Respiratory Sensitization: Chemistry-based Identification

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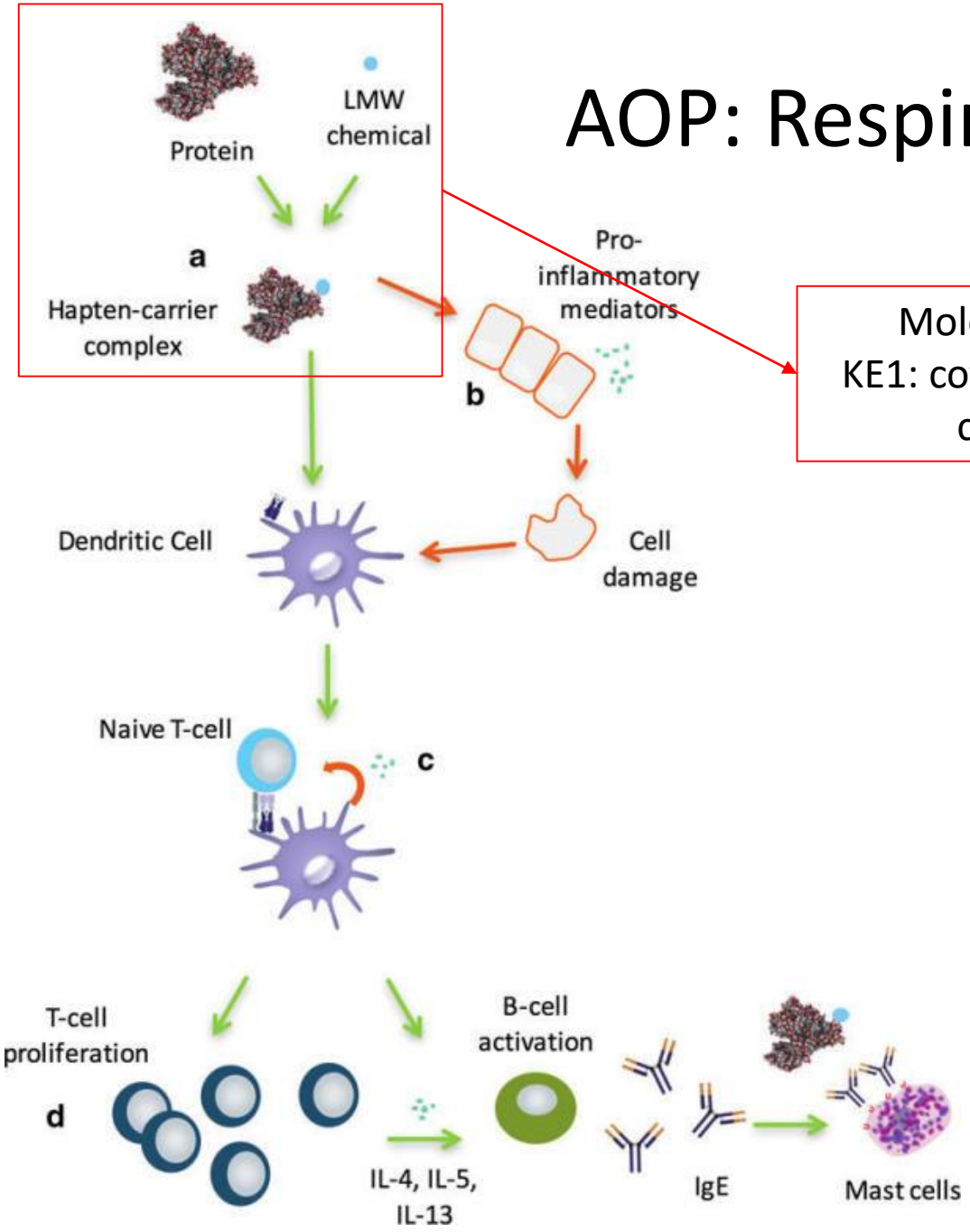
Respiratory Sensitization

- A range of LMW organic chemicals can cause respiratory sensitization e.g. isocyanates
- No suitable animal or *in-vitro* model – limited data
- Majority of reported respiratory sensitizers are from human case studies (occupational asthma)
- LLNA often used; however, not all skin sensitizers are also respiratory sensitizers

AOP: Respiratory Sensitization



Molecular Initiating Event / Key Event 1[1]
KE1: covalent binding of low-molecular-weight chemicals to lung or skin proteins

[1]Sullivan et al (2017) Applied In Vitro Toxicology 3, p213

Available Human Data

- 104 organic chemicals identified as being linked with occupational asthma[2]
- Chemicals identified from clinical reports
- Not all chemicals had confirmed bronchial challenge test data (considered the gold standard by physicians)
- Analysis of the dataset showed a number of clear irritants (e.g. acids)
- A set of 82 control chemicals were also identified

[2]Enoch et al (2012) Chemical Research in Toxicology 25, p2490

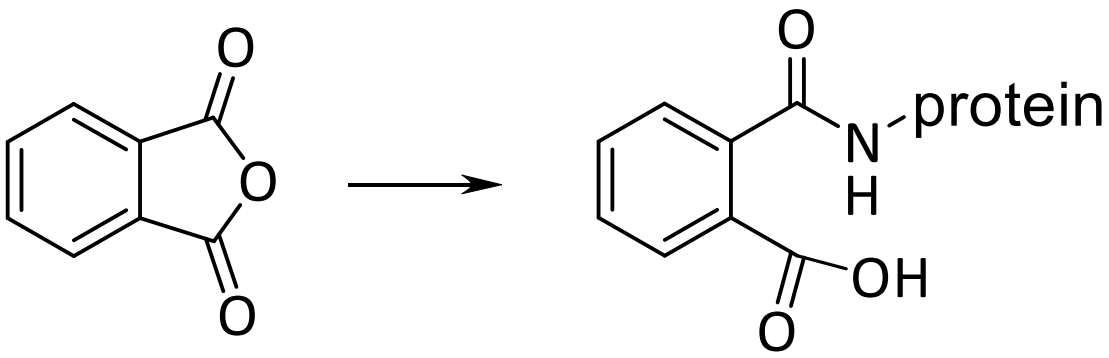
Structural Alerts: Respiratory Sensitization

- Clinical data have been used to develop structural alerts for respiratory sensitization
- It is based on the MIE being covalent protein binding (mainly to lysine)
- It can be used to identify chemicals likely to cause protein binding in the lung
- However, many of the alerts are developed from low numbers of chemicals
- The alerts are available in the OECD QSAR Toolbox

Structure-Activity Relationship

Low reactivity
No cross-linking

Low reactivity
Protein cross-linking



High reactivity
No cross-linking

Structural Alert Summary

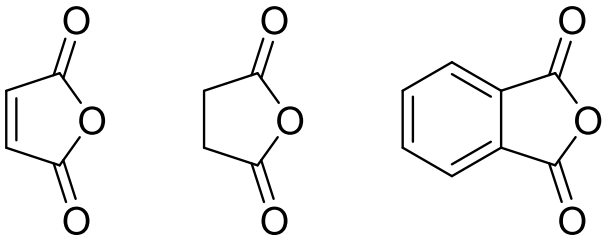
Mechanistic domain	Number of alerts
Acylation	7
Michael addition	16
Schiff base formation	16
S_N2	9
S_NAr	4
Total	52

	Alert	No alert
Respiratory sensitizer (104)	95	9
Control (82)	7	75

Structural Alert Confidence

Ring opening acylation

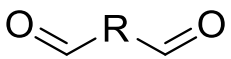
Anhydrides



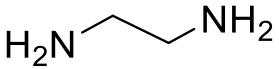
Cross-linking Schiff base

Di-aldehydes (Glyoxal, glutaraldehyde etc.)

Ethylenediamines



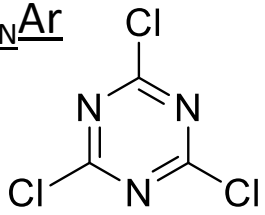
Ethanolamines



Piperazine

Extremely reactive S_NAr

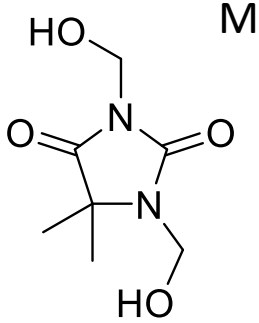
Cyanuric chloride



Formaldehyde releasers

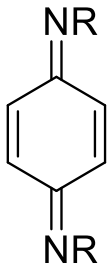
Hydroxymethyl imidazolidine-2.4-diones

Methenamine



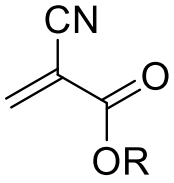
Extremely reactive Michael acceptors

Reactive dyes (quinones, quinone-imines etc.)



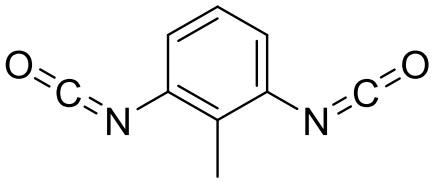
Cyano-acrylates

Azocarbonamide



Cross-linking acylation

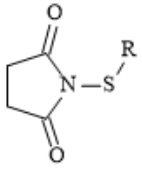
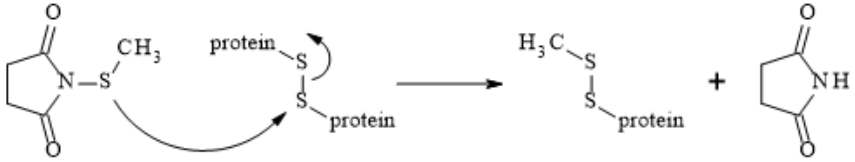
Di-isocyanates

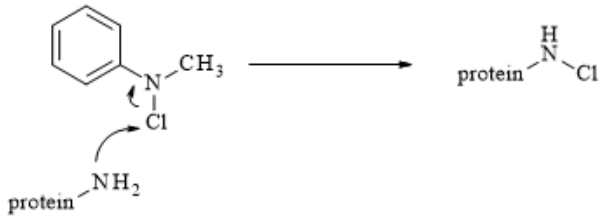


OECD QSAR Toolbox Meta Data

- All of the structural alerts are implemented in the Toolbox
- Importantly, the meta-data allows you to inspect the supporting evidence for an alert
- This is especially useful for alerts that have been developed from a single chemical
- As an example, consider two the S_N2 alerts:

  N-alkylthiosuccinamides and chloro-nitrogens

Individual profile/alert/boundaries/other info applicable for defining categories within a profiler	
Name	N-Alkylthiosuccinamides
Type of profile/alert	Structural alert
Description/ applicability domain	 R = sp^3 carbon Organic chemicals with a molecular weight less than 1000g/mol
Mechanism	Chemicals containing a N-alkylthiosuccinamides moiety have been suggested to undergo an S_N2 reaction with a di-sulphide linkage in proteins in the lung. It is important to note that in this reaction the exogenous chemical is acting as the nucleophile and the protein di-sulphide linkage as the electrophile [1].
	
Set of chemicals used for profile development (local training set)	The dataset from which the profiler was developed contained one chemical containing this alert, which has been reported as being a respiratory sensitiser in humans. [hyperlink to Excel file containing the data]
Data/Knowledge used for profile development	There has been a single peer-reviewed report of this alert causing respiratory sensitisation in humans in the work place [2].
Profile/alert analysis	Profile/alert: N-Alkylthiosuccinamides; Number chemicals analysed (Total/Sensitisers): 1/1
References	[1] Enoch SJ et al (2012) *Development of mechanism-based structural alerts for respiratory hazard identification.* Chemical Research in Toxicology, 25, p2490-2498. [2] Royce S et al (1993) *Occupational asthma in a pesticides manufacturing worker.* Chest, 103, p295-296.

Individual profile/alert/boundaries/other info applicable for defining categories within a profiler	
Name	Chloro nitrogen
Type of profile/alert	Structural alert
Description/ applicability domain	$R_2N—Cl$ R = any carbon, hydrogen Organic chemicals with a molecular weight less than 1000g/mol
Mechanism	Chemicals containing a chloro nitrogen moiety have been suggested to be oxidised to an epoxide which can then undergo a ring opening S_N2 reaction [1].
	
Set of chemicals used for profile development (local training set)	The dataset from which the profiler was developed contained one chemical containing this alert, which has been reported as being a respiratory sensitiser in humans. [hyperlink to Excel file containing the data]
Data/Knowledge used for profile development	There have been several peer-reviewed reports of this alert causing respiratory sensitisation in humans in the work place [2-5].
Profile/alert analysis	Profile/alert: Chloro nitrogen; Number chemicals analysed (Total/Sensitisers): 1/1
References	[1] Enoch SJ et al (2012) *Development of mechanism-based structural alerts for respiratory hazard identification.* Chemical Research in Toxicology, 25, p2490-2498. [2] D'Alo S et al (2012) *Chloramine-induced anaphylaxis while showering: a case report.* Journal of medical case reports, 6, p324-324. [3] Quirce S et al (2010) *Cleaning Agents and Asthma.* Journal of Investigational Allergology and Clinical Immunology, 20, p542-550. [4] Sartorelli P et al (2010) *Asthma induced by Chloramine T in nurses: case report.* Medicina Del Lavoro, 101, p134-138. [5] Siracusa A et al (2013) *Asthma and exposure to cleaning products - a European Academy of Allergy and Clinical Immunology task force consensus statement.* Allergy, 68, p1532-1545.

Structural Alerts: Biological Confidence

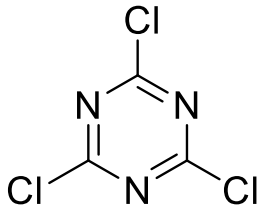
- The alerts were developed from clinical human data
- Work is on-going to develop biological support for the alert set – this is based on identifying AOP evidence
- It is envisaged that this will lead to a revised, more predictive, set of structural alerts

Episuite – Vapour Pressure

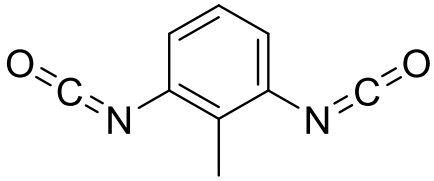
- We have previously rationalised the relatively low skin sensitization potency of acrylates in terms of their volatity[3]
- It might be assumed that chemicals need to be volatile to be respiratory sensitizers
- This turns out not to be the case

[3]Ebbrell et al (2017) Chemical Research in Toxicology 30, p604

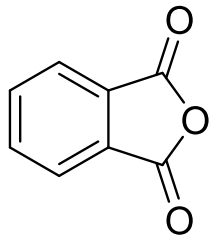
Episuite – Vapour Pressure



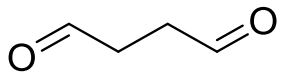
0.24 mmHg (-1.63)



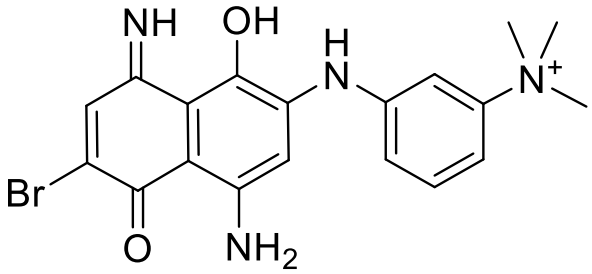
0.15 mmHg (-0.84)



0.00036 mmHg (-3.43)



5.23 mmHg (0.72)

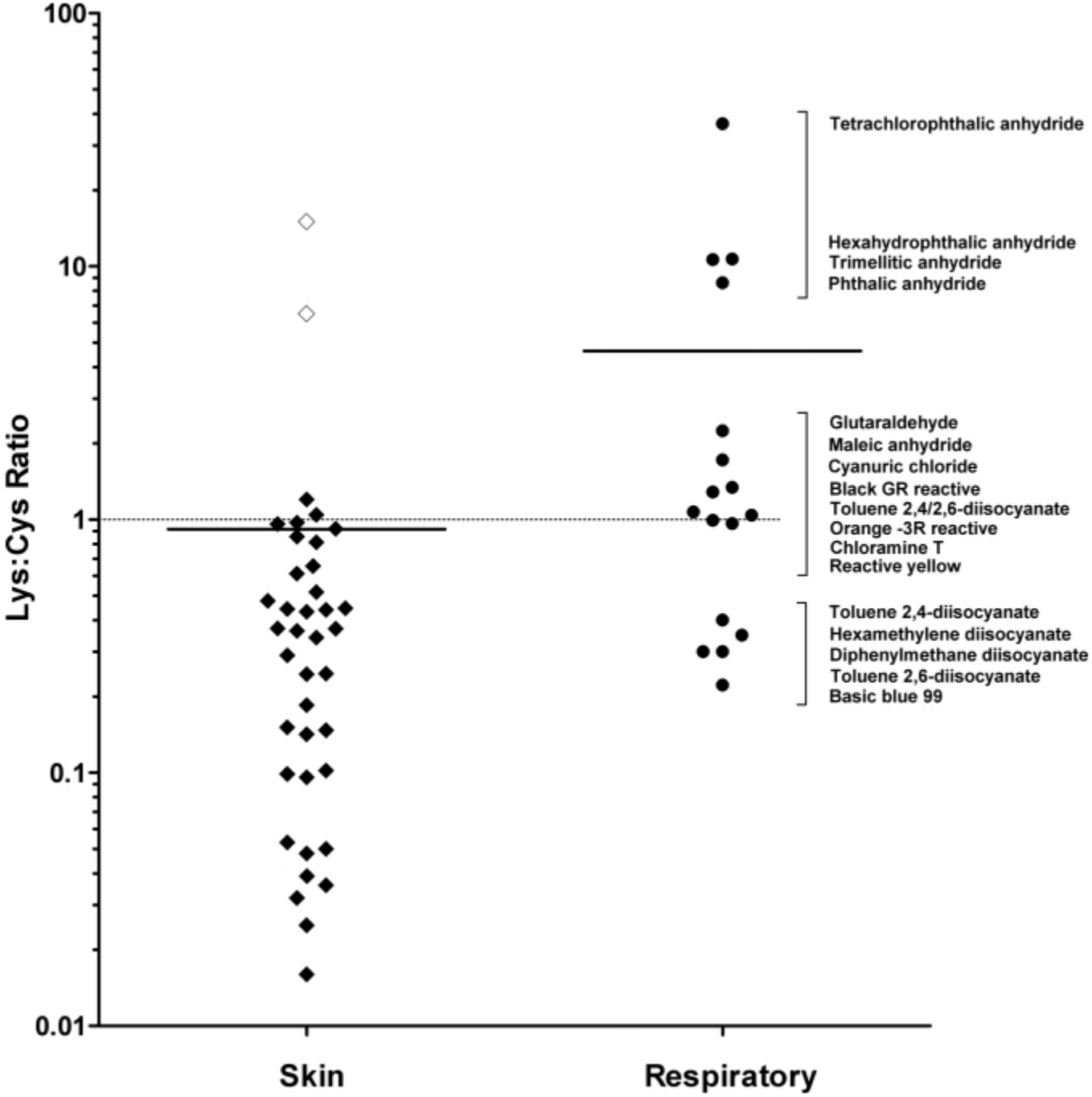


7.41x10^{-19} mmHg (-18.13)

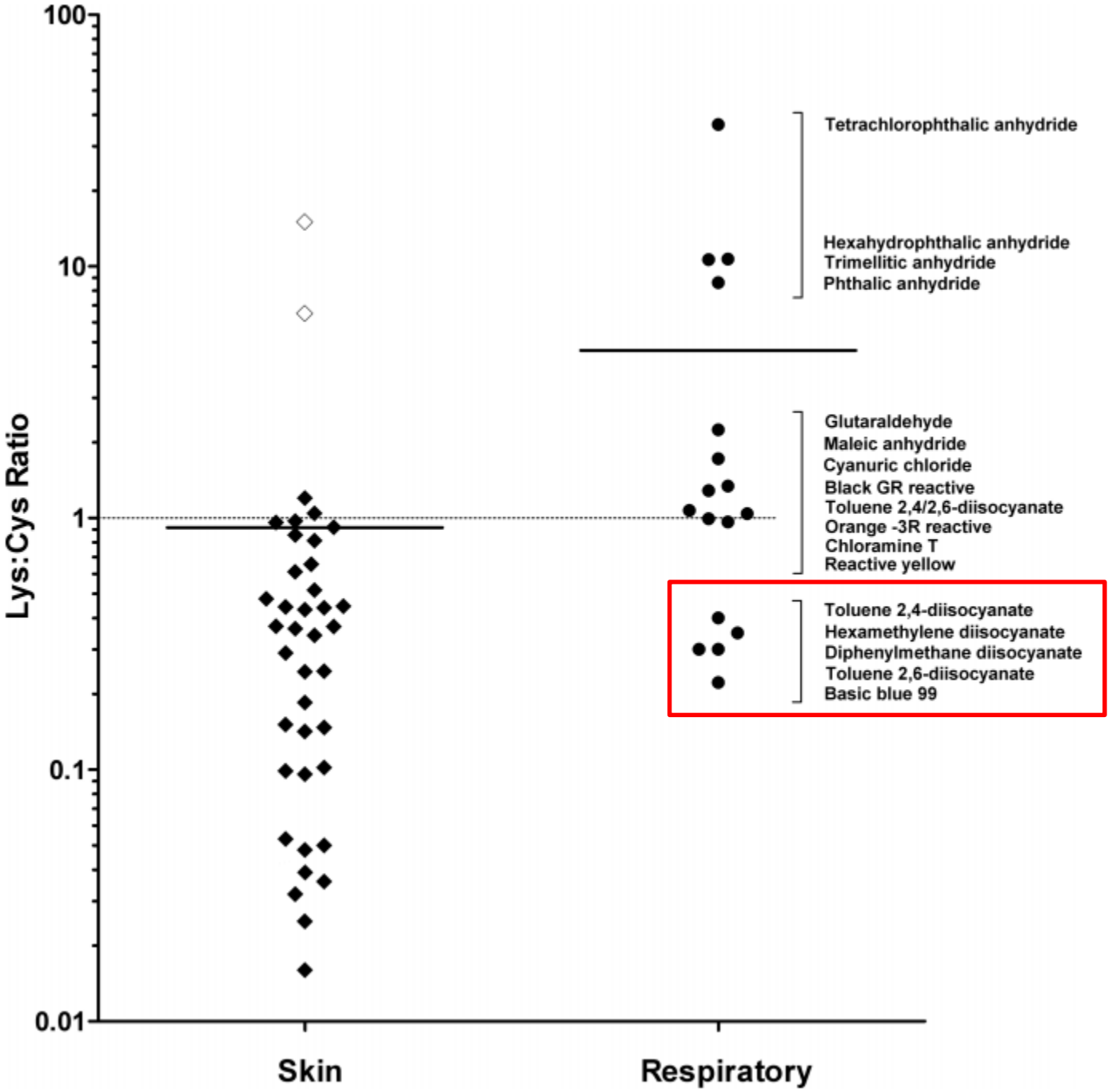
In Chemico: DPRA

- DPRA data can be used to identify direct acting respiratory sensitizers
- Lalko et al[4] have shown the Lys/Cys ratio to be a useful discriminator
- Reaction conditions: 0.5 mM peptide to 5 mM (Cys, pH = 7.4) or 25 mM (Lys, pH = 10.2) of test material. Assay time = 24 hours

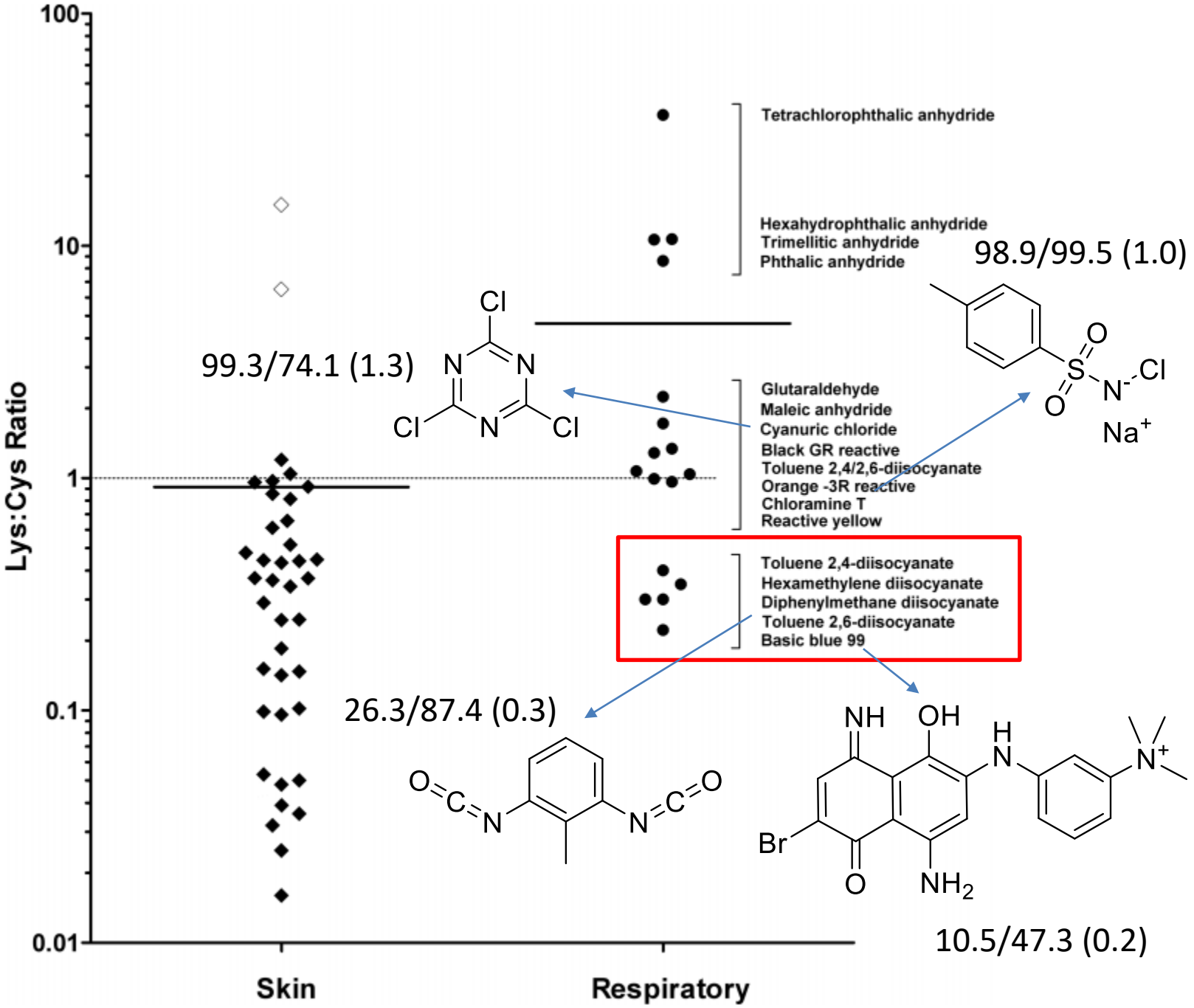
[4]Lalko et al (2012) Toxicological Sciences 129, p421



[4]Image from Lalko et al (2012) Toxicological Sciences 129, p421

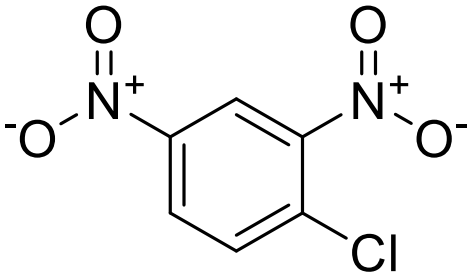


[4]Image from Lalko et al (2012) Toxicological Sciences 129, p421

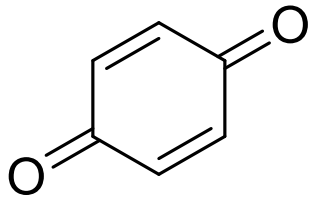


[4]Image from Lalko et al (2012) Toxicological Sciences 129, p421

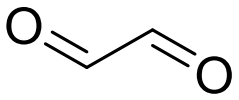
Structural Alerts and *In Chemico* Data



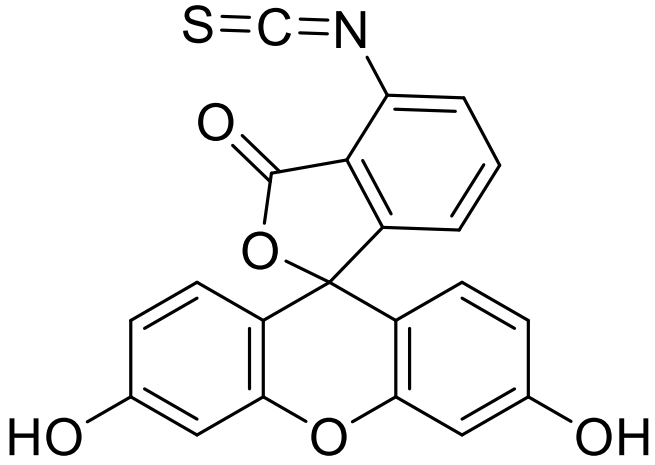
2,4-DNCB
Cys: 100%
Lys: 15%
Lys:Cys = 0.2
Alert: No



p-Benzoquinone
Cys: 99%
Lys: 91%
Lys:Cys = 0.9
Alert: Yes



Glyoxal
Cys: 57%
Lys: 68%
Lys:Cys = 1.2
Alert: Yes



Fluorescein isothiocyanate
Cys: 100%
Lys: 61%
Lys:Cys = 0.6
Alert: No

Conclusions

- A set of structural alerts exist for respiratory sensitization (encoded in the OECD QSAR TB)
- The alerts have varying levels of confidence (which can be assessed using the metadata)
- Respiratory sensitizers do not need to be highly volatile
- *In chemico* (DPRA) data can be used to identify potential respiratory sensitizers
- Combining the structural alerts (and SAR knowledge) with DPRA data enables respiratory sensitizers to be identified based on their MIE

References

1. Sullivan et al (2017) Applied In Vitro Toxicology 3, p213
2. Enoch et al (2012) Chemical Research in Toxicology 25, p2490
3. Ebbrell et al (2017) Chemical Research in Toxicology 30, p604
4. Lalko et al (2012) Toxicological Sciences 129, p421

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