Carbamates, QSAR predictions and ICH M7 classification; making use of expert knowledge.

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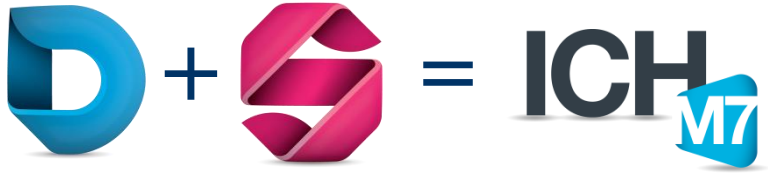
Overview

- Introduce the ICH M7 guidelines
- Outline a workflow used to assign an ICH M7 classification
- Show the impact of having an alert for *in vitro* mutagenicity for carbamates
- Discuss the prevalence of mutagens in proprietary datasets containing carbamate protecting groups
- Conclusions

The ICH M7 guidelines

- ICH M7[1] outlines regulatory guidelines to assess and control mutagenic impurities in pharmaceuticals to limit potential carcinogenic risk.

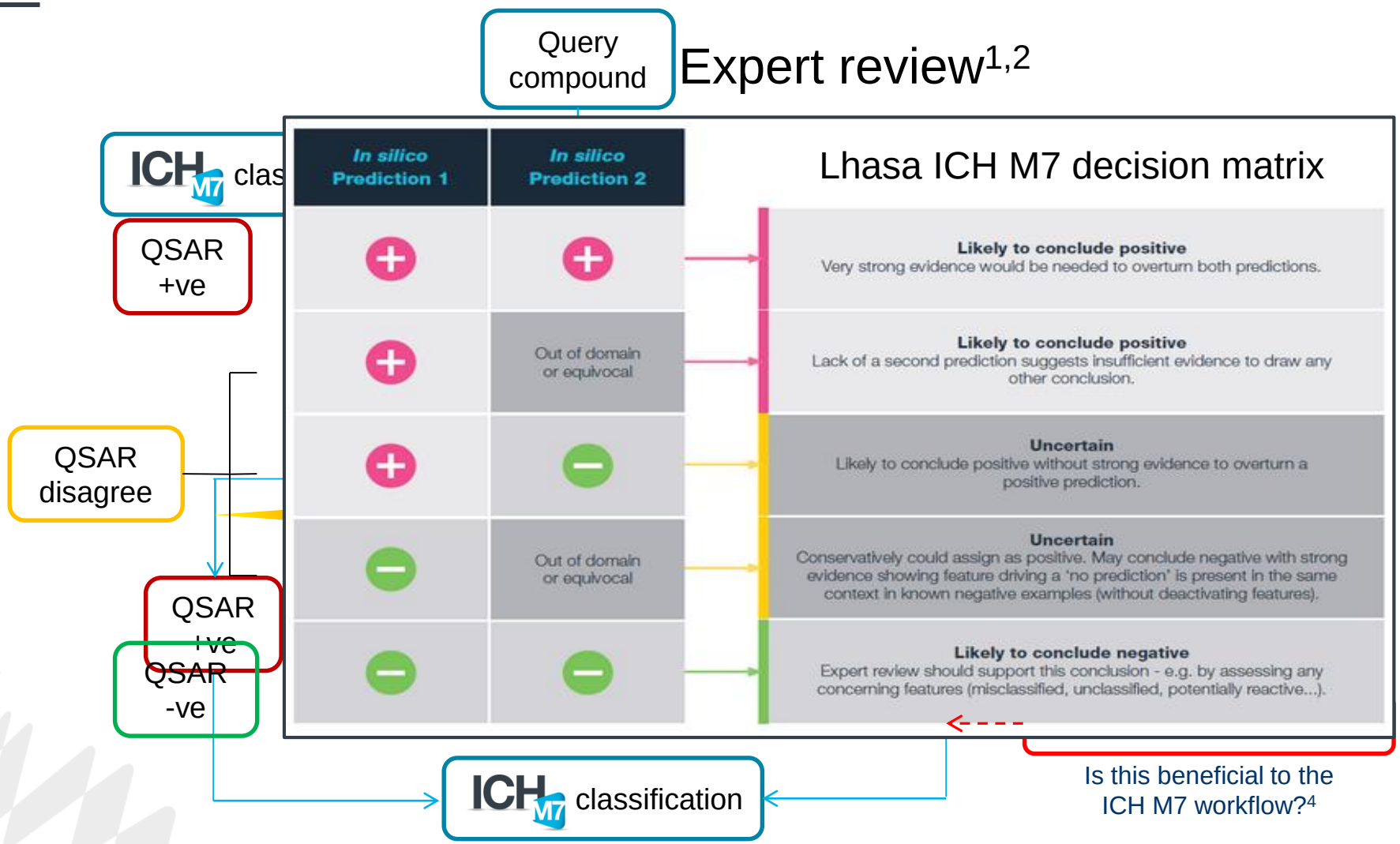
- ICH M7 allows the use of two complementary QSAR systems (one expert-rule based, one statistical based) for predicting the outcome of *in vitro* mutagenesis for a given impurity.



- This QSAR assessment (along with expert review) is then used to assign an ICH M7 classification to the impurity, which will impact the level at which this impurity can be present in the API, if at all.

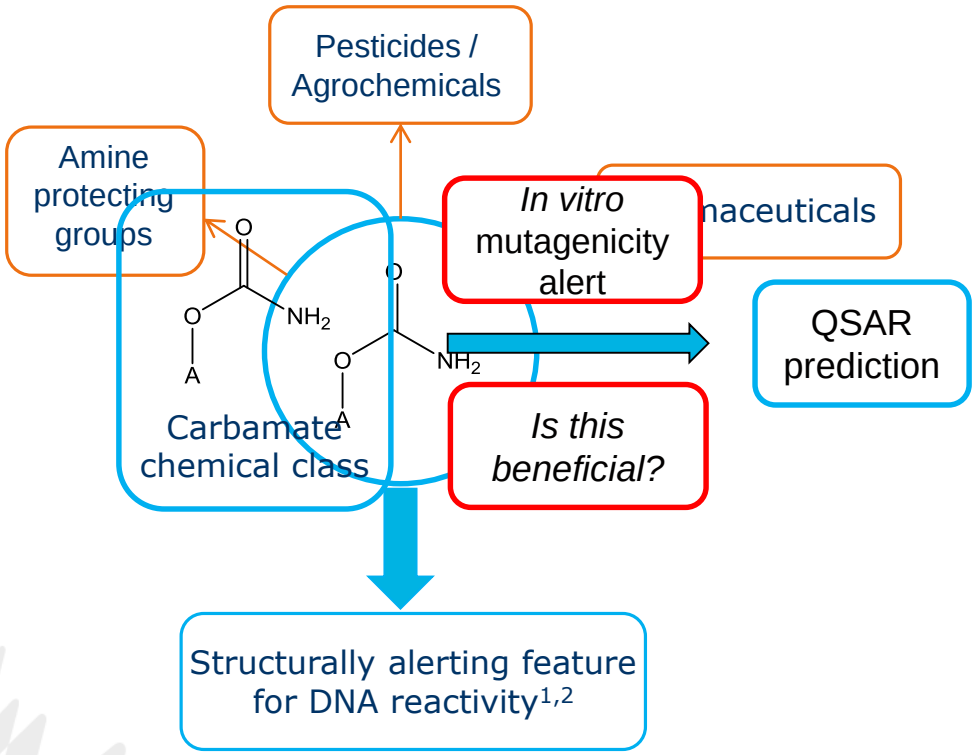
1. http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html

An example of an ICH M7 workflow

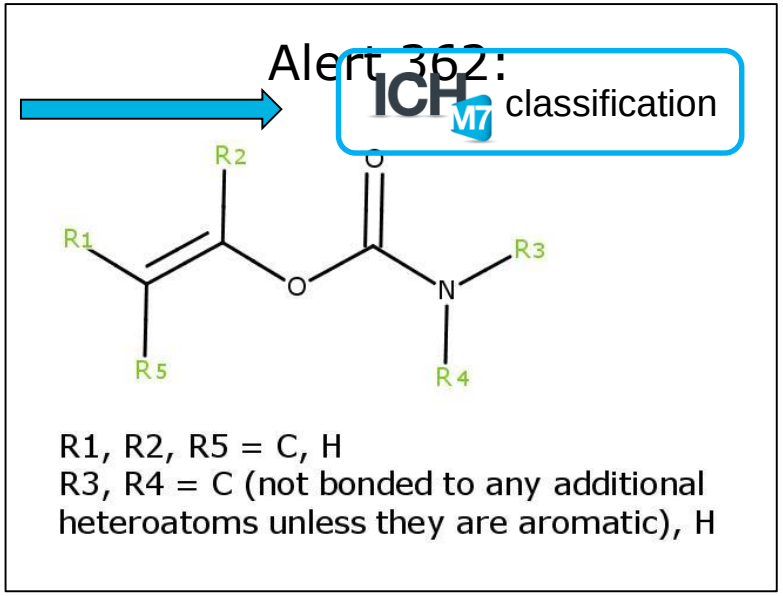


1. Barber, C. *et al*., *Regul .Toxicol. Pharmacol*., 2015, **73**, 367-377.
2. Amberg et al., *Regul. Toxicol. Pharmacol*., 2016, **77**, 13-24.
3. Powley, M. W., *Regul. Toxicol. Pharmacol.*, 2015, **71,** 295-300.
4.Jolly, R. *et al*., *Regul. Toxicol. Pharmacol.*, 2015, **71,** 388-397.

Carbamates: is an *in vitro* mutagenicity alert required?



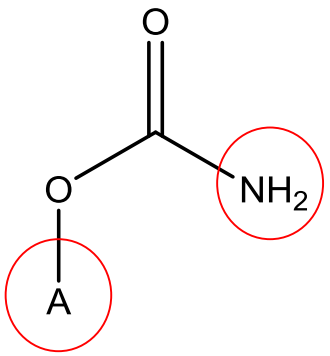
Currently Derek Nexus fires an alert for *in vitro* mutagenicity for vinyl carbamates (at a probable level, Derek KB 2015.1.0).



1. Ashby, J., Tennant, R.W., *Mutat. Res.*, 1988, **204**, 17-115.
2. Müller, L. *et al.*, *Regul. Toxicol. Pharmacol.*, 2006, **44,** 198-211.

Structural definitions

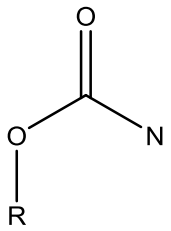
A



A = alkyl, aryl or H

A = A carbamate structural alert from the literature[1]

B



R = C (alkyl), C (aryl)

Alkyl - singly bonded and not attached to additional heteroatoms
Aryl - contains aromatic bonds

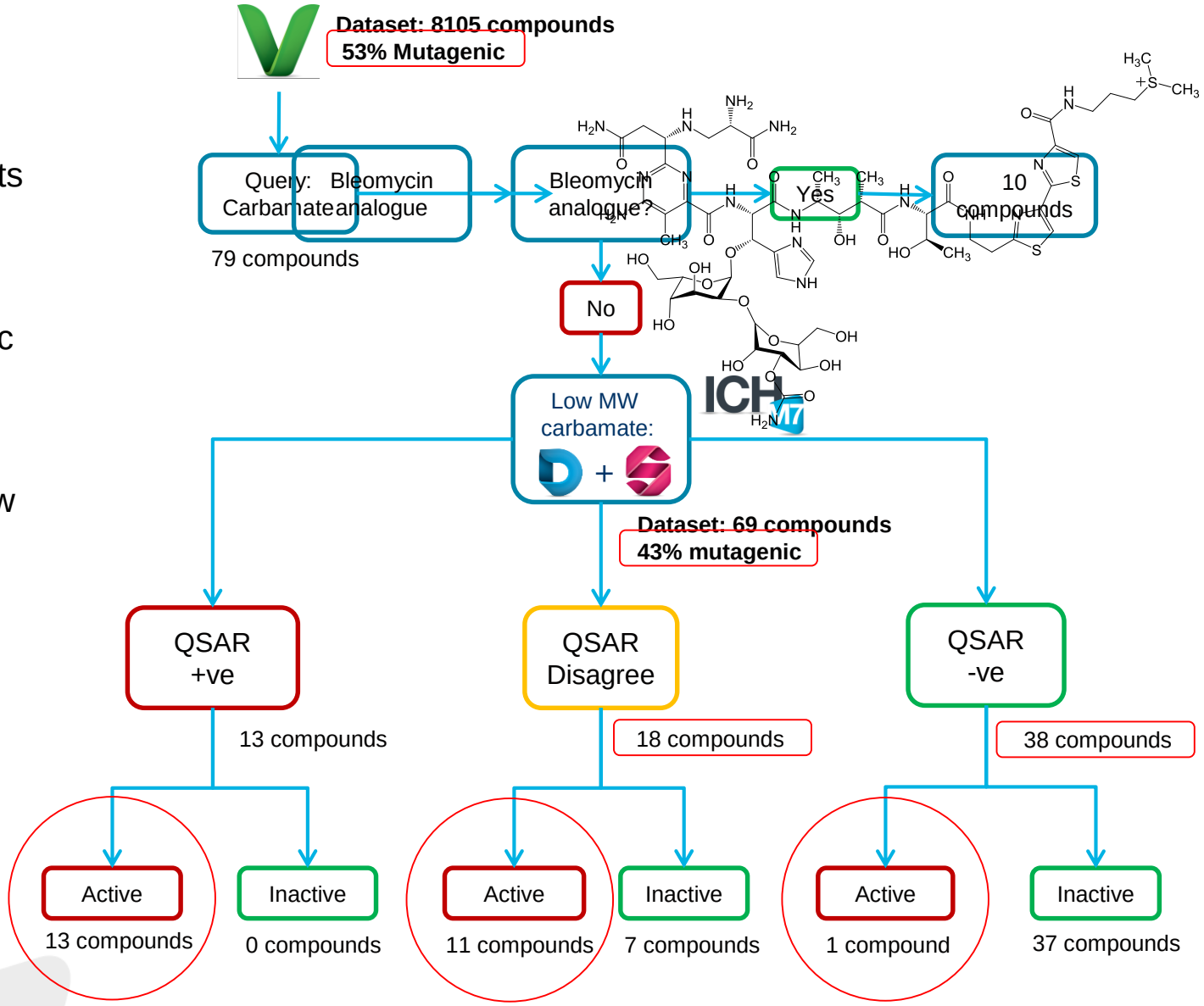
N atom cannot be attached to additional heteroatoms and only contains single bond

B = Refined definition of a carbamate structural alert

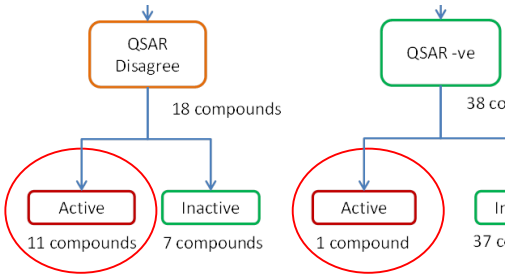
1. Müller, L. *et al.*, *Regul. Toxicol. Pharmacol.*, 2006, **44,** 198–211.

Workflow: QSAR predictions for the *in vitro* mutagenicity of carbamates

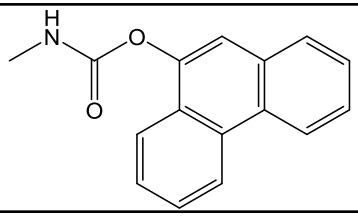
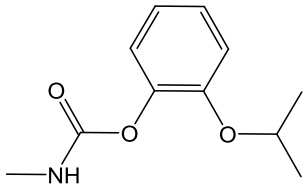
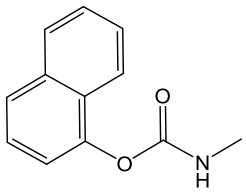
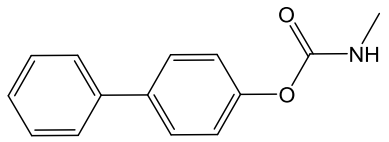
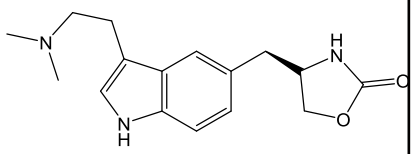
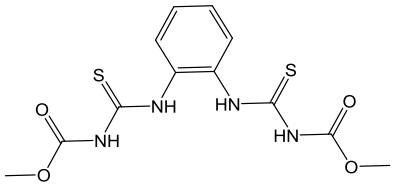
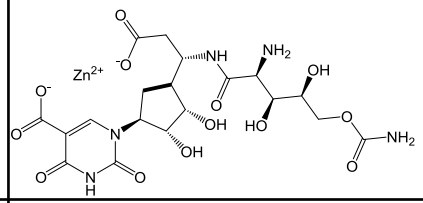
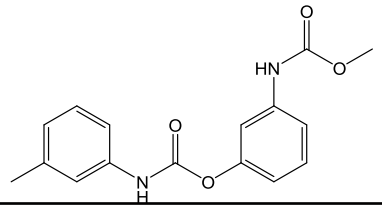
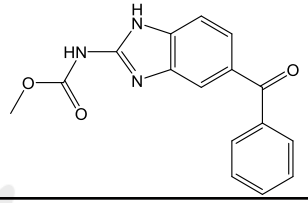
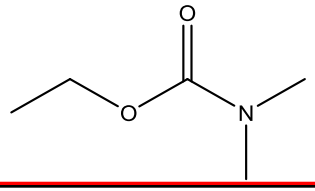
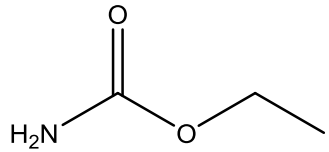
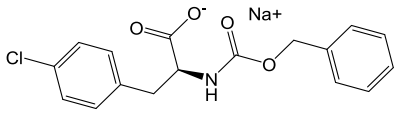
- Compounds from public Ames datasets were extracted from Vitic Nexus v2.6.0
- Activity = Lhasa Vitic overall call
- Carbamate B was used in this workflow
- Derek Nexus v4.1.0 and Sarah Nexus v1.2.0 were used



Expert review

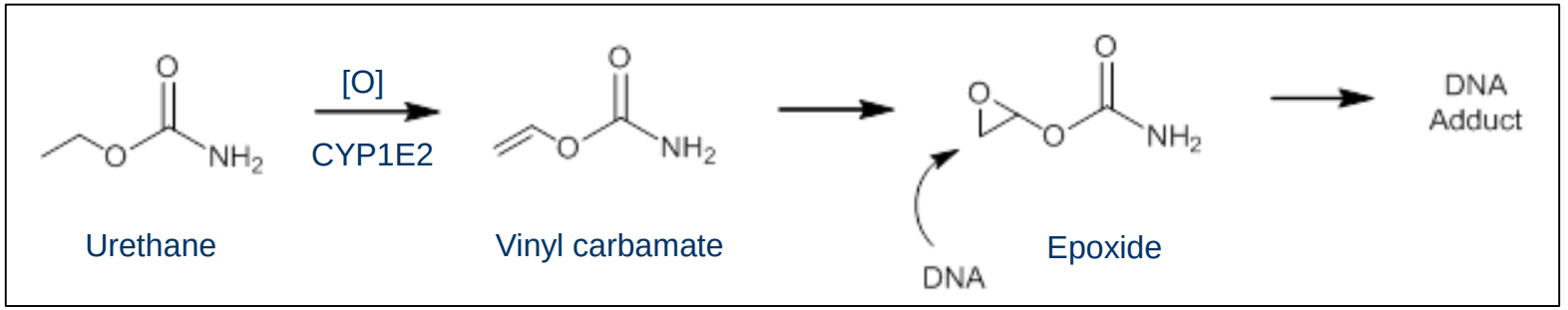


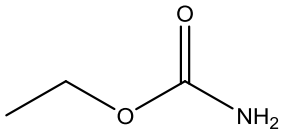
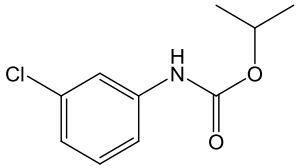
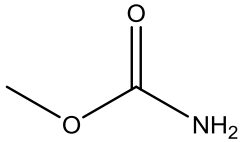
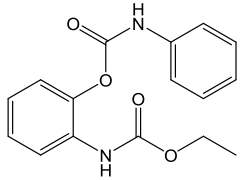
Summary of **Ames active** compounds: 11 QSAR disagree, 1 QSAR negative

			
TA1537, 30% hS9	TA1535	TA1538, mS9	TA1537, 30% hS9
DX -ve / SX +ve	DX -ve / SX +ve	DX -ve / SX +ve	DX -ve / SX +ve
			
TA98, +S9	TA98 and TA100, 30% rS9	TA100, ±S9	TA98, 30% hS9
DX –ve / SX +ve	DX –ve / SX +ve	DX –ve / SX ood	DX –ve / SX +ve
			
TA98 and TA100, +S9	TA1535, 30% rS9, +S9	TA1535, ±S9	TA100 and TA1535
DX –ve / SX +ve	DX –ve / SX +ve	DX –ve / SX +ve	DX –ve / SX -ve

DX = Derek Nexus, SX = Sarah Nexus, ood = out of domain, r = rat, h = hamster, m = mouse

Proposed mechanism of action



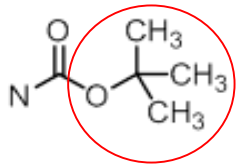
	Ames active		Ames inactive
	Ames inactive		Ames inactive

- Compounds with additional steric bulk on the *O*-ethyl group were found to be non-mutagenic, as were compounds with additional N-substitutions.

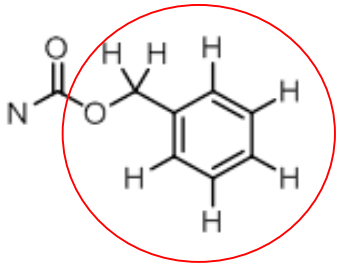
In general, carbamates are inactive in the Ames test, bar a few exceptions; notably urethane which undergoes a specific DNA reactive mechanism.

Carbamate protecting groups

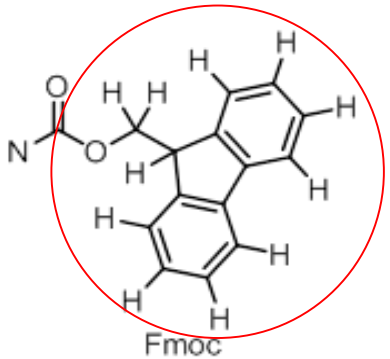
Using proprietary data donated by our members, the common carbamate protecting groups; N-Boc, Cbz and Fmoc were examined for their *in vitro* mutagenic potential.



N-Boc



CbZ



Fmoc

Nitrogen atom can only be singly bonded and only attached to H, C atoms

- Steric bulk of the protecting groups means the site of metabolism is blocked
- Unlikely to go through mechanism outlined previously (for urethane)

Carbamate protecting groups

Public and proprietary datasets were obtained for each protecting group

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

- The prevalence of mutagenic carbamates across all 3 datasets is low
- Only 6 out of 92 non-alerting compounds were Ames active
- Member 2 donated 1261 compounds in total (p = 20%)

n.b datasets are too small to attribute any statistical significance

Conclusions

- Carbamates have been highlighted in the literature as a functional group associated with DNA reactivity.
- 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.
- Having an alert for *in vitro* mutagenicity to cover the whole carbamate chemical class would be misleading and detrimental to the predictive performance of a QSAR system and ultimately an ICH M7 classification.
- This investigation has resulted in a no alert report for *N*-methyl-*O*-aryl carbamates and identified extension of two existing *in vivo* chromosome damage alerts for vinyl and alkyl carbamates to the mutagenicity *in vivo* endpoint.

Disclaimer – The results presented are for discussion purposes only and should not be used for ICH M7 classification and/or submission to regulators.

Acknowledgements

Special thanks to:

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Thank you for your attention