxic carcinogenicity.
- Potential *S. typhimurium* TA100 mutagen based on QSAR.
- Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR.
- Potential carcinogen based on QSAR.
- Unlikely to be a carcinogen based on QSAR.
- For a better assessment a QSAR calculation could be applied.

13. Definitions for these results are provided in Annex D, Table D1.

ToxMic module

14. Structural alerts for the *in vivo* micronucleus assay in rodents are estimated via the ToxMic module. The following outcomes are reported by the system (Istituto Superiore di Sanita, 2008):

- Class 1 (At least one positive structural alert for the micronucleus assay).
- Class 2 (No positive alert for the micronucleus assay).

Details of data sets

Benigni / Bossa rulebase

15. Benigni *et al.* (2008) state the following statistics for the mutagenicity structural alert SAR model (Table 1) and the QSAR model (Table 2). Details statistics for each structural alert are publically available in Benigni *et al.* (2008). Further information on the statistics of the QSAR models and their applicability domain are provided in Annex E, Table E1.

Table 1 Statistics for the structural alert model (Benigni *et al.*, 2008)

Endpoint	Sensitivity	Specificity	Accuracy
Mutagenicity	0.85	0.72	0.78

Table 2 Statistics for the QSAR model (Benigni *et al.*, 2008)

QSAR	Size of dataset	External validation		
		Acc. (%)	Sens. (%)	Spec. (%)
Mutagenic activity of aromatic amines in *Salmonella typhimurium* TA100 (with S9 metabolic activation)	64 (mutagens) 47 (non-mutagens)	81	86	72
Mutagenic activity of αβ-unsaturated aliphatic aldehydes in *Salmonella typhimurium* TA100 (without S9 metabolic activation)	17 (mutagens) 3 (non-mutagens)	100	-	-

Acc: Accuracy Sens: Sensitivity Spec: Specificity

ToxMic module

16. Istituto Superiore di Sanita (2008) report that ToxMic includes structural alerts for 35 functional groups.

17. The ToxMic module has a specificity of 0.57 (based on 547 negatives) and a sensitivity of 0.65 (out of 182 positives), corresponding to an overall accuracy of 0.59 (Benigni *et al.*, 2009). The true positive rates for each structural alert within the model following is reported in Benigni *et al.* (2009).

Adherence to OECD principles for the validation of QSAR models

18. Toxtree does not report adherence to OECD principles. Based on use of the model, the following conclusions can be made with regards to its adherence to those principles (Table 3).

Table 3 Adherence to OECD principles

OECD principle	Adheres to the principle?	
	Benigni / Bossa rulebase	**ToxMic module**
A defined endpoint	Yes	Yes
An unambiguous algorithm	Partially; based on combination of structural alerts and defined QSAR equations	No; based on structural alerts
A defined domain of applicability	Yes (for QSAR elements)	Not reported
Appropriate measure of goodness-of fit, robustness and predictivity	Yes	Yes
A mechanistic interpretation (if possible)	Structural alerts do not provide a mechanistic interpretation of a biological mode of action	Structural alerts do not provide a mechanistic interpretation of a biological mode of action

Strengths/limitations of models

Strengths:

- Simple structural alert system.
- User can develop new structural alert trees.

Limitations:

- Structural alerts can be useful in identifying potential toxicity, but cannot be used to make conclusions on non-toxicity.
- Where QSARs can be applied, these are applied in a rigid manner.
- The micronucleus assay has low sensitivity.

Input and output of the model

19. Input of structural data into Toxtree is undertaken by use of SMILES, structural data files, or drawing of the chemical structure. The model will produce an overall prediction, and the structural alert that has been identified can be viewed on-screen. However, the rationale behind these alerts is not readily available to the user. Toxtree does not produce any output reports or statistical tests of the prediction.

TOPKAT

Name of model, developer, latest version number and release date

20. TOPKAT is a proprietary model developed by Accelrys Inc (now Biovia), and is now part of the "BIOVIA Discovery Studio" (Biovia, 2014). As a commercial model, there are limited data on the current version in the public domain. Some information on legacy versions is available; notably, a brief review was included in the JRC review by Serafimova *et al.* (2010).

Endpoint(s) covered by the model and the size of the data set

21. TOPKAT includes predictive models for a range of toxicological and ecotoxicological endpoints; however, the model only includes genotoxicity predictions for Ames mutagenicity (no further details reported) (Biovia, 2014).

Details of data sets

22. No details on the size of the data sets within the current version of TOPKAT were located within the public domain.

Adherence to OECD principles for the validation of QSAR models

23. No details on adherence to OECD principles within the current version of TOPKAT were located within the public domain.

Strengths/limitations of models

24. The publically available information is insufficient to provide an assessment of the strengths and limitations of this model.

Ease of use and transparency of the model

25. The publically available information is insufficient to provide an assessment of use and transparency of this model.

Input and output of the model

26. The publically available information is insufficient to provide an assessment of input and output of this model.

DEREK Nexus

Name of model, developer, latest version number and release date

27. Nexus is commercially available software produced by Lhasa Ltd (Lhasa Ltd, 2017a); it consists of several modules, with DEREK and SARAH of relevance to genotoxicity endpoints. The current version of Nexus, version 2.2 was published in December 2017 (Lhasa Ltd, 2018). Nexus version 2.2 includes DEREK Nexus 6.0.

28. The latest update to Nexus occurred whilst this scoping paper was in preparation. Therefore much of the data presented relate the previous version of DEREK Nexus (version 5.0). However, where possible, these data have been supplemented with publically available information on DEREK Nexus 6.0.

29. DEREK Nexus is a knowledge-based model; SARAH Nexus is a statistically-based model and is discussed below.

Endpoint(s) covered by the model and the size of the data set

30. DEREK Nexus 5.0 includes predictions for the following genotoxicity endpoints:

- Chromosomal damage (*in vitro* / *in vivo*).
- Photo-induced chromosomal damage (*in vitro* / *in vivo*).
- Mutagenicity (*in vitro* / *in vivo*).
- Photo-induced mutagenicity (*in vitro* / *in vivo*).
- Non-specific genotoxicity (*in vitro* / *in vivo*).
- Photo-induced non-specific genotoxicity (*in vitro* / *in vivo*).

Positive predictions

31. In a training presentation, Lhasa Ltd (year unknown) stated that positive results within DEREK Nexus 5.0 are reported as the following ‘likelihood levels’. Definitions of these levels are provided in Annex F, Table F1. No data were located on the numerical thresholds for these likelihood levels:

- Certain.
- Probable.
- Plausible.
- Equivocal.
- Doubted.
- Improbable.
- Impossible.

32. DEREK Nexus 5.0 identifies structural alerts, which are provided with the nomenclature ‘toxicophores’, and applies ‘reasoning rules’ to provide an output result. DEREK Nexus 5.0 includes a total 852 structural alerts (Lhasa Ltd,

year unknown). DEREK Nexus 6.0 contains 845 structural alerts, with 132 alerts relating to mutagenicity and 98 alerts for chromosomal damage. Reasoning rules appear to apply mechanistic considerations within the prediction; for example if a chemical operates via a rodent-specific mode of action, even if a structural alert is triggered, a result of 'Impossible' will be provided for a bacterial prediction.

Negative predictions

33. The materials provided by Lhasa Ltd (year unknown) state that DEREK Nexus 5.0 will provide negative predictions for bacterial mutagenicity. Three types of negative prediction are included within the software:

- Inactive (no misclassified or unclassified features).
- Inactive (contains misclassified features).
- Inactive (contains unclassified features).

34. According to marketing material for DEREK Nexus 6.0, a negative result that contains no misclassified or unclassified features is a "highly confident negative prediction". Misclassified features have been derived from chemicals in the dataset that have positive results but have not triggered a structural alert. Unclassified features are chemical structures that have not been identified in the dataset (Lhasa Ltd, 2018). Therefore, the latter two negative results have lower confidence than the first result.

Details of data sets

35. The "Ames test reference set" contains 4630 positive and 4880 negative results. It is unclear how this reference set relates to the endpoints described above or how many of the data points within in this reference set relate to the training set and how many relate to the test set. No information with regards to other data sets is available.

Adherence to OECD principles for the validation of QSAR models

36. The Lhasa website states that QSAR predictions "*Both Derek Nexus and Sarah Nexus have been designed independently to meet the OECD validation principles*". (Lhasa Ltd, 2017b). Based on use of the tool, the following conclusions can be made with regards to its adherence to those principles (Table 4).

Table 4 Adherence to OECD principles

OECD principle	Adheres to the principle?
A defined endpoint	Yes
An unambiguous algorithm	No; based on structural alerts
A defined domain of applicability	Yes
Appropriate measure of goodness-of fit, robustness and predictivity	Yes
A mechanistic interpretation (if possible)	Yes

Strengths/limitations of models

Strengths:

- Combines structural and mechanistic interpretations to provide an overall prediction.
- Can examine the structures and the experimental data upon which the prediction is based.
- Predictions can be customised for specific species.
- Some data include hyperlinks to original sources.
- DEREK can generate reports in several formats.
- DEREK can provide negative predictions and includes a rationale for such predictions.
- Subscription includes support services from Lhasa Ltd.

Limitations:

- The basis of each prediction must be examined in detail, for example, if a rodent-specific mode of action is triggered, but all mammalian species are selected as the basis of the prediction, the tool will not make a distinction between those species; only a mammalian prediction will be presented and may present a positive result. If the prediction were to be run a second time excluding the rodent species, a prediction of 'impossible' will be returned.

Input and output of the model

37. DEREK Nexus allows the input of chemical data via a number of mechanisms, including use of SMILES, input of structural data files and drawing of the chemical structure. Each structural alert can be reviewed and a summary assessment of the alert, the test chemicals upon which this alert is based and a reference to source data are provided. Some references are also hyperlinked to allow the user to access the original paper. Reasoning rules are provided and a prediction report can be automatically generated in several formats. This report includes all of the above mention information.

Statistical-Based QSARs

Danish QSAR Database

Name of model, developer, latest version number and release date

38. The Danish QSAR database was published as a freely available on-line tool in 2004, and is available via http://qsar.food.dtu.dk/. It is not clear when the current version of the database was published. The database was developed by the National Food Institute, Technical University of Denmark, with support from the Danish Environmental Protection Agency, the Nordic Council of Ministers and the European Chemicals Agency (DTU Food, 2016).

Endpoint(s) covered by the model and the size of the data set

39. The Technical University of Denmark (DTU Food, 2016) has reported that the model repository consists of >600 000 substances covering approximately 200 QSAR models with various endpoints. Genotoxicity endpoints have been modelled in the three software systems; Leadscope, CASE Ultra and SciQSAR. In addition, an overall "battery prediction" can be made within the tool, which combines the results from all three models (DTU Food, 2016). It is stated that in many cases, using this battery algorithm can improve the accuracy of any predictions and expand the applicability domain (DTU Food, 2016). The extent by which this approach improves the prediction is not reported.

40. The Danish QSAR database contains the following models for genotoxicity (DTU Food, 2016):

- Ashby structural alerts.
- Ames assays.
    - Bacterial reverse mutation test (Ames test in *S. typhimurium in vitro*).
    - Direct acting Ames mutagens (without S9).
    - Base pair Ames mutagens.
    - Frame shift Ames mutagens.
    - Potent Ames mutagens, reversions ≥ 10 times controls.
- Other *in vitro* assays.
    - Chromosome aberrations in CHO cells.
    - Chromosome aberrations in CHL cells.
    - Mutations in thymidine kinase locus in mouse lymphoma cells.
    - Mutations in HGPRT locus in CHO cells.
    - UDS in rat hepatocytes.
    - Syrian hamster embryo cell transformation.
- *In vivo* assays.
    - Sex-linked recessive lethal test in Drosophila.

- Micronucleus test in mouse erythrocytes.
- Dominant lethal mutations in rodents.
- Sister chromatid exchange in mouse bone marrow cells.

- Comet assay in mouse.

Details of data sets

41. Details on the size of the training sets and validation statistics extracted from (DTU Food, 2016) are presented in Table 5.

Table 5 Training sets and validations statistics for the Danish QSAR database

Model endpoint for genotoxicity	Training set	Model	Model validation results		
			Sens.	Spec.	Con.
Structural alerts					
Ashby structural alerts	782	CASE Ultra	89.7 %	95.1 %	91.9 %
		Leadscope	87.5 %	90.7 %	88.5 %
		SciQSAR	81.7 %	80.6 %	81.1 %
Ames tests					
Bacterial reverse mutation test (Ames test in *S. typhimurium in vitro*)	4102	CASE Ultra	83.9 %	89.1 %	86.4 %
		Leadscope	84.3 %	85.7 %	84.9 %
		SciQSAR	79.3 %	79.1 %	79.2 %
Direct acting Ames mutagens (without S9)[a]	388	CASE Ultra	63.5 %	90.4 %	79.5 %
		Leadscope	66.9 %	78.9 %	74.0 %
		SciQSAR	56.5 %	72.9 %	68.6 %
Base pair Ames mutagens[a]	204	CASE Ultra	52.8 %	88.4 %	71.9 %
		Leadscope	70.2 %	66.4 %	68.4 %
		SciQSAR	68.6 %	67.7 %	68.1 %
Frame shift Ames mutagens[a]	309	CASE Ultra	73.5 %	84.1 %	78.9 %
		Leadscope	74.4 %	78.6 %	76.6 %
		SciQSAR	68.3 %	78.2 %	73.8 %
Potent Ames mutagens, reversions ≥ 10 times controls[a]	187	CASE Ultra	73.7 %	87.7 %,	81.2 %
		Leadscope	68.9 %	70.0 %	69.8 %
		SciQSAR	75.0 %	74.7 %	74.9 %
Other *in vitro* endpoints					
Chromosome aberrations in CHO cells	233	CASE Ultra	40.4 %	94.5 %	74.4 %
		Leadscope	54.1 %	79.3 %	68.8 %
		SciQSAR	50.5 %	84.3 %	70.3 %
Chromosome aberrations in CHL cells	600	CASE Ultra	63.3 %	86.7 %	76.4 %
		Leadscope	74.6 %	75.2 %	74.9 %
		SciQSAR	73.0 %	72.8 %	72.9 %
Mutations in thymidine kinase locus in mouse lymphoma cells	555	CASE Ultra	76.5 %	86.3 %	81.2 %
		Leadscope	85.1 %	83.8 %	84.4 %
		SciQSAR	79.1 %	80.5 %	79.8 %
Mutations in HGPRT locus in CHO cells	239	CASE Ultra	75.4 %	84.5 %	78.9 %
		Leadscope	81.7 %	78.4 %	80.5 %
		SciQSAR	80.0 %	73.0 %	76.5 %
UDS in rat hepatocytes	415	CASE Ultra	60.6 %	87.0 %	74.1 %
		Leadscope	74.1 %	70.1 %	72.4 %
		SciQSAR	69.6 %	72.5 %	71.1 %
Syrian hamster embryo cell transformation	363	CASE Ultra	50.8 %	86.9 %	70.4 %
		Leadscope	71.6 %	76.5 %	74.5 %
		SciQSAR	76.1 %	66.5 %	71.3 %
In vivo					
Sex-linked recessive lethal test in	367	CASE Ultra	75.4 %	92.0 %	83.6 %

Model endpoint for genotoxicity	Training set	Model	Model validation results		
			Sens.	Spec.	Con.
Drosophila		Leadscope	79. %	80.3 %	79.6 %
		SciQSAR	74.2 %	78.3 %	76.2 %
Micronucleus test in mouse erythrocytes	357	CASE Ultra	31.2 %	95.2 %	75.7 %
		Leadscope	64.1 %	77.6 %	72.3 %
		SciQSAR	52.1 %	83.3 %	69.7 %
Dominant lethal mutations in rodents	191	CASE Ultra	42.4 %	92.7 %	73.7 %
		Leadscope	61.5 %	80.4 %	71.8 %
		SciQSAR	57.7 %	81.4 %	71.7 %
Sister chromatid exchange in mouse bone marrow cells	265	CASE Ultra	97.8 %	94.8 %	93.9 %
		Leadscope	88.6 %	95.9 %	94.0 %
		SciQSAR	73.7 %	93.2 %	86.8 %
Comet assay in mouse	286	CASE Ultra	60.1 %	93.1 %	82.9 %
		Leadscope	86.6 %	80.8 %	83.0 %
		SciQSAR	82.4 %	82.0 %	82.2 %

Sens: Sensitivity Spec: Specificity Con: Concordance

a. The guidance for this model states that this model should only be applied to identify chemicals that will produce positive results and fall within the applicability domain (DTU Food, 2016). The rationale for this statement is not stated within the model. However, the model only considers chemicals with no unknown structural fragments to be within the applicability domain, except for chemicals predicted 'positive' where a single unknown fragment will be accepted.

Adherence to OECD principles for the validation of QSAR models

42. The Danish QSAR database does not report adherence to OECD principles. Based on use of the tool, the following conclusions can be made with regards to its adherence to those principles (Table 6).

Table 6 Adherence to OECD principles

OECD principle	Adheres to the principle?
A defined endpoint	Yes
An unambiguous algorithm	Unknown, whilst the guidance document implies that there are unambiguous algorithms within the model, they are not reported in the prediction documentation. This is likely due to the commercial nature of some of the models included within the database
A defined domain of applicability	Yes, although the domain boundaries are not reported
Appropriate measure of goodness-of fit, robustness and predictivity	Yes, see above
A mechanistic interpretation (if possible)	No

Strengths/limitations of models

Strengths:

- Large dataset of experimental data
- Uses several models and combines results in a 'battery algorithm'
  Can combine search criteria, such as partial chemical structure matches, endpoint data, structural similarity and 'AND', 'OR' and 'NOT' algorithms.

Limitations:

- Although the Danish QSAR database appears to follow OECD principles, it is not transparent in application of those principles
- Cannot modify the predictions within the database

Input and output of the model

43. The Danish QSAR database allows the input of chemical data via a number of mechanisms, including CAS and EC number and drawing of the chemical structure. A Word report is produced that details all data for that chemical within the database.

SARAH Nexus

Name of model, developer, latest version number and release date

44. Nexus is commercially available software produced by Lhasa Ltd (Lhasa Ltd, 2017a); it consists of several modules, with DEREK and SARAH of relevance to genotoxicity endpoints. The current version of Nexus, version 2.2 was published in December 2017 (Lhasa Ltd, 2018). Nexus version 2.2 includes SARAH Nexus 3.0.

45. The latest update to Nexus occurred whilst this scoping paper was in preparation. Therefore the data presented in this paper are based on use of the previous version of SARAH Nexus (version 2.0). However, where possible, these data have been supplemented with publically available information on SARAH Nexus 3.0.

46. SARAH Nexus is a statistically-based model; DEREK Nexus is a knowledge-based model and is discussed above.

Endpoint(s) covered by the model and the size of the data set

47. SARAH Nexus 2.0 provides a statistical model for the prediction of "Ames mutagenicity". This terminology is not defined further within the available marketing material. Results are reported as a result (e.g. positive) and a "confidence" related to this result. Confidence relates to the accuracy of the prediction; a high confidence is correlated to a high accuracy (Lhasa Ltd, 2016).

Details of data sets

48. SARAH Nexus 2.0 contains 9507 structures, consisting of 4628 positive and 4879 negative data points (Lhasa Ltd, year unknown). Data are not available for all five strains of *S. typhimurium* for each of these structures (Lhasa Ltd, year unknown); it is not reported how many structures do have experimental data for all five strains. The Lhasa website reports that SARAH Nexus 3.0 contains 9882 structures (Lhasa Ltd, 2017b). However, it is not stated how many of these additional structures are positive or negative structures or whether the different number of structures between version 2.0 and 3.0 represent 'new' structures or refinement of the existing dataset. No data are available on the statistical validation of the model.

Adherence to OECD principles for the validation of QSAR models

49. The Lhasa website states that QSAR predictions "*Both Derek Nexus and Sarah Nexus have been designed independently to meet the OECD validation principles*". (Lhasa Ltd, 2017b). Based on use of the model, the following conclusions can be made with regards to its adherence to those principles (Table 7).

Table 7 Adherence to OECD principles

OECD principle	Adheres to the principle?
A defined endpoint	Yes
An unambiguous algorithm	Yes
A defined domain of applicability	Yes
Appropriate measure of goodness-of fit, robustness and predictivity	Yes
A mechanistic interpretation (if possible)	Yes

Strengths/limitations of models

Strengths:

- User can assign thresholds for frequency of equivocal predictions and sensitivity of the model.
- Can examine the structures and experimental data upon which the prediction is based.
- Some data include hyperlinks to original sources.
- SARAH Nexus can generate reports in several formats.

Limitations:

- Interpretation of prediction is difficult.

Input and output of the model

50. SARAH Nexus allows the input of chemical data via a number of mechanisms, including use of SMILES, input of structural data files and drawing of the chemical structure. The data can be reviewed and references to source data are provided. A prediction report can be generated in several formats.

Hybrid QSARs

Case Ultra

Name of model, developer, latest version number and release date

51. Case Ultra is produced by MultiCASE as commercial software and is currently available as version 1.5.2.0 (MultiCASE Inc, 2017a). As commercial software, detailed information for elements of this model could not be obtained from publically available sources. It is considered a hybrid QSAR as it will provide rule-based and statistical-based models (MultiCASE Inc, 2017a).

52. Elements of this model have been included in the Danish QSAR database and the OECD QSAR Toolbox.

Endpoint(s) covered by the model and the size of the data set

53. Case Ultra contains several mutagenicity and genotoxicity models that are licensed in 'bundles'.

54. The following models are included in the Bacterial Mutagenicity Models bundle (MultiCASE Inc, 2017b).

- Main ICH M7 Models.
  - Expert rules for mutagenicity.
  - Mutagenicity for 7 major strains of *S. typhimurium*, FDA data source.
  - Mutagenicity by A-T site mutation in E. coli / TA102, FDA data source.
  - Aggregated *Salmonella* mutagenicity from public and proprietary sources.
  - Aggregated *E. coli* mutagenicity from public and proprietary sources.
- Supporting ICH M7 Models.
  - *Salmonella* mutagenicity, GENETOX data source.
  - *Salmonella* mutagenicity, NTP data source.
  - Mutagenicity in *E. coli*, CCRIS data source.
- Strain specific *Salmonella* mutagenicity models.

        - TA97 mutation with and without S9.
        - TA98 mutation with and without S9.
        - TA100 mutation with and without S9.
        - TA102 mutation with and without S9.
        - TA104 mutation with and without S9.
        - TA1535 mutation with and without S9.
        - TA1537 mutation with and without S9.
        - TA1538 mutation with and without S9.
    - Site-specific *Salmonella* mutagenicity models.
        - HISC3076 mutation with and without S9
        - HISD3052 mutation with and without S9
        - HISG46 mutation with and without S9
        - HISG428 mutation with and without S9
        - HISO1242 mutation with and without S9.

55. These models are based on experimental data from Ames tests and are consistent with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M7 document "*Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*" (MultiCASE Inc, 2017b).

56. Case Ultra also contains the following models in the Genotoxicity Models bundle (MultiCASE Inc (2017b). Research Cooperation Agreement (RCA) models are based on US Food and Drug Administration (FDA) data (details on the nature of the RCA are not reported) (MultiCASE Inc, 2017b):

    - RCA Genotoxicity Models.
        - Expert rules for mutagenicity.
        - Mutagenicity for 7 major strains of S. *typhimurium.*
        - Mutagenicity by A-T site mutation in E. *coli* / TA102.
        - Clastogenicity, *in vitro*, chromosome aberrations, CHO cells.
        - Clastogenicity, *in-vitro*, chromosome aberrations, CHL cells.
        - Clastogenicity, micronucleus, mouse.
        - Gene mutations, *in-vitro*, mouse lymphoma L5178Y cells.
    - Research Only Genotoxicity Models.
        - Yeast mutagenicity.
        - Drosophila mutagenicity.
        - Mammalian mutagenicity, *in vivo.*
        - Mammalian mutagenicity *in vitro*, CHO V79 HGPRT loci.
        - DNA effects, unscheduled DNA synthesis.
        - Clastogenicity, *in vitro*, sister chromatid exchange.
    - Additional Genotoxicity Models.
        - Drosophila mutation.

- Mouse lymphoma L5178Y (NTP, GENETOX, CCRIS data sources).
- Chromosomal aberration, NTP data source.
- Micronuclei induction.
- Sister chromatid exchange, NTP data source.
- Aneuploidy in yeast.
- SOS chromotest.

Details of data sets

57. The training sets with CASE Ultra are proprietary and commercially available from MultiCASE Inc. These data have not been purchased for the purposes of this scoping paper.

58. A recent review by Plosnik *et al.* (2016) states that all the models within CASE Ultra, with the exception of Ames mutagenicity, which is rule-based, use statistical approaches to provide predictions.

Adherence to OECD principles for the validation of QSAR models

59. Plosnik *et al.* (2016) has reported that CASE Ultra adheres to the OECD principles for the validation of QSAR models.

Strengths/limitations of models

60. The publically available information is insufficient to provide an assessment of the strengths and limitations of this model.

Input and output of the model

61. The publically available information is insufficient to provide an assessment of the input and output of this model.

VEGA

Name of model, developer, latest version number and release date

62. VEGA was developed by Istituto di Ricerche Farmacologiche, Mario Negri and Kode Chemoinformatics. The current version, 1.1.4, was published in February 2017 (Mario Negri, 2017a).

63. VEGA is not explicitly stated to be a hybrid model; however, the CONSENSUS model (described below) combines three knowledge-based models (CAESAR, SarPy/IRFMN and ISS) with a statistical model (KNN/Read-across).

Endpoint(s) covered by the model and the size of the data set

64. VEGA covers a range of endpoints; those relevant to genotoxicity are:

- Mutagenicity (Ames test) CONSENSUS model – v 1.0.2.
- Mutagenicity (Ames test) model (CAESAR) – v 2.1.13.
- Mutagenicity (Ames test) model (SarPy/IRFMN) – v 1.0.7.
- Mutagenicity (Ames test) model (ISS) – v 1.0.2.
- Mutagenicity (Ames test) model (KNN/Read-Across) – v 1.0.0.

65. Mario Negri (2017b) reports that the CONSENSUS model evaluates the results from the CAESAR, SarPy/IRFMN, ISS and KNN/Read-Across models and uses an applicability domain assessment of each model's prediction and its weight to provide an overall prediction (Mario Negri, 2017b).

Details of data sets

CAESAR

66. CAESAR was originally a stand-alone model, but has subsequently been integrated into VEGA. The CAESAR data set consists of a training set of 3367 chemicals and a test set of 837 chemicals. The data set in VEGA consists of two types of data; the original model dataset (training set of 3253 chemicals, test set of 798 chemicals) and compounds that have structural alerts for suspect mutagenicity (training set of 114 chemicals, test set of 39 chemicals) (Mario Negri, 2017c).

67. The model initially checks the original dataset, which includes a set of twelve structural alerts (detailed below in Table 8, column A). If one or more chemical fragments are found within the chemical under investigation, the chemical is predicted as a 'mutagen' (Mario Negri, 2017c).

68. If no structural alerts are found, the model runs the structural alerts for 'suspect mutagenicity'; the second dataset detailed in Table 8, column B. Identification of one or more chemical fragments returns a prediction for 'suspect mutagenicity'. It should be noted that the authors report that the structural alerts for suspect mutagenicity "*have a moderate rate of false positive in the training set of the model*" (no formal definition of 'moderate' is provided) (Mario Negri, 2017c).

Table 8 The CAESAR data sets within the VEGA model

Column A	Column B
Structural Alerts for mutagen compounds (Original dataset)	**Structural Alerts for suspect mutagen compounds (second dataset)**
SA 1: Acyl halides SA 6: Propiolactones or propiosultones SA 12: Quinones SA 13: Hydrazine SA 14: Aliphatic azo and azoxy SA 16: alkyl carbamate and thiocarbamate SA 18: Polycyclic Aromatic Hydrocarbons SA 21: alkyl and aryl N-nitroso groups SA 22: Azide and triazene groups SA 25: Aromatic nitroso group SA 28bis: Aromatic mono- and dialkylamine SA 29: Aromatic diazo	SA 7: Epoxides and aziridines SA 8: Aliphatic halogens SA 19: Heterocyclic Polycyclic Aromatic Hydrocarbons SA 27: Nitro-aromatic

SA numbers refer to the numbers applied in the Benigni/Bossa rulebase (see Toxtree for further details).

69. Mario Negri (2017c) reports the following statistics for the "Structural Alerts for mutagen compounds (Original dataset)" (Table 9). No statistical data are reported for the "Structural Alerts for suspect mutagens" (second dataset). However, the size of this dataset is reported (Table 10).

Table 9 Statistics for the "Structural Alerts for mutagen compounds (Original dataset)" (Mario Negri, 2017c)

Data set	N	Sensitivity	Specificity	Accuracy
Training set	3367	0.97	0.86	0.92
Test set	837	0.90	0.83	0.74

Table 10 Number of chemicals contained within the "Structural Alerts for suspect mutagen compounds (second dataset)" (Mario Negri, 2017c)

Data set	Mutagen compounds (n)	Non-mutagen compounds (n)
Training set	18	96
Test set	19	20

SarPy/IRFMN

70. According to Mario Negri (2017d), the SarPy/IRFMN dataset consists of a training set of 3367 chemicals and a test set of 837 chemicals. SarPy/IRFMN consists of two sets of rules; rules identifying structural alerts for mutagenicity (112 rules) and rules for non-mutagenicity (93 rules). If one or more chemical fragments matching the structural alerts are found within the chemical under investigation, the chemical is predicted as a mutagen. If no rules match the chemical, it is classed as possible non-mutagen. The full list of structural rules are detailed in Mario Negri (2017d). The statistics for this data set are presented in Table 11.

Table 11 Statistics for the SarPy/IRFMN dataset (Mario Negri, 2017d)

Data set	N	Sensitivity	Specificity	Accuracy
Training set	3367	0.86	0.77	0.82
Test set	837	0.86	0.76	0.81

ISS

71. This model implements the ISS Benigni / Bossa rulebase, as included in Toxtree and described above (Mario Negri, 2017e). The statistics for this data set are presented in Table 12.

Table 12 Statistics for the ISS dataset (Mario Negri, 2017e)

Data set	N	Sensitivity	Specificity	Accuracy
Training set	670	0.89	0.68	0.79

KNN/Read-Across

72. The KNN/Read-Across data set consists of 5570 chemicals (5764 predicted compounds and 6 non-predicted compounds) and provides predictions on the basis of a structural similarity index. This structural similarity index considers the number of atoms, cycles, heteroatoms, halogen atoms and the presence of functional groups. The index value ranges from 1 (maximum similarity) to 0 (Mario Negri, 2017f). The statistics for this data set are presented in Table 13.

Table 13 Statistics for the KNN/Read-Across data set (Mario Negri, 2017f)

Data set	N	Sensitivity	Specificity	Accuracy
Training set	5570	0.83	0.76	0.80

Adherence to OECD principles for the validation of QSAR models

73. VEGA does not report adherence to OECD principles. Based on use of the model, the following conclusions can be made with regards to its adherence to those principles (Table 14).

Table 14 Adherence to OECD principles

OECD principle	Adheres to the principle?			
	CAESAR	SarPy/IRFMN	ISS	KNN/Read-Across
A defined endpoint	Yes	Yes	Yes	Yes
An unambiguous algorithm	No, based on structural alerts	No, based on structural alerts	No, based on structural alerts	It is based on structural similarity equations but the equations are not stated; therefore it cannot be considered unambiguous
A defined domain of applicability	Yes	Yes	Yes	Yes
Appropriate measure of goodness-of fit, robustness and predictivity	Yes	Yes	Yes	Yes
A mechanistic interpretation (if possible)	Structural alerts do not provide a mechanistic interpretation of a biological mode of action	Structural alerts do not provide a mechanistic interpretation of a biological mode of action	Structural alerts do not provide a mechanistic interpretation of a biological mode of action	Unclear

Strengths/limitations of models

Strengths:

- Simple structural alert system.
- Provides assessment of relatability of prediction, including whether the chemical is within or outside of the applicability domain and indexes of applicability.
- Provides summary of chemicals used as a basis of comparison.

Limitations:

- Structural alerts can be useful in identifying potential toxicity, but cannot be used to make conclusions on non-toxicity.
- Structural alerts for suspect mutagenicity within CAESAR "*have a moderate rate of false positive in the training set of the model*".
- Aspects of the model are not transparent, for example, it is not clear how the four predictions combine to form the CONSENSUS model.

- Rigid application of tool; the dataset cannot be adapted.

Input and output of the model

74. VEGA allows the input of chemical data via SMILES or input files (.smi or .txt format). A PDF report is produced that details each prediction, its reliability and measures of fit to the applicability domain.

OECD QSAR Toolbox

Name of model, developer, latest version number and release date

75. The OECD QSAR Toolbox for Grouping Chemicals into categories was developed by LMC Oasis. The current version, 4.1.1, was published in September 2017 (LMC, 2017).

76. The approach taken within the OECD QSAR Toolbox is highly flexible, as users can choose their own 'profilers' (chemical grouping mechanism) by which chemicals are identified for the purposes of data gap filling. As such, the predictions developed by the OECD QSAR Toolbox are driven by the user, rather than fixed coded algorithms.

77. The OECD QSAR Toolbox is not explicitly stated to be a hybrid model; however, it utilises a combination of knowledge-based and statistical-based profilers to develop QSAR predictions. Therefore, it is considered a hybrid model in this report.

Endpoint(s) covered by the model and the size of the data set

78. The database contains nearly 80 000 chemicals, covering over 2 million studies (Table 15) (ECHA, 2017).

Table 15 Summary of the overall size of the OECD QSAR Toolbox (ECHA, 2017)

	Chemicals	Data points
Physico-chemical properties	45,238	177,258
Environmental fate and transport	9,446	97,469
Ecotoxicology	17,649	856,473
Human health	30,447	912,687

79. Data are arranged in levels, with each sub-level offering more specificity in the endpoint. The complete list of endpoints contained within the database is not visible to the user; the QSAR development process is user-driven, and as such, specific endpoints are displayed based on the choices of the user. A partial example of the typical sub-levels that may be seen for the genotoxicity endpoints is provided below; (LMC, 2017):

- Human health

- Genetic toxicity
  - *In vitro*
    - Test type (e.g. Bacterial Reverse Mutation Assay, Micronucleus assay etc)
      - Endpoint (e.g. Gene mutation, Chromosome aberration etc)
        - Test organism (e.g. *S. typhimurium*, Chinese hamster lung fibroblasts)
          - Metabolic activation (e.g. With S9)
            - Strain (e.g. TA 100)
  - *In vivo*
    - Test type (e.g. Chromosome aberration assay)
      - Endpoint (e.g. Chromosome aberration)
        - Test organism (e.g. Rat)
          - Undefined metabolic activation
            - Strain (e.g. Fischer 344)

80. The level of specificity in the measured endpoint is defined by the user; it is possible to derive a QSAR prediction for a very specific endpoint, such as an Ames assay in *S. typhimuirum* TA98 with S9, or a QSAR prediction for a more general endpoint, such as for all strains of S. *typhimurium* with and without S9. The size of the data set to be used in the prediction is therefore partially based on the 'level' to which a user applies the data-gap filling approach.

Details of data sets

81. Data within the OECD QSAR Toolbox have been supplied from a range of commercial and publically available sources. Data from these sources include profilers (mechanisms by which structurally similar chemicals can be identified), experimental data sets and QSAR equations. These tools are detailed in Annex G, Table G1 (adapted from OECD (2017)).

82. The OECD QSAR toolbox also includes databases for observed and simulated metabolic processes; these are detailed in Annex H, Table H1 (LMC, 2017).

Adherence to OECD principles for the validation of QSAR models

83. Details on the adherence of the OECD QSAR toolbox to the principles for validation of QSARs are presented in Table 16.

Table 16 Adherence to OECD principles

OECD principle	Adheres to the principle?
A defined endpoint	Yes
An unambiguous algorithm	Yes; the user generates an algorithm specific to their predicted chemical endpoint based on their choice of profilers. The choices of the user are recorded to ensure transparency.
A defined domain of applicability	Yes
Appropriate measure of goodness-of fit, robustness and predictivity	Yes
A mechanistic interpretation (if possible)	Defined by user action

Strengths/limitations of models

Strengths:

- Contains a large database of profilers and experimental data.
- User-derived approaches to QSAR development, rather than 'hard-coded' algorithms.
- Each profiler contains a brief description of its function and database size.
- The database includes tools for observed and simulated metabolic processes.
- Automatic generation of QSAR prediction report, structured to demonstrate adherence to the OECD principles for the validation of QSAR models.

Limitations:

- Limited restrictions in place to prevent the selection of profilers inappropriate to the endpoint in question; for example, there is no restriction on the application of skin irritation/corrosion rules to profile genotoxic endpoints.
- Requires significant understanding of the principles prior to use.

Input and output of the model

84. The OECD Toolbox allows the input of chemical data via a number of mechanisms, including use of SMILES, input of structural data files and drawing of the chemical structure. Data can be reviewed on-screen and references to experimental data are provided. A prediction report can be automatically generated in PDF format.

Leadscope Model Applier

Name of model, developer, latest version number and release date

85. The Leadscope Model Applier version 2.2 is a series of commercially available models developed by Leadscope Inc. The Model Applier consists of two models of relevance to genotoxicity; Genetox Expert Alerts Suite (version 3.0) and the Non-human Genetic Toxicity Model Suite (current version unknown) (Leadscope Inc., 2012a; Leadscope Inc., 2012b; Leadscope Inc., 2012c; Leadscope Inc., 2016). The current version of the Non-human Genetic Toxicity Model Suite is not stated within the publically available materials for this model. Genetox Expert Alerts Suite is a knowledge-based model, while the Non-human Genetic Toxicity Model Suite is statistically-based. Therefore, the Leadscope Model Applier is considered a hybrid model.

86. Elements of this model have been included in the Danish QSAR database (detailed above).

Endpoint(s) covered by the model and the size of the data set

87. The Genetox Expert Alerts Suite uses a rule-based system to assess genotoxicity in the Ames assay. Version 2.0 of the database contains experimental data for 10,295 chemicals and 290 validated alerts (Leadscope Inc., 2012b). It is not known how many chemicals are included within version 3.0.

88. The Non-human Genetic Toxicity Model Suite contains QSAR models for the following endpoints (Leadscope Inc., 2012c):

- *Salmonella* mutagenicity.
- *E. coli* mutagenicity.
- Mouse lymphoma.
- *In vitro* chromosome aberrations.
- *In vivo* micronucleus.

89. No details on the size of the databases were available in the publically available documents.

Details of data sets

90. The white paper for the Genetox Expert Alerts Suite states the following statistics. The model was validated against data for 14,404 chemicals (data provided for version 3.0) (Leadscope Inc., 2016):

- True positives: 4527
- False negatives: 996
- True negatives: 7041
- False positives: 1001

- Concordance: 85.4 %
- Sensitivity: 82.4 %
- Specificity: 87.5 %
- Positive predictivity: 81.9 %
- Negative predictivity: 87.9 %

91. Details of some of the validation data for the Non-human Genetic Toxicity Model Suite are provided in the Frequently Asked Questions document (Leadscope Inc., 2017). However, this is an incomplete record, and uses these data to compare to other commercially available models. Therefore, they are not reproduced here.

Adherence to OECD principles for the validation of QSAR models

92. Based on the information provided in marketing materials, the following conclusions can be made with regards to the adherence of the Leadscope Model Applier to OECD principles (Table 17).

Table 17 Adherence to OECD principles

OECD principle	Adheres to the principle?	
	Genetox Expert Alerts Suite	**Non-human Genetic Toxicity Model Suite**
A defined endpoint	Yes	Yes
An unambiguous algorithm	No; based on structural alerts	Yes
A defined domain of applicability	Not reported	Yes
Appropriate measure of goodness-of fit, robustness and predictivity	Yes	Yes
A mechanistic interpretation (if possible)	Unclear	Unclear

Strengths/limitations of models

93. The publically available information is insufficient to provide an assessment of the strengths and limitations of this model. The model was developed to follow ICH M7 guidelines for impurities and is therefore intended for use in the pharmaceutical industry. It is unclear from the available documentation whether it can be applied to other types of chemicals.

Input and output of the model

94. The publically available information is insufficient to provide an assessment of the input and output of this model.

ToxRead

Name of model, developer, latest version number and release date

95. ToxRead has been developed by the Instituto di Ricerche Farmacologiche, Mario Negri and is currently undergoing beta testing (version 0.9) (Anon, year unknown; Mario Negri, 2017). As the model is currently in development, only a partial assessment was possible.

Endpoint(s) covered by the model and the size of the data set

96. ToxRead presently undertakes predictions for Ames and Bioconcentration Factors (Anon, year unknown). The Ames assay is predicted using four rulesets; Benigni/Bossa, SARpy rules, IRFMN rules and CRS4 rules. Substances are identified based on similarity to the target using the similarity index implemented within VEGA (Anon, year unknown).

97. The model presently contains 6055 records of experimental Ames data, 784 records of carcinogenicity classifications, 9959 records of octanol-water co-efficient and 857 records of BCFs.

Details of data sets

98. No details of training or validation sets are explicitly stated within the model or the available guidance documents.

Adherence to OECD principles for the validation of QSAR models

99. ToxRead does not report adherence to OECD principles. Based on use of the tool, the following conclusions can be made with regards to its adherence to those principles (Table 18).

Table 18 Adherence to OECD principles

OECD principle	Adheres to the principle?
A defined endpoint	Partially
An unambiguous algorithm	Not at the present time
A defined domain of applicability	Not at the present time
Appropriate measure of goodness-of fit, robustness and predictivity	Fisher tests are employed to indicate the rules that have less statistical significance (and therefore, lower reliability), but the prediction itself is not tested
A mechanistic interpretation (if possible)	No

Strengths/limitations of models

100. As the model is presently only available as a beta test version, rather than a complete model, it is not appropriate to provide a summary of its present strengths and limitations.

Input and output of the model

101. Data are only input via SMILES. Output is an on-screen representation of structurally similar chemicals with experimental results and a score of similarity to the target chemical.

Questions for the Committee:

- Have (Q)SAR models advanced sufficiently to warrant COM reviewing their use in Stage 0?
- Are there any other models members are aware of which should be included?
- Are there any other aspects which should be reviewed (such as ease of use)?
- Do the member's wish to review these models in more detail and if so which aspects should we focus on?

WRc under contract supporting the PHE COM Secretariat

Date January 2018

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OECD QSAR principles

Principle 1 - A defined endpoint: This is intended to provide clarity in the endpoint being predicted by providing details on the specific effect within a specific organ/tissue under specific conditions, for example, a bacterial reverse mutation test (Ames test) in *S. typhimurium* TA98 with S9 would be considered an appropriately defined endpoint.

Principle 2 - An unambiguous algorithm: This is intended to ensure that the model algorithm is transparent and is based on information on chemical structure and/or physicochemical properties

Principle 3 - A defined domain of applicability: There will be limitations within QSAR models with regards to the types of chemical structures, physicochemical properties and mechanisms of action for which a reliable prediction can be generated. This limitation is the domain of applicability of the model, and must be described to provide reassurance of the reliability of the prediction.

Principle 4 - Appropriate measure of goodness-of fit, robustness and predictivity: This is a set of principles by which the prediction is statistically measured to assess its reliability.

Principle 5 - A mechanistic interpretation (if possible): For example, if a prediction states that a chemical is irritating, is there a clear method by which it binds to proteins on the skin to cause irritation.

(OECD, 2007)

Definition of terms used in this scoping paper

Training sets and test sets

Training sets represent the input data used to establish the model. Ideally, a 'test set' of data is also used as an external validation technique to check the predictability and applicability of the model. However, such approaches are not always possible. As a result, training sets are often divided into two reduced data sets, with one of the reduced training sets serving as the input data to establish the model, and the second reduced set serving as the external validation.

Sensitivity

Sensitivity represents the true positive rate, i.e. for those chemicals which are known to be positive in the experimental genotoxicity assay, the model correctly predicts a positive result for that same assay.

Specificity

Specificity represents the true negative rate, i.e. the proportion of chemicals that the model predicts to be negative that have also been experimentally determined to be negative in the genotoxicity assay.

Concordance

Concordance represents the amount of 'agreement' between two measures; these measures are typically the model that is applied within the QSAR and a 'gold standard' measure, which is the best approach for measuring the same endpoint. This gold standard may be an experimental assay or it may represent an alternative model.

Accuracy

Accuracy represents the precision of the software and is a ratio between the correctly predicted true positives and the true negatives.

Positive predictivity

Positive predictivity is the probability of a positive outcome from the model to be correctly positive, i.e.

$$\frac{True\ positive}{(True\ positive + False\ positive)}$$

Negative predictivity

Negative predictivity is the probability of a negative outcome from the model to be correctly negative, i.e.

$$\frac{True\ negative}{(True\ negative + False\ negative)}$$

Additional models identified but not reviewed within this scoping paper

Lazar was an open source database for producing toxicity predictions, including mutagenicity predictions. However, it appeared to no longer be available at the time of preparation of this scoping paper, and therefore, was not considered further. Previous investigations have indicated that using the Leave-one-out cross-validation strategy and external validation, *Salmonella* mutagenicity can be predicted with 85% accuracy for compounds within the applicability domain (Helma, 2006).

SciQSAR (previously known as MDL-QSAR) was identified as genotoxicity predictive model that was previously commercially available. However, this model appears to no longer be available and was therefore not considered further. This mutagenicity data set was also used to create a statistically-based SciQSAR-Hansen mutagenicity model. Previous investigations have indicated that using a 10% leave-group-out internal cross validation resulted in specificity of 71 %, sensitivity of 83 %, concordance of 77 % and false negative rate of 17 % (Contrera, 2013).

OASIS is a commercial database with models for Ames mutagenicity (*Salmonella* strains TA97, 98, 100, 1535 and 1537), in vitro chromosomal aberration (Chinese hamster lung and ovary cells), mouse lymphoma and *in vivo* micronucleus assays. Much of these data has been integrated into the OECD QSAR toolbox, and the OASIS website navigates users to a download page for the OECD Toolbox, rather than a subscription page to OASIS. Therefore, due to commercial licensing issues of accessing the OASIS database, and its availability via alternative means, it is not considered separately within this scoping paper.

The following are definitions, based on information reported in Benigni *et al.* (2008), explain the results of the Benigni / Bossa rulebase (Table D1).

Table D1 Definitions of the results of the Benigni / Bossa rulebase

Result	Definition
Structural alert for *S. typhimurium* mutagenicity	The target chemical contains one or more functional groups that match one or more structural alerts within the database that have been identified to operate via a genotoxic mode of action.
No alerts for *S. typhimurium* mutagenicity	The functional groups of the target chemical do not match any structural alerts within the database
Potential *S. typhimurium* TA100 mutagen based on QSAR	Target chemical has been assigned this output on the basis of the output of QSAR6 or QSAR13; these QSAR models are applied to aromatic amines or αβ-unsaturated aldehydes.
Unlikely to be a *S. typhimurium* mutagen based on QSAR	Target chemical has been assigned this output on the basis of the output of QSAR6 or QSAR13
No alerts for carcinogenic activity	The functional groups of the target chemical do not match any structural alerts within the database
Structural alert for non-genotoxic carcinogenicity	The target chemical contains one or more functional groups that match one or more structural alerts within the database that have been identified to operate via a non-genotoxic carcinogenic mode of action.
Potential carcinogen based on QSAR	The target chemical has been assigned this result according to the output of a QSAR that is applied to aromatic amines.
Unlikely to be a carcinogen based on QSAR	The target chemical has been assigned this result according to the output of a QSAR that is applied to aromatic amines
For better assessment a QSAR calculation could be applied	The target chemical has been identified as having functional groups that are appropriate to the application of a QSAR, but due to the user-defined settings, the QSAR model has not been applied.

The following, based on information reported in Benigni *et al.* (2008), provides further information on the statistics and applicability domain of the QSAR model (Table E1).

Table E1 Statistics and applicability domain of the QSAR model

QSAR	Squared Canonical Correlation	Applicability domain
QSAR6: Mutagenic activity of aromatic amines in *Salmonella typhimurium* TA100 (with S9 metabolic activation)	0.52 (accuracy: 87.4 %; specificity: 95.7 %; sensitivity: 81.3 %)	Model applies only to homocyclic amines, and excludes aromatic amines containing aromatic nitro groups as well. QSAR also applies to chemicals containing diazo, isocyanate and immine groups that are considered as precursor of the corresponding aromatic amine.
QSAR13: Mutagenic activity of αβ-unsaturated aliphatic aldehydes in *Salmonella typhimurium* TA100 (without S9 metabolic activation)	0.61 (The equation correctly reclassified 100 % of the compounds). Leave-One-Out cross-validation resulted in 85 % accuracy.	The QSAR applies to linear aldehydes.

Data presented below are extracted and adapted from a training presentation on Derek Nexus 5.0 provided by Lhasa Limited (Lhasa Ltd, year unknown).

Table F1 Definition of the results of DEREK Nexus

Result	Definition
Certain	There is proof that the proposition is true
Probable	There is at least one strong argument that the proposition is true and there are no arguments against it
Plausible	The level of likelihood indicates the weight of evidence supports the proposition
Equivocal	There is equal weight of evidence for and against the proposition
Doubted	The weight of evidence opposes the proposition
Improbable	There is at least one strong argument that the proposition is false and there are no arguments that it is true
Impossible	There is proof that the proposition is false

Table G1 Tools and databases contained within the OECD QSAR Toolbox

Source	Tool or database provided	Description
Databases		
US EPA	Aquatic US-EPA ECOTOX	Large database on adverse effects of single chemical stressors to ecologically relevant aquatic species.
	Terrestrial US-EPA ECOTOX	Large database on adverse effects of single chemical stressors to ecologically relevant terrestrial species.
	Biota-Sediment Accumulation Factor	Dataset of approximately 20000 biota-sediment accumulation factors.
	Phys-Chem EPISUITE	Experimental results on physical chemical properties as accessed from EPISUITE; extracted from the PHYSROP database maintained at Syracuse Research Corporation.
	ToxRefDB	Chronic developmental and reproductive toxicity studies on cancer of more than 300 pesticides.
Istituto Superiore de Sanita,	Carcinogenicity Mutagenicity ISSCAN	Experimental results for genotoxicity and carcinogenicity.
Environment Canada	Bioaccumulation Canada	Database of bioaccumulation data.
	kM Database	Database of bioconcentration factors and total elimination rate constants for fish.
Danish Environmental Protection Agency	Danish EPA Database	Estimation results for numerous properties and effects based on QSAR models.
RIVM, the Netherlands	Skin Irritation	Primary Skin Irritation Indices for skin irritation tests.
Ministry of the Environment, Government of Japan	Aquatic Japan MoE	Aquatic toxicity data from the Japanese Existing Chemicals Programme.
Ministry of Health, Labour and Welfare, Japan	Toxicity Japan MHLW	Results from single dose toxicity tests and mutagenicity tests from the Japanese Existing Chemicals Programme.
European Centre for Ecotoxicology of Chemicals (ECETOC)	Aquatic ECETOC	Aquatic toxicity data.
	Eye Irritation ECETOC	Eye irritation data.
	Skin Sensitisation ECETOC	Skin and respiratory sensitisation data.
European Chemical Industry Council (CEFIC)	Bioaccumulation fish CEFIC-LRI	Fish bioaccumulation data.
Fraunhofer Institute of Toxicology and Experimental Medicine, Germany	RepDose Fraunhofer ITEM	Subacute to chronic repeated dose toxicity studies conducted with rodents.
New Energy and Industrial Technology Development Organization (NEDO), Japan	Repeat Dose Toxicity NEDO	Repeated dose toxicity of 82 industrial chemicals.
Laboratory of Mathematical Chemistry (LMC), Bulgaria	ERBA OASIS	Estrogen Receptor Binding Affinity (ERBA) data expressed as relative binding affinities in comparison with the estradiol affinity.

Source	Tool or database provided	Description
	Genotoxicity OASIS	Data on bioaccumulation in aquatic organisms.
	Micronucleus OASIS	Data on 577 chemicals for *in vivo* bone marrow and peripheral blood micronucleus tests.
Istituto Superiore de Sanita, Italy & Office of Public Health, Switzerland	Micronucleus ISS MIC	*In vivo* micronucleus mutagenicity assay data in rodents.
Laboratory of Mathematical Chemistry (LMC), Bulgaria and Ministry of Economy, Trade and Industry (METI), Japan	OASIS Biodegradation	Experimental biodegradation results from the Japanese Existing Chemicals Programme.
Laboratory of Mathematical Chemistry (LMC), Bulgaria, US EPA, University of Tennessee, Knoxville and Ministry of Economy, Trade and Industry (METI), Japan	Aquatic OASIS	Aquatic toxicity data.
Laboratory of Mathematical Chemistry (LMC), Bulgaria, Ministry of Economy, Trade and Industry (METI), Japan and Exxon Mobil	OASIS Bioaccumulation	Bioaccumulation data from the Japanese Existing Chemicals Programme as well as results generated by Exxon Mobil.
Laboratory of Mathematical Chemistry (LMC), Bulgaria, Unilever, Exxon Mobil, and P&G	Skin Sensitisation	Skin sensitisation data gathered by LMC, Unilever, Exxon Mobil, P&G and OECD.
International QSAR Foundation, Unilever and University of Tennessee, Knoxville	GSH Experimental EC50	Abiotic thiol reactivity expressed by the *in chemico* RC50 value for electrophiles.
Profilers		
US EPA	Aquatic toxicity classification by ECOSAR	Profiler classifies chemicals into chemical classes for which structure activity relationships have been developed for aquatic toxicity.
	Bioaccumulation-metabolism alerts	Structural fragments from the BCF-BAD model in EPISuite version 4.0.
	Bioaccumulation-metabolism half-lives	Groups chemicals into very slow, slow, moderate, fast and very fast biotransformation rates.
	Biodegradation fragments (BioWIN MITI)	Categorisation scheme based on the structural fragments used by the MITI Biodegradation Probability Models.
	Organic functional groups (US-EPA)	645 structural fragments and correction factors used in the enhanced Organic Functional Groups derived from the KOWWIN fragment library from

Source	Tool or database provided	Description
		EPISuite
	US-EPA New Chemical categories	The rules reproduce the categories cited in the document "TSCA New Chemicals Program (NCP)/Chemical Categories".
Istituto Superiore de Sanita, Italy	Mutagenicity/Carcinogenicity alerts by Benigni/Bossa	This rulebase for mutagenicity and carcinogenicity was developed as a module to the Toxtree software. The structural alerts (SAs) from the rulebase have been included as a profiler in the Toolbox.
	Micronucleus alerts by Benigni/Bossa	35 structural alerts for a preliminary screening of potentially in vivo mutagens based on the ToxMic rulebase from Toxtree.
European Commission	Acute aquatic toxicity classification by Verhaar	Defines chemicals into classes of inert, less inert, reactive and specifically-acting chemicals for an acute toxicity to fish.
	Toxic hazard classification by Cramer	TEstimation of a Threshold of Toxicological Concern (TTC)
Laboratory of Mathematical Chemistry (LMC), Bulgaria	Chemical Elements	All chemical elements from Periodic table organised in 18 groups.
	DNA Binding by OASIS	DNA binding categorisation scheme based on the model of Ames mutagenicity developed by LMC.
	Organic functional groups	227 organic functional groups, specific groups of atoms that are responsible for the characteristic chemical reactions of those molecules.
German Federal Institute for Risk Assessment (BfR)	Eye irritation/corrosion exclusion rules by BfR	Exclusion rules for eye irritation/corrosion based on physico-chemical cut-off values to identify chemicals that do not exhibit eye irritation or corrosion potential.
	Eye irritation/corrosion inclusion rules by BfR	Structural inclusion rules to identify chemicals that show potential for eye irritation and corrosion.
	Skin irritation/corrosion exclusion rules by BfR	Exclusion rules for skin irritation/corrosion based on physico-chemical cut-off values to identify chemicals that do not exhibit skin irritation or corrosion potential.
	Skin irritation/corrosion inclusion rules by BfR	Structural alerts for positive classification of chemicals causing irritation, corrosion or the combination irritation/corrosion depending on their mechanisms.
University of Vienna, Austria	Organic functional groups, Norbert Haider (checkmol)	204 organic functional groups recognized by "Checkmol" program which was developed by Dr Haider, University of Vienna.
Laboratory of Mathematical Chemistry (LMC), Bulgaria and US EPA	Acute aquatic Toxicity MOA by OASIS	Classifies chemicals for their acute aquatic toxicity mode of action, which was developed by the US EPA.
	Oncologic primary classification	This profiler consists of molecular definitions developed by the US-EPA to mimic the structural criteria of chemical

Source	Tool or database provided	Description
		classes of potential carcinogens covered by the US-EPA's OncoLogic™ Cancer Expert System for Predicting Carcinogenicity Potential.
Laboratory of Mathematical Chemistry (LMC), Bulgaria, L'Oréal, Exxon Mobil, Unilever, Dow Chemical and Research Institute for Fragrance Materials (RIFM)	Protein Binding by OASIS	Structural alerts on protein binding developed by industry consortia with the LMC.
QSARs		
US EPA	ECOSAR	Model to estimate acute and chronic toxicity to aquatic organisms.
Multicase Inc.	Model for the Prediction of the Octanol-Water Partition Coefficient	None reported
	Model for the Estimation of the Aqueous Solubility of Organic Molecules by the Group Contribution Approach	None reported
	Model of estimating estrogen receptor (ER) binding	None reported
	Model for estimating the toxicity to microorganisms (Vibrio Fischeri)	None reported
	Model for estimating Human Intestinal Absorption	None reported
ChemAxon	Model for estimating pKa	pKa predictions for 150000 chemicals.

Table H1 Metabolism tools contained within the OECD QSAR Toolbox

Metabolism	Database details
Documented	
Observed mammalian metabolism	Metabolic pathways for 100 chemicals from 630 different mammalian studies. Includes aliphatic amines, alkyl and aryl halides, ethers, esters, carbamates, carboxylic acid esters and multifunctional compounds. Around 50% of the *in vivo* studies are for oral administration.
Observed Microbial metabolism	Degradation pathways for 551 chemicals. The database includes C1-compounds, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulfonates, nitrates, nitro-derivatives, nitriles, and compounds containing more than one functional group. Most of data are for aerobic degradation.
Observed Rat *In vivo* metabolism	Metabolic pathways for 647 chemicals. This database includes aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, alcohols and phenols, carbonyl compounds, carboxylic acids and esters, nitro compounds, amines, organic sulphides, heterocyclic and, mostly, multi-functional chemicals. Fields of applications include industrial chemicals, solvents, monomers, pharmaceuticals, pesticides, some phytochemicals and azo chemicals.
Observed rat liver metabolism with quantitative data	No details reported in the OECD QSAR Toolbox
Observed Rat Liver S9 metabolism	Metabolic pathways for 261 chemicals from *in vitro* systems such as rodent (mostly rat) liver microsomes and S9 fraction. This database includes aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, carboxylic acids and esters nitro compounds, amines, heterocyclic and multi-functional chemicals.
Simulated	
Autoxidation simulator	Training set of 140 chemicals (terpenes, simple aliphatic and polyethyleneglycol ethers, aldehydes, aminophenols).
Autoxidation simulator (alkaline medium)	Training set of 133 chemicals (terpenes, simple aliphatic and polyethyleneglycol ethers, aldehydes, aminophenols).
Dissociation simulator	No details reported in the OECD QSAR Toolbox
Hydrolysis simulator (acidic)	The training set includes epoxides, aziridines, esters, carbamates, halomethanes, selected alkyl halides, anhydrides, dithiocarbamates, isocyanates, isothiocyanates, sulfonyl chloride, lactones, nitriles, amides, N-halamines, carbamates, diketenes and organic peroxide.
Hydrolysis simulator (basic)	The training set includes sulfonyl halides, organophosphorus compounds, epoxides, aziridines, esters, carbamates, halomethanes, selected alkyl halides, anhydrides, dithiocarbamates, isocyanates, isothiocyanates, sulfonyl chloride, lactones, nitriles, amides, N-halamines, carbamates, diketenes, organic peroxides.
Hydrolysis simulator (neutral)	The training set includes discrete organic chemicals, epoxides, aziridines, esters, carbamates, halomethanes, selected alkyl halides, anhydrides, dithiocarbamates, isocyanates, isothiocyanates, sulfonyl chloride, lactones, nitriles, amides, N-halamines, carbamates, diketenes, organic peroxides.
In vivo Rat metabolism simulator	The simulator represents a set of 609 structurally generalised, hierarchically arranged abiotic and enzymatic transformation reactions. The simulator contains 479 enzymatic phase I transformations, including aliphatic C-oxidation, aromatic C-hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide hydrolysis, carbonyl group reduction, nitro and azo group reduction, N-hydroxylation, oxidative deamination, β-oxidation, ring cleavage, hydrolytic cleavage, aromatization, decarboxylation and dehalogenation. The simulator contains 104 enzymatic phase II transformations, including glucuronidation, sulphation, glutathione conjugation and N-acetylation.

Microbial metabolism simulator	Multiple pathway catabolism is simulated using the abiotic and enzyme-mediated reactions.
Rat liver S9 metabolism simulator	509 structurally generalised, hierarchically arranged biotransformation reactions for *in vitro* metabolism in rodent (mostly rat) liver microsomes and S9 fraction. The organic compounds in the training set include single and fused-ring arenes, phenols, haloalkanes and haloarenes, aromatic and aliphatic amines, nitroarenes, alkanes and cycloalkanes, alkenes, ethers, carboxylic acids and their derivatives, halogenated hydrocarbons, alcohols, epoxides, N-nitrosoamines and azo chemicals. The simulator contains 450–470 enzymatic phase I transformations and 15–20 enzymatic phase II transformations.
Skin metabolism simulator	Due to the lack of reported skin metabolism data and the hypotheses that skin enzymes can metabolise xenobiotics via reactions analogous to those in the liver, the simulator was developed as a simplified mammalian liver metabolism simulator.
Tautomerism simulator	The simulator has been developed for generation of all possible tautomeric forms of a target chemical.