Expert review of the mutagenicity of carbamates: using (Q)SAR predictions for ICH M7 classification

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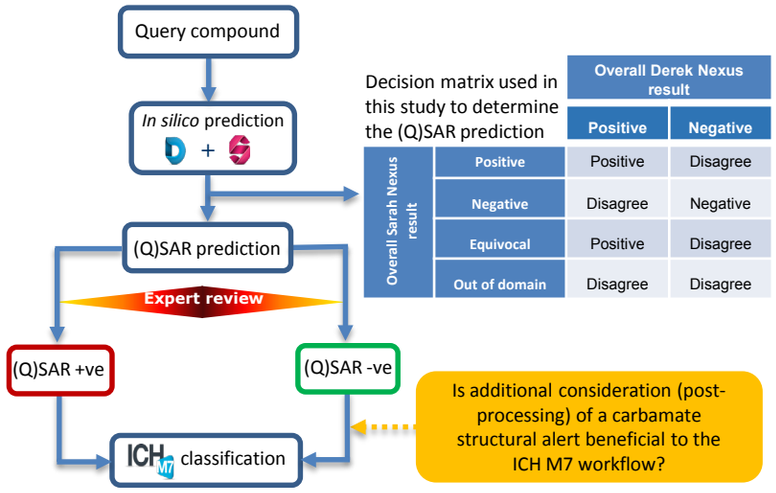
Introduction

The ICH M7 guidelines[1] aim to assess and control mutagenic impurities in pharmaceuticals. These guidelines allow the use of two complementary *in silico* systems (one expert rule-based and one statistical-based) to predict the bacterial mutagenicity of a given impurity. Assessment of the combination of results produced from two *in silico* predictions has received a lot of attention, a situation which is complicated when also considering confidence and domain of applicability. The need for additional levels of scrutiny for negatively predicted compounds in certain chemical classes has been suggested in the literature.[2] Carbamates have been highlighted as a chemical class of concern for mutagenicity.[3, 4] but they are versatile and well used, particularly as protecting groups. Derek Nexus contains an *in vitro* mutagenicity alert for vinyl carbamates based on data and mechanistic knowledge harvested over 30 years.

This study outlines the impact applying additional scrutiny to the carbamate chemical class in a workflow aimed at achieving ICH M7 classifications.

ICH M7 classification workflow (for this study)



Decision matrix used in this study to determine the (Q)SAR prediction

Overall Sarah Nexus result	Overall Derek Nexus result: Positive	Overall Derek Nexus result: Negative
Positive	Positive	Disagree
Negative	Disagree	Negative
Equivocal	Positive	Disagree
Out of domain	Disagree	Disagree

Figure 1. Workflow for assigning an ICH M7 classification of an impurity using the expert rule-based system Derek Nexus (D) and statistical-based system Sarah Nexus (S).

- To achieve an ICH M7 classification in the absence of available relevant data, the outputs from two complementary *in silico* systems have to be resolved to a single (Q)SAR prediction (Figure 1).
- A high degree of confidence can be taken when the two predictions are in agreement.[5]
- For the purposes of this study when the systems agreed the prediction was accepted, predictions which disagreed where kept separate to easily judge the affect of an additional alert for carbamates.

The carbamate query

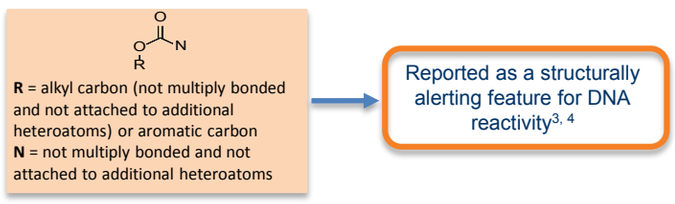


Figure 2. Description of the carbamate query used in this study.

Method, Results and Expert review

1. A robust structural definition for carbamates was created (Figure 2).
2. A dataset of compounds with Ames data were extracted from Vitic Nexus and queried for compounds containing a carbamate (Figure 3).
3. Bleomycin derivatives were removed as toxicity cannot be associated to one structural feature.
4. The resulting 69 carbamates were then processed through Derek and Sarah Nexus to provide (Q)SAR predictions (Figure 1-decision matrix).

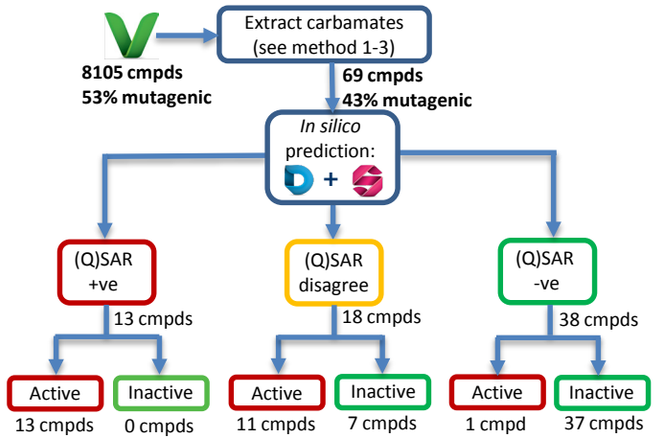


Figure 3. QSAR prediction for a series of carbamates extracted from Vitic Nexus.

- High sensitivity was observed as 24 out of 25 actives were detected by at least one *in silico* system, only 1 Ames active compound (**12**) was not detected.
- Negative predictivity was also high as 37 out of 38 non-mutagens were correctly predicted as negative.
- **The addition of a carbamate alert would create 37 false positives and one true positive (cmpd 12).**

Strain profiles of 11 QSAR disagree and 1 QSAR negative that are experimentally Ames positive

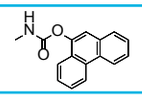
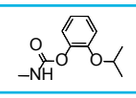
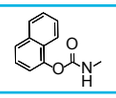
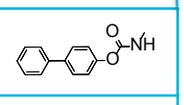
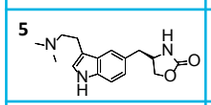
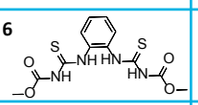
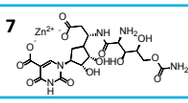
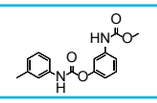
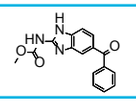
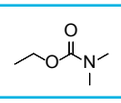
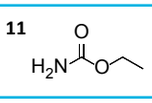
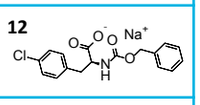
1 	2 	3 	4 
TA1537, 30% hS9	TA1535, -S9	TA1538, +mS9	TA1537, 30% hS9
5 	6 	7 	8 
TA98, +rS9	TA98 and TA100	TA100, ±rS9	TA98, 30% hS9
9 	10 	11 	12 
TA98 and 100, +rS9	TA1535, 30% ±rS9	TA1535, +rS9	TA100 and 1535, -S9

Table 1. Strain profile data of Ames positive carbamates not predicted as mutagenic by both systems. Positive results were seen in the strains outlined for each compound. r = rat, h = hamster, m = mouse.

- Analysis of the strain profiles for compounds **1-12** found no common mechanism to explain the activity seen in the Ames test.
- Literature reports suggest that the Ames test is a poor predictor of mutagenicity for, among others, the carbamate chemical class.[6]

Mechanism of action

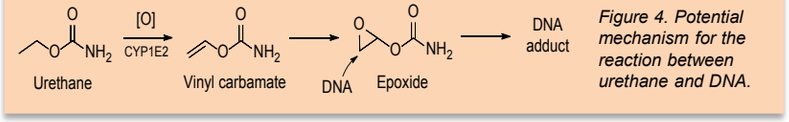


Figure 4. Potential mechanism for the reaction between urethane and DNA.

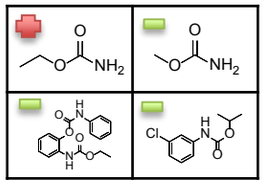


Table 2. Activity of urethane derivatives in the Ames test.

Urethane's mutagenic mode of action is believed to be via vinyl carbamate (Figure 4). Analysis of the structural-activity relationships indicates that only low molecular weight derivatives of urethane may be able to proceed through this mechanism (Table 2). Published structural alerts are likely to have been derived from the activity of low molecular weight carbamate derivatives capable of forming DNA reactive epoxides, but this mechanism is not applicable to the whole chemical class.

Carbamate protecting groups

Mining public and proprietary data sources, including data donated by two Lhasa members, furnished small datasets for Cbz, Boc and Fmoc protected carbamates (Table 3, Figure 5). Compounds which contained known toxicophores were removed to allow assessment of the carbamate functionality only. The prevalence of mutagenic Cbz, Boc and Fmoc containing carbamates is low. Only 6 out of 92 non-alerting compounds were Ames active.

Dataset	Protecting group	Alerting compounds removed: Total	Ames +ve	Ames -ve
Lhasa (Public data)	Cbz	6	1	5
	Boc	18	0	18
	Fmoc	2	0	2
Member 1	Cbz	4	0	4
	Boc	13	0	13
	Fmoc	3	0	3
Member 2	Cbz	8	1	7
	Boc	38	4	34
	Fmoc	0	0	0
	Total	92	6	86

Table 3. Ames activity of compounds containing a carbamate protecting group and have been processed in Derek Nexus.

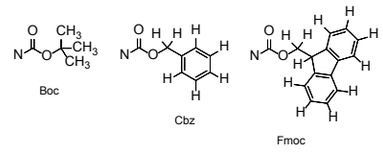


Figure 5. Structural definitions of carbamate protecting groups used in this study. The nitrogen cannot be multiply bonded or attached to additional heteroatoms.

The 5 Ames active compounds in member 2's data contain no alerting features and are currently under investigation to elucidate this activity.

Conclusions

- Assessment of public and proprietary data has shown that, in general, carbamates are inactive in the Ames test, with a few notable exceptions; namely urethane and some small molecule derivatives.
- Subsets of the carbamate chemical class are covered in Derek and Sarah Nexus and (Q)SAR predictions perform well for this difficult area of chemical space.
- Addition of an alert for *in vitro* mutagenicity to cover the whole carbamate chemical class would be detrimental to the predictive performance of a (Q)SAR system and ultimately an ICH M7 classification by increasing time spent on expert review as well as unnecessary testing and/or control measures of impurities.

References

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