Abiotic transformation developments: pre-hapten activation and consumer products

David W Roberts

IDEA Workshop 16-17th June 2015

Pro- and pre- and propre or prepro haptens

Basically 3 –types

1. Metabolised to reactive hapten – non-variable potency Pro

2. Abiotically converted to reactive hapten – non-variable potency Pre

3. Abiotically converted to reactive hapten – variable potency Pre

In many cases we don't know whether 1 or 2 Propre? Prepro?

Some chemicals can be both 1 and 3 (eg cinnamic alcohol)

Collaboration with RIFM and LMC

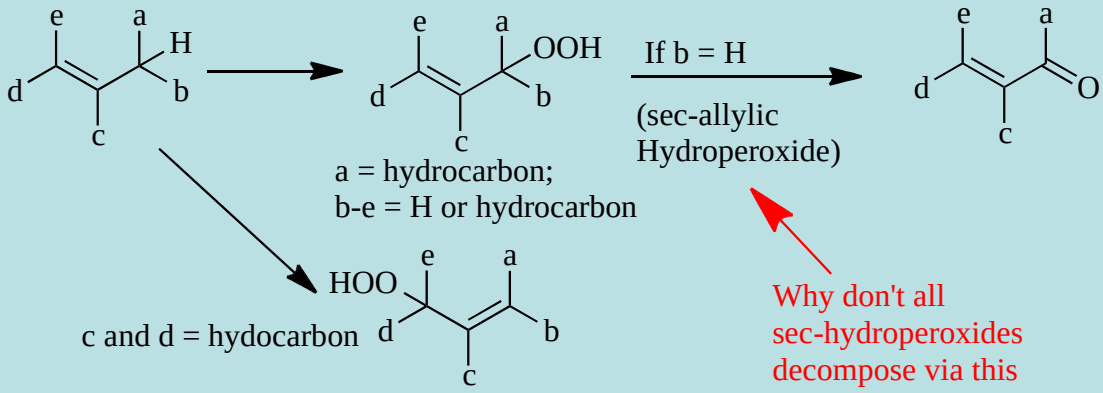
- Defining structural alerts for pro-, pre-, and propre
- Mechanistic modelling of potency:
  - SAR
  - QMM (quantitative mechanistic modelling)

Chemical types

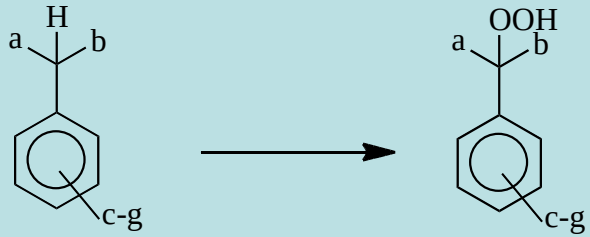
- Hydroperoxides — From Pre- but not pro-
- Aliphatic amines — Pre- or Pro-?
- Aldehydes and ketones — Direct and from Pre- and Pro-
- Epoxides — Direct and from Pre- and Pro-
- Quinone(-like) — Pre- and/or Pro- and/or Pre+Pro

Hydroperoxide alerts

Secondary and tertiary allylic hydroperoxide formation



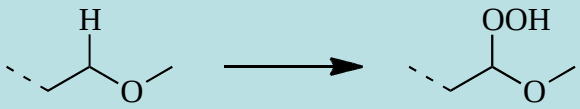
Tertiary benzylic hydroperoxide formation



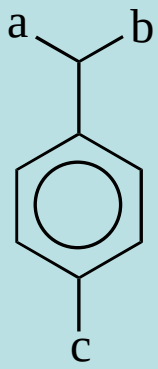
e.g. cumene hydroperoxide,
a = b = Me; c = p-Me; d-g = H

a an b = alkyl; c-g = H or any group

Ether hydroperoxides

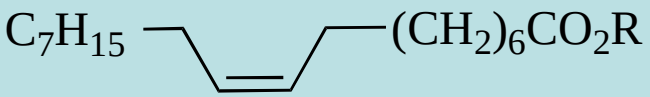


Some alert failures



a and b = alkyl, total C10-C14

c = H or SO_3Na



R = H, + in LLNA, - in GPMT

R = Me, biodiesel

R = glyceryl, olive oil

Hydroperoxide questions

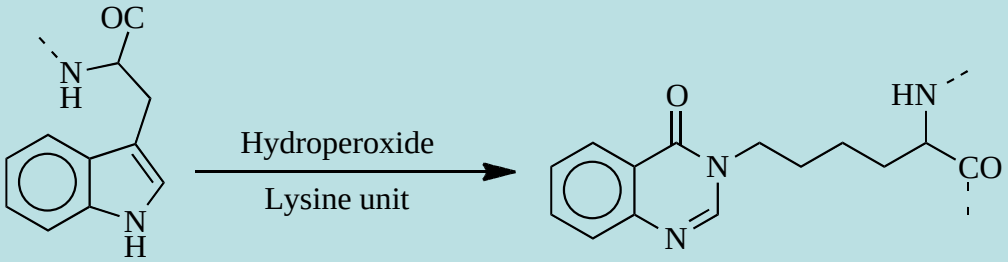
Structure-potency relationships?

Structure-stability relationships

Pro-hapten structure-oxidation chemistry relationships

Reaction chemistry with self (dimerisation) and other olefinic compounds

Can they sensitize by a non-specific “virtual hapten” pathway?
Whereby a tryptophan side group rearranges and transfers to a lysine unit:



Natsch et al, *Chem. Res. Toxicol.,* 2015, 28 (6), 1205–1208, based on Karlsson et al, Chem. Res. Toxicol. 2014, 27, 1294−1303

Alcohol oxidation to aldehydes/ketones

Applies to allylic and propargylic OH groups

Not to saturated alcohols

Benzylic alcohols – probably occurs, but many aromatic aldehydes are weak/NS

Would ortho-$HOCH_2C_6H_4OH$ sensitize as a pro-atranol-type?

Competing activation pathways in some cases

Pre- and pro- mechanisms not mutually exclusive

Comparing EC3 values of cinnamic aldehyde (EC3 = 0.75%) with cinnamic alcohol containing ca 2% (by DPRA) cinnammic aldehyde (EC3 of sample = 22%), about 1% gets oxidised by the pre-hapten route

Aliphatic amines

In many cases can be predicted from reactivity of resulting aldehyde or ketone and logP of parent amine:

CH-N → C=O (cf amino acid metabolism)

Pre- or pro- or both?

Exclusion rule: CH_3-N is not a precursor for CH_2O.

Epoxides

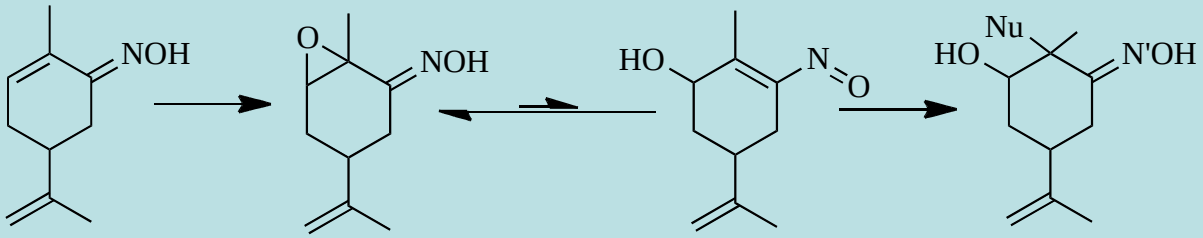
Common as intermediate metabolites in eg liver

Less common in skin (benz[a]pyrene is one example).

Shown to be formed from pro-hapten α,β-unsat alcohols and aldehydes

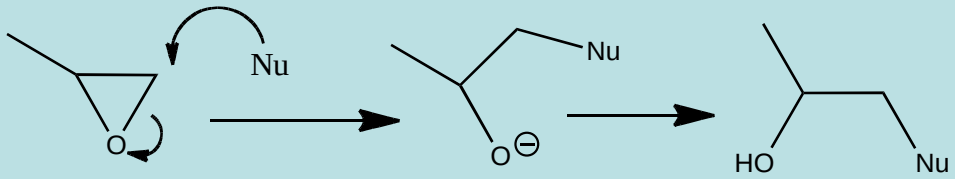
Also from conjugated dienes with at least one of the double bonds in a ring

Involved in unsaturated oxime sensitisation – probably via nitroso-tautomers



Bergstrom et al. Chem. Res. Toxicol., 2007, 20 (6), 927–936

Epoxides – SAR principles for potency



Epoxides are S_N2 electrophiles (maybe some S_N1)

Same chemistry principles apply as for other S_N2 electrophiles:

1. Primary more reactive than secondary

2. Allylic (and heteroallylic) and benzylic more reactive than saturated

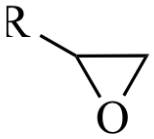
3. Electronegative groups that stabilise negative charge on O increase reactivity

4. Neighbouring group effects can increase reactivity

Sensitisation potency should depend on a combination of reactivity and hydrophobicity

Epoxides (mainly glycidyl)

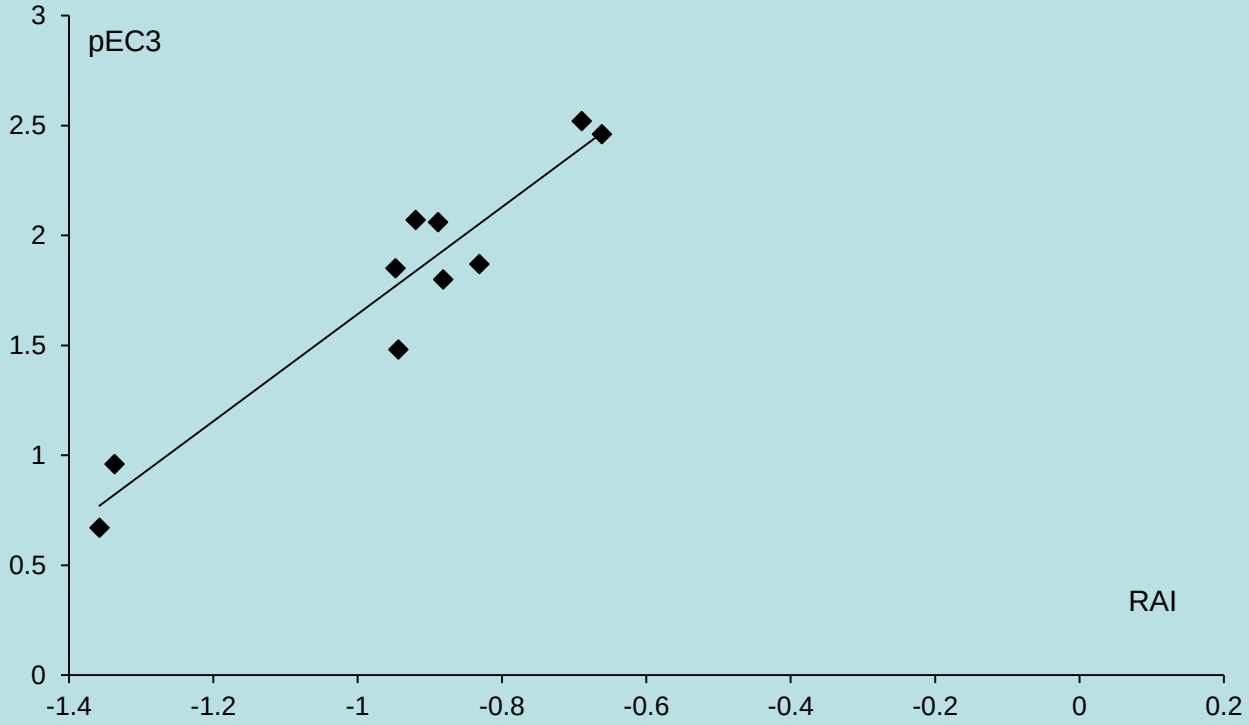
Reactivity to H-Pro-His-Cys-Lys-Arg-Met-OH
(Niklasson et al. Chem. Res. Toxicol. 2009, 22, 1787–1794)

	**Log*k***	**LogP**	**RAI**	**EC3**
$PhOCH_2$	-1.31	1.62	-0.66	0.46
$PhCH_2OCH_2$	-1.54	1.64	-0.88	2.5
$PhOCH_2CH_2$	-1.62	1.97	-0.83	2.3
c-HexylOCH_2	-1.62	1.69	-0.94	5.2
$PhCH_2CH_2OCH_2$	-1.62	1.75	-0.92	1.5
$BuOCH_2$	-1.85	1.24	-1.36	28
$CH_3CH{=}CHCH_2OCH_2$	-1.72	0.96	-1.34	14
$PhSCH_2$	-1.54	2.12	-0.69	0.5
$PhCH_2CH_2$	-1.82	2.19	-0.95	2.1
$PhNHCH_2$	-1.41	1.3	-0.89	1.3

$RAI = \log k + 0.4\log P$

Epoxides – S_N2 domain

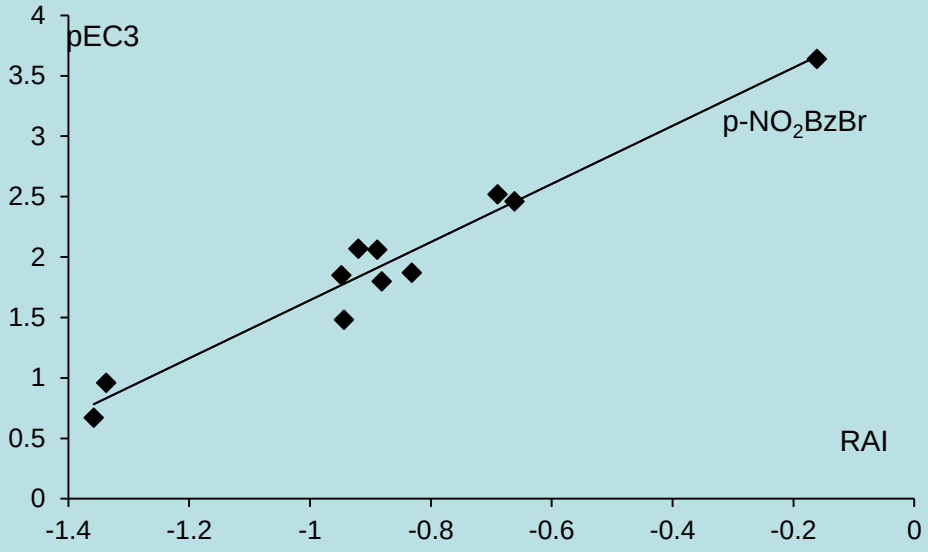
RAI = log*k* + 0.4 logP



pEC3 = 2.44RAI+4.08; R^2 = 0.9163

Prediction for p-nitrobenzylbromide, EC3 = 0.44 (observed 0.05)

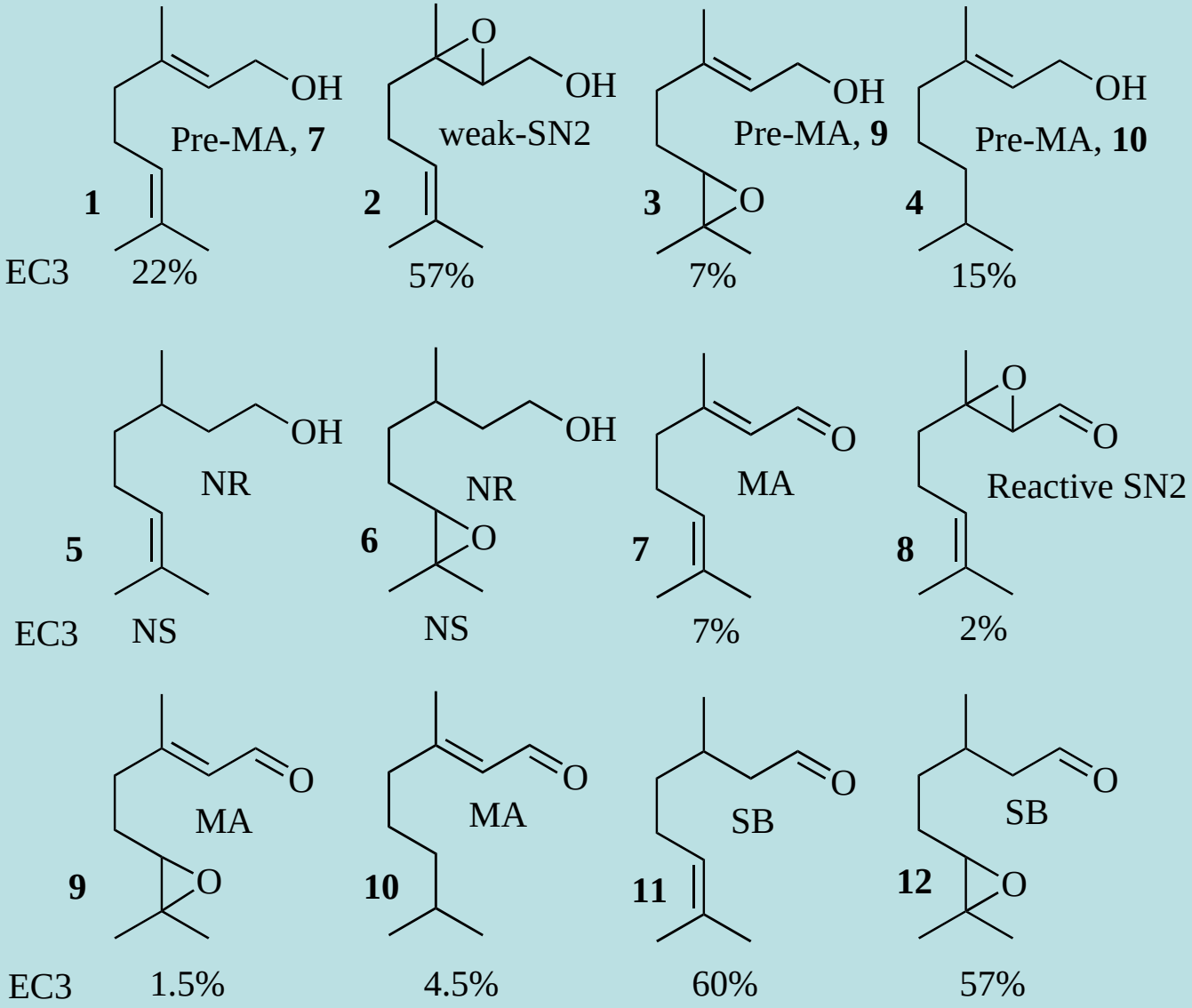
Epoxides with p-NO_2BzBr



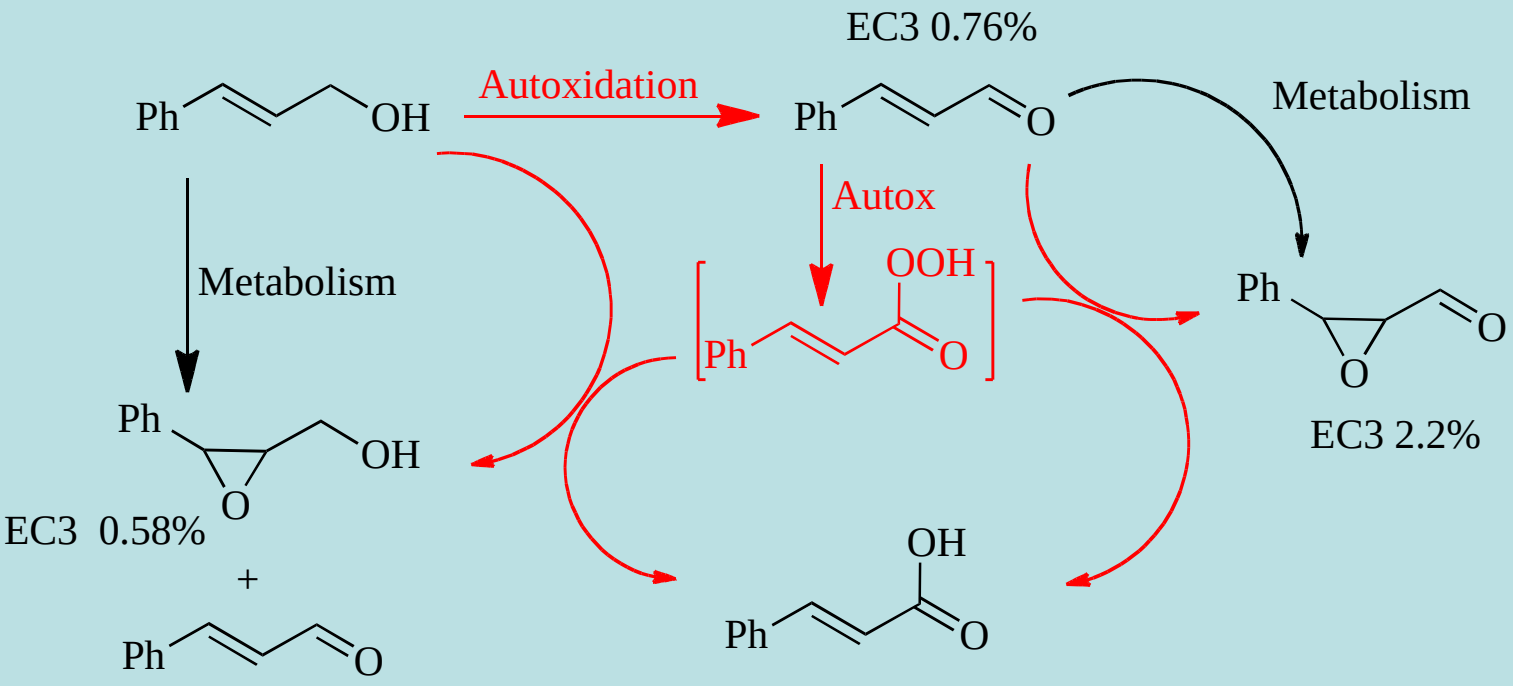
pEC3 = 2.41RAI + 4.05; R^2 = 0.9579 (2.44RAI + 4.08 without p-NO_2BzBr)

Geraniol, geranial, and their epoxides

Delaine et al. Chem. Res. Toxicol. 2014, 27, 1860−1870



Cinnamic alcohol – prepro-hapten

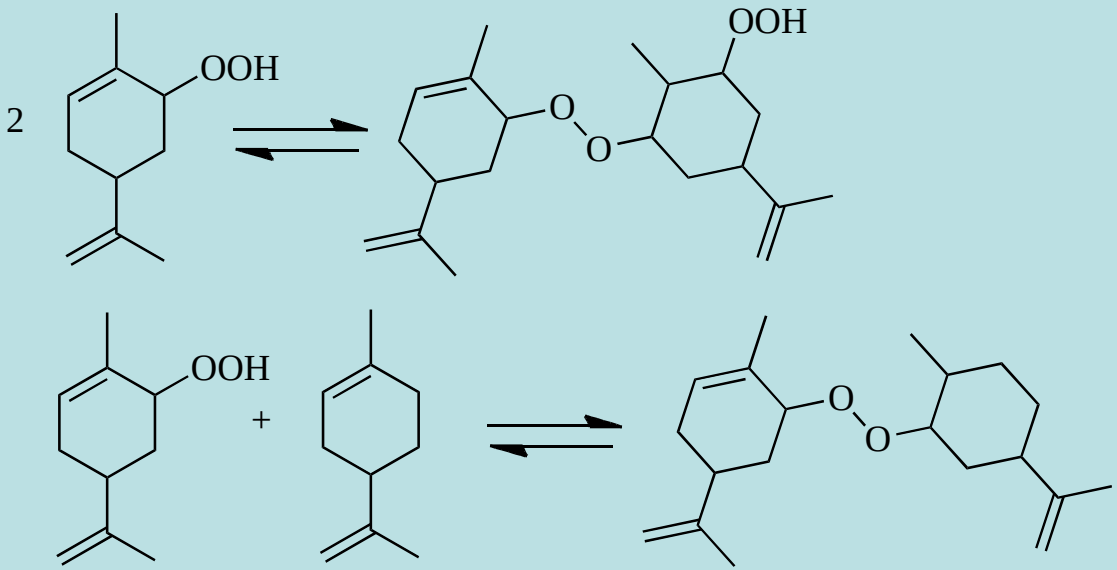


Niklasson, I. B., Ponting, D. J., Luthman, K., and Karlberg, A.-T. (2014) Bioactivation of cinnamic alcohol forms several strong skin sensitizers. Chem. Res. Toxicol. 27, 568−575.

Conclusions

What don't we know enough about?

Hydroperoxide chemistry: structure-stability; structure-potency



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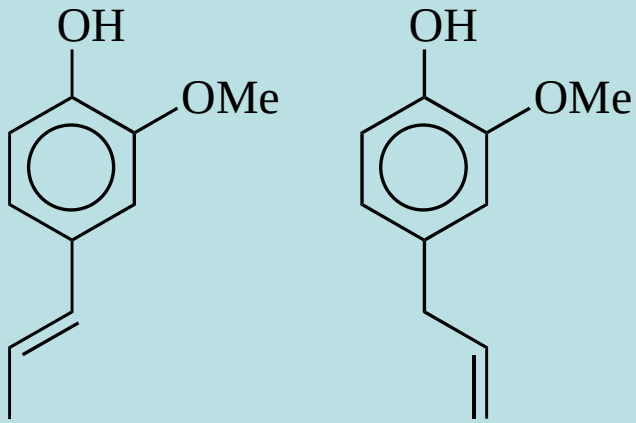
Relative potency of monomer H-peroxide, dimers, (trimers)?
Similar addition of hydroperoxides to other olefins?
Potency of H-peroxide/parent adduct?
k_{rel} and K for addition of H-peroxide to itself, parent, other olefins?

Main area of uncertainty

Complex aromatics (complex means >2 groups, at least one not H-carbon)

A thought experiment: suppose these were new compounds:



- Differences between types of pre-/pro and frequent uncertainty about which applies

- Basically 3 –types
- 1. Metabolised to reactive hapten – has a non-variable potency Pro
- 2. Abiotically converted to reactive hapten – has a non-variable potency Pre
- 3. Abiotically converted to reactive hapten – variable potency Pre

- 1 and 2 cannot always be distinguished
- 1 and 3 can sometimes both apply (eg cinnamic alcohol)

- Hydroperoxides from hydrocarbons – confident pre-hapten mechanism
- Rules for tendency to form – allylic or benzylic tertiary and secondary C-H (no primary known)
- Why don't all secondaries decompose immediately?
- LAB and LAS examples where the rules fail
- General hydroperoxide sensitization (Natsch et al) by "negative hapten"
- Implications – sensitization by any one hydroperoxide produces sensitivity to all. Potency in sensitization and elicitation is additive.

- Epoxides – common as intermediate metabolites in eg liver, less common in skin (benz[a]pyrene is one example). Shown as pro-haptens from a,b-unsat alcohols and aldehydes
- Epoxides – "rules" for formation,
- Rules for potency: SN2 electrophiles, same chemistry principles apply as for other SN2. QMMfor datset including 4-NO2BzX
- Epoxides – involved in oxime sensitization – via nitroso-tautomers?

Aromatic di-NH2 (PPD and related) and di-OH (eg hydroquinone)

Are they pro- or fast activated steady state pre?

Aliphatic amines – in many cases can be predicted from reactivity of resulting aldehyde and logP of parent amine. Exclusion rule – CH3-N is not a precursor for CH2O.

Comparing H-quinone with BQ, assuming HQ acts by conversion to BQ, estimate that ca 10% of the HQ gets activated to BQ in LLNA (show calc)

Allylic OH to a,b-unsat aldehyde (eg ci=nnam. Alc) Evidence for both pre- (variable) and pro- leading to a similar mixture of reactive species – Cinn ald + cinn alc epoxide + cinn ald epoxide. Comparing EC3s and DPRA, already about 2% in commercial, extra ca 1% by metabolism

Benzylic and propargylic alcohols similarly (but benzylic alcohols would give benzaldehydes, vw or NS in LLNA. Q – Would ortho-HOCH2C6H4OH sensitize as a pro-atranol-type?

Who I am and what I do

David/Dave Roberts

PhD Manchester 1965, Organic Chemistry

Unilever Research Port Sunlight 1967-2003

1975 Sultone sensitizers as impurities in surfactants

↓

Project to understand manufacturing by-products:

How to control /suppress them

How to know whether they're sensitizers

↓

Dual career at chemistry/biology and chemistry/chem. eng interfaces

What I do nowadays

Consultant in Manufacturing and Toxicological Chemistry

Honorary Researcher at Liverpool JM University

Major activity in CD

Quantitative Mechanistic Modelling (QMM), i.e.

How can we use chemistry to decide if a chemical:

- is a sensitizer or not
- how potent it is, if at all

The difference between pro-haptens and pre-haptens

Pro-haptens

– metabolically activated to reactive haptens *in cutaneo*

Pre-haptens

– abiotically activated *ex cutaneo*

Can we always/ever be sure?

A different difference

Intrinsically allergenic

- If not directly reactive, sensitizes via conversion to a reactive species under test or exposure conditions
- Has a reproducible potency (eg EC3)

Potential allergen precursor

- Not significantly activated under test/exposure conditions, but has a tendency to form sensitizing impurities.
- Does not have a reproducible potency (eg EC3 depends on storage/handling history)

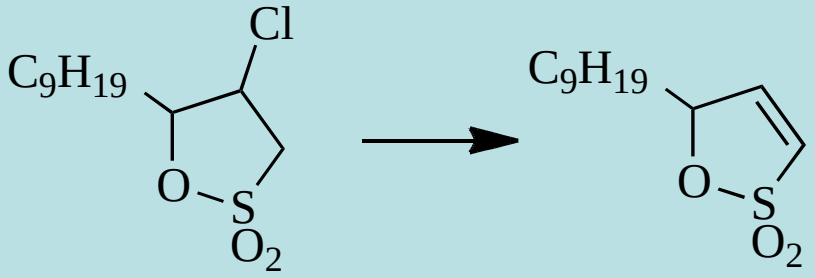
Activation reactions

Oxidation/autoxidation

- C-H to Allylic/benzylic hydroperoxides
- C=C to Reactive epoxides
- CHOH to C=O
- hydroquinones and catechols to quinones
- etc

Hydrolysis

Dehydrohalogenation



Formation of allergens by autoxidation – how much and how fast?

Several situations to consider:

Reactivity-limited

Mass-transfer-limited

Oxygen availability limited

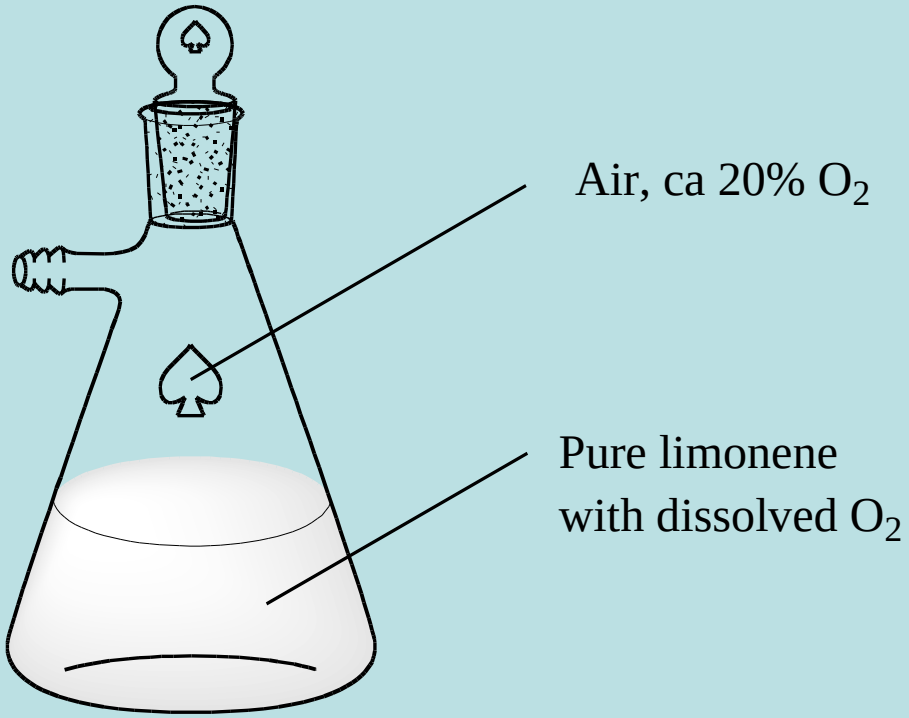
Limited by stability of allergenic autoxidation products

Slow reaction, long time

Lab model of a half-full storage tank, 25° C

$S(O_2)$ ca. 20mmol/L

From original dissolved O_2, 0.25% hydroperoxides



From O_2 in original head-space + air intake, 0.14%

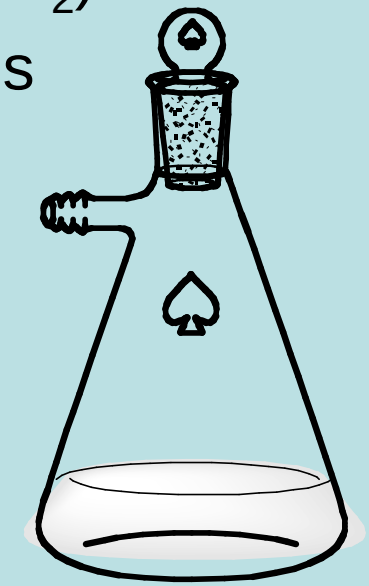
Total maximum hydroperoxide level, 0.39%

Slow reaction, longer time

Remove half the liquid in the tank

The removed volume is replaced by air (20% O_2)

Potential to form further 0.14% hydroperoxides



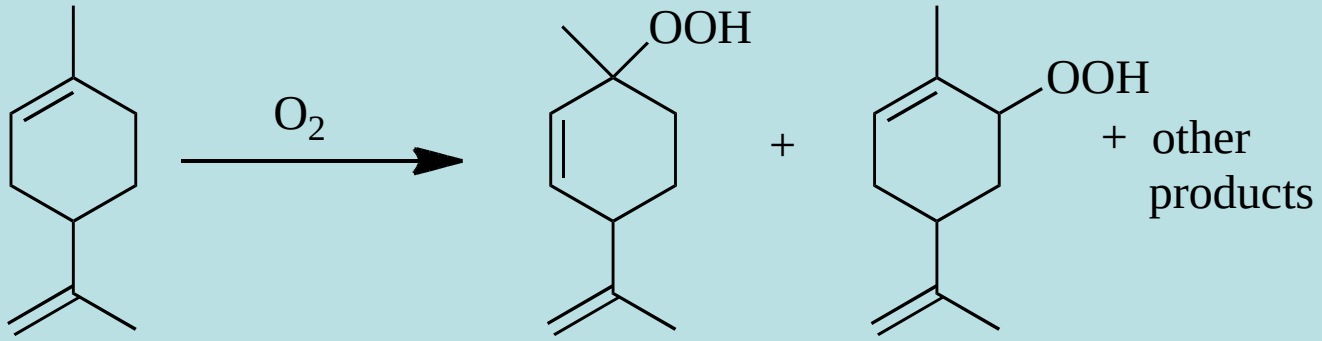
Total maximum hydroperoxide level now 0.53%

Further removal of liquid

Tank level	Max. % oxidation products
Half full	0.39
1/4 full	0.53
1/8 full	0.67
1/16 full	0.71

What does this mean for potency?

Limonene autoxidation



EC3, 0.33%
pEC3, 2.6

EC3, 0.83%
pEC3, 2.2

Worst case assumptions:

Only these hydroperoxides, no decomposition, fully cross-reactive, EC3 = 0.33%

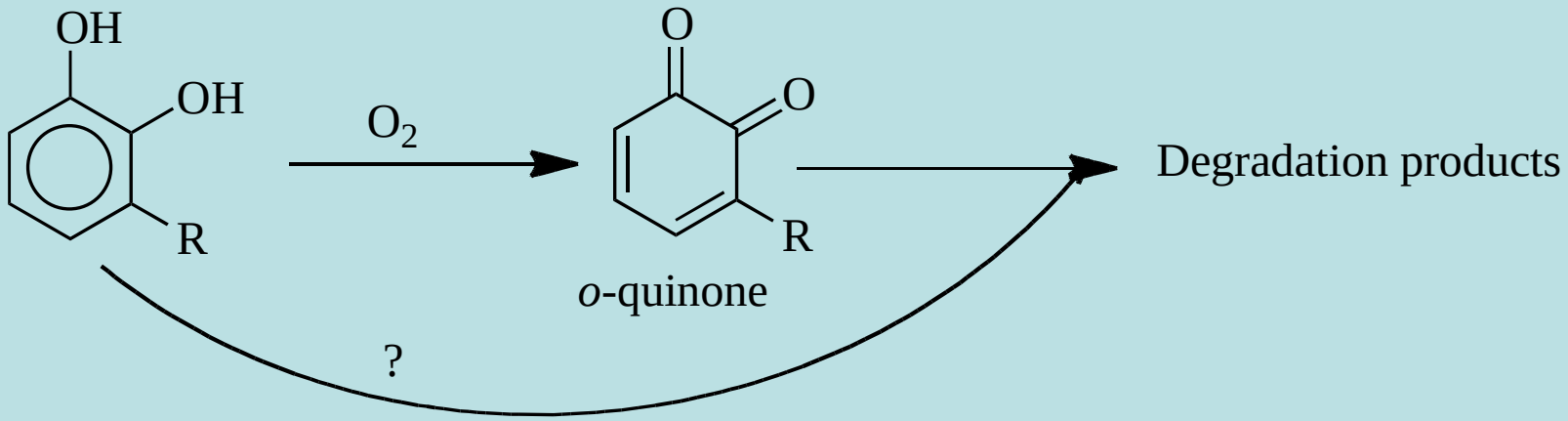
Prolonged storage, occasional removal of liquid

Tank level	Max. % oxidation products	EC3 of air-exposed limonene
Half full	0.39	85%
1/4 full	0.53	62%
1/8 full	0.67	49%
1/16 full	0.71	46%

Fast reaction, O_2 mass-transfer limited, short-lived reactive allergen

Example – poison ivy as a pre-hapten

Oxidised to a short-lived ortho-quinone – protein reactive



d[quinone]/dt = $k_1[O_2]_{air}$[AESA/V] – k_2[poison ivy][quinone] = 0 at steady state

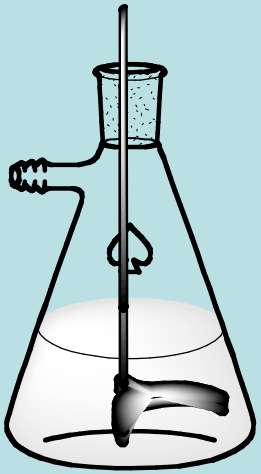
AESA = air exposed surface area; V = volume

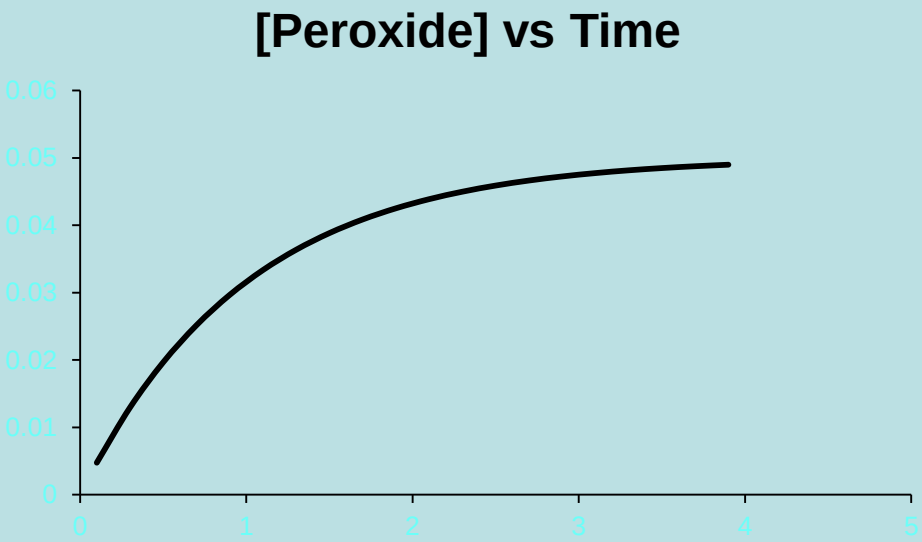
Steady state concentration of quinone = (k_1/k_2) $[O_2]_{air}$[AESA/V]/[poison ivy]

Slow reaction, through current of air

$[O_2]$ remains steady at ca. 20 mmol/L

$d[ROOH]/dt = k_1[O_2][RH] - k_2[ROOH] - k_3[ROOH][RH]$





Mixture chemistry and kinetics

In mixtures and formulations there is competition for O_2, and some components will react more readily than others with hydroperoxides

How competitive are aldehyde O=C-H against allylic C=C-C-H?

How competitive are, e.g., limonene and linalool for O_2?

Relative reactivities of limonene peroxides and linalool peroxides in epoxidation of linalool and limonene?

Mixture potency considerations

If several allergens are present, to what extent is their potency:

Additive or…independent

By analogy with mixture toxicity in ecotox:

If compounds A, B, C…are fully cross-reactive, potency is additive: $(1/EC3)mix = f_A/EC3_A + f_B/EC3_B + f_C/EC3_C$…

(f_A = fraction of A in mixture, etc)

If they aren't cross-reactive, $EC3_{mix} = EC3_A/f_A$ where A is the component closest to its EC3

Esters, R^1-CO.O-R^2

Depending on R^1 and R^2 the -**CO.O-** group may:

Be directly electrophilic – acyl transfer agent

Activate reaction of a group in R^1

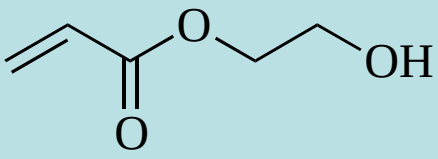
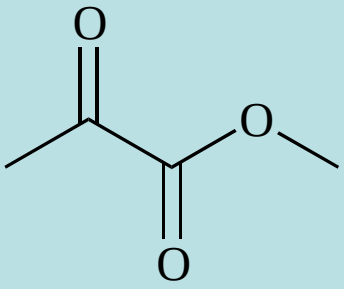
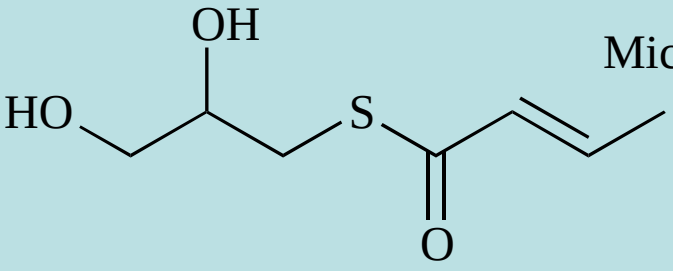
Be involved in reaction in R^2 (S_N2 leaving group)

Get hydrolysed:

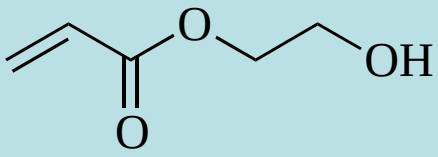
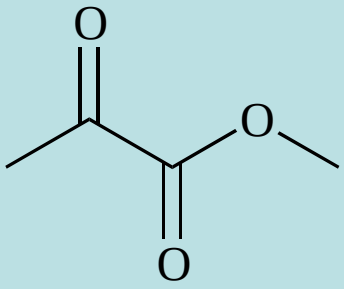
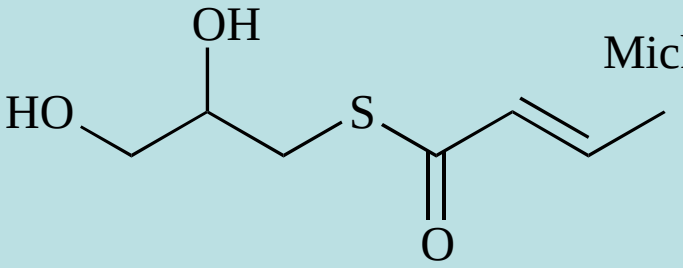
Releasing an allergenic R^2OH, or…

Losing reactivity in R^1, losing acyl transfer reactivity

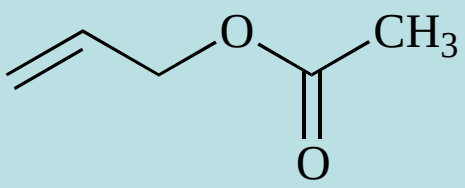
Some esters

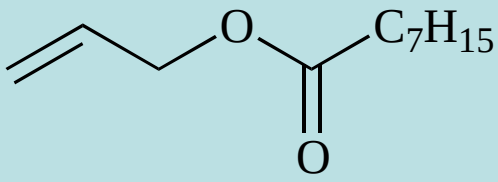
Ester	Mechanistic domain
	Michael acceptor
	Schiff base
	Michael acceptor, acyl

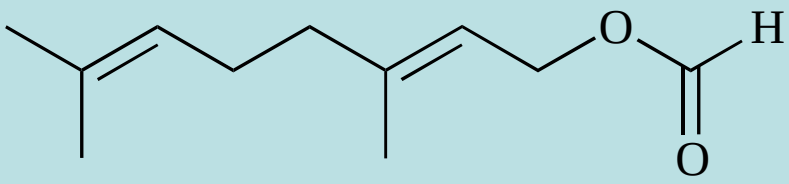
Some esters

Ester	Mechanistic domain	EC3 (%)
	Michael acceptor	1.4
	Schiff base	2.4
	Michael acceptor, acyl	NS

Some more esters

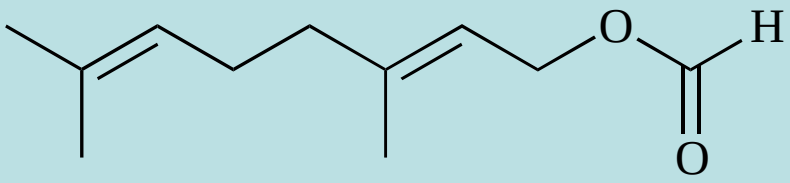


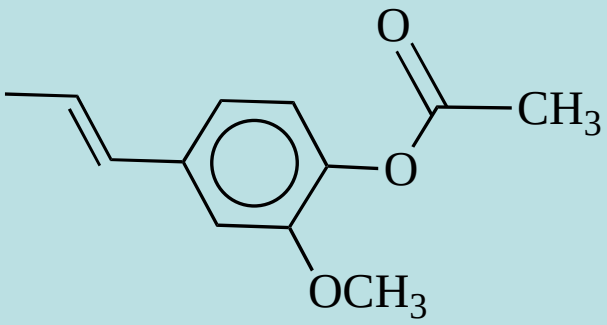


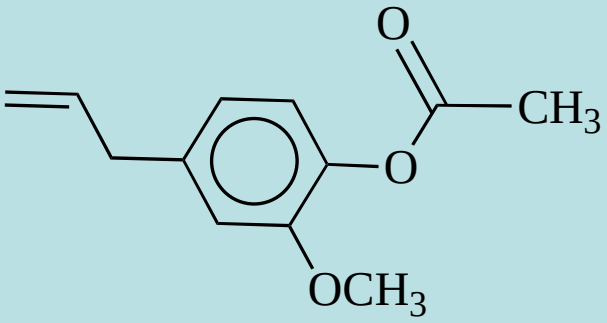


	Mechanistic domain	EC3 (%)
	SN2	NS
	SN2	6.4
	SN2, pro-geraniol	NS

And two more







	Mechanistic domain	EC3 (%)
	SN2, pro-geraniol	NS
	Acyl, pro-isoeugenol	NS
	Acyl, pro-eugenol	NS

Key knowledge gaps – as I see it

Extent of oxidation that is likely in common practice: storage/handling of "pure" materials

Levels of potent sensitizers formed in model "typical" formulation mixtures in realistically simulated manufacturing, handling and storage conditions

Mixture chemistry, relative rates, relative potencies.

Mixture toxicity as applied to skin sensitization

- Cross- reactive
- Non-cross reactive

Relative rates of oxidation of "classical" prehaptens vs other fragrance ingredients (eg aldehydes)

Stability of key hydroperoxides etc.

RECENT DEVELOPMENTS IN STRUCTURE-ACTIVITY RELATIONSHIPS FOR SKIN SENSITISATION FOR NON-ANIMAL BASED PREDICTION OF ALLERGENIC POTENTIAL

David W Roberts

Liverpool John Moores University

School of Pharmacy and Biomolecular Sciences

Liverpool, UK

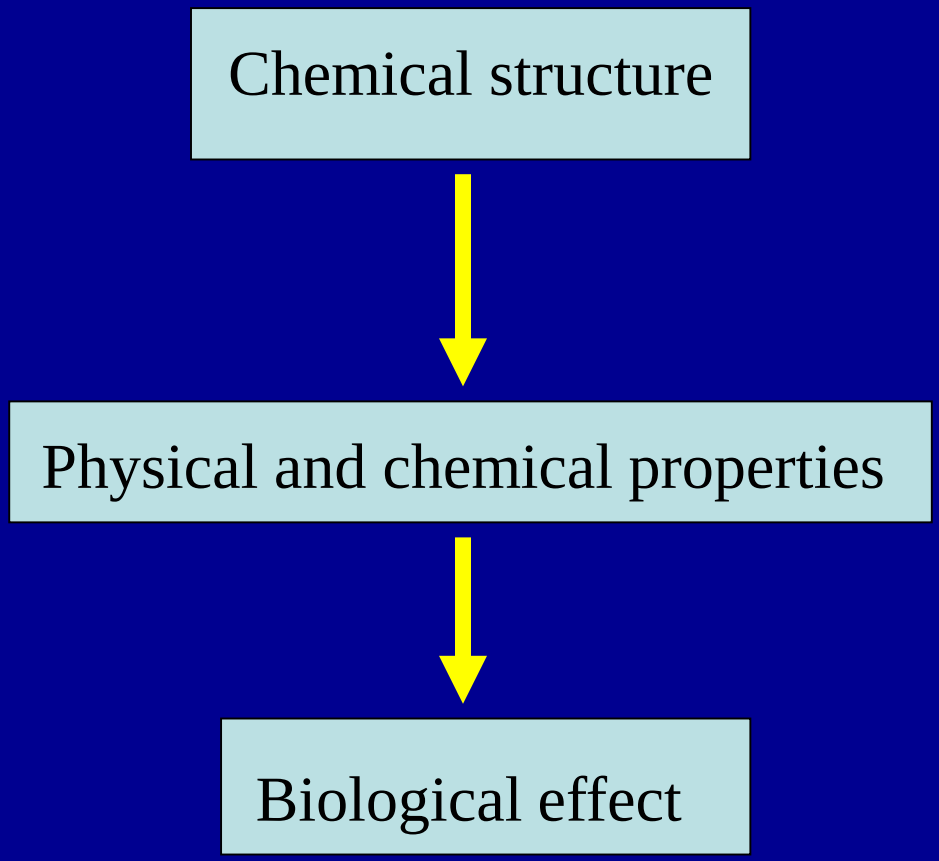
What this is about

Quantitative Mechanistic Modelling (QMM), i.e.

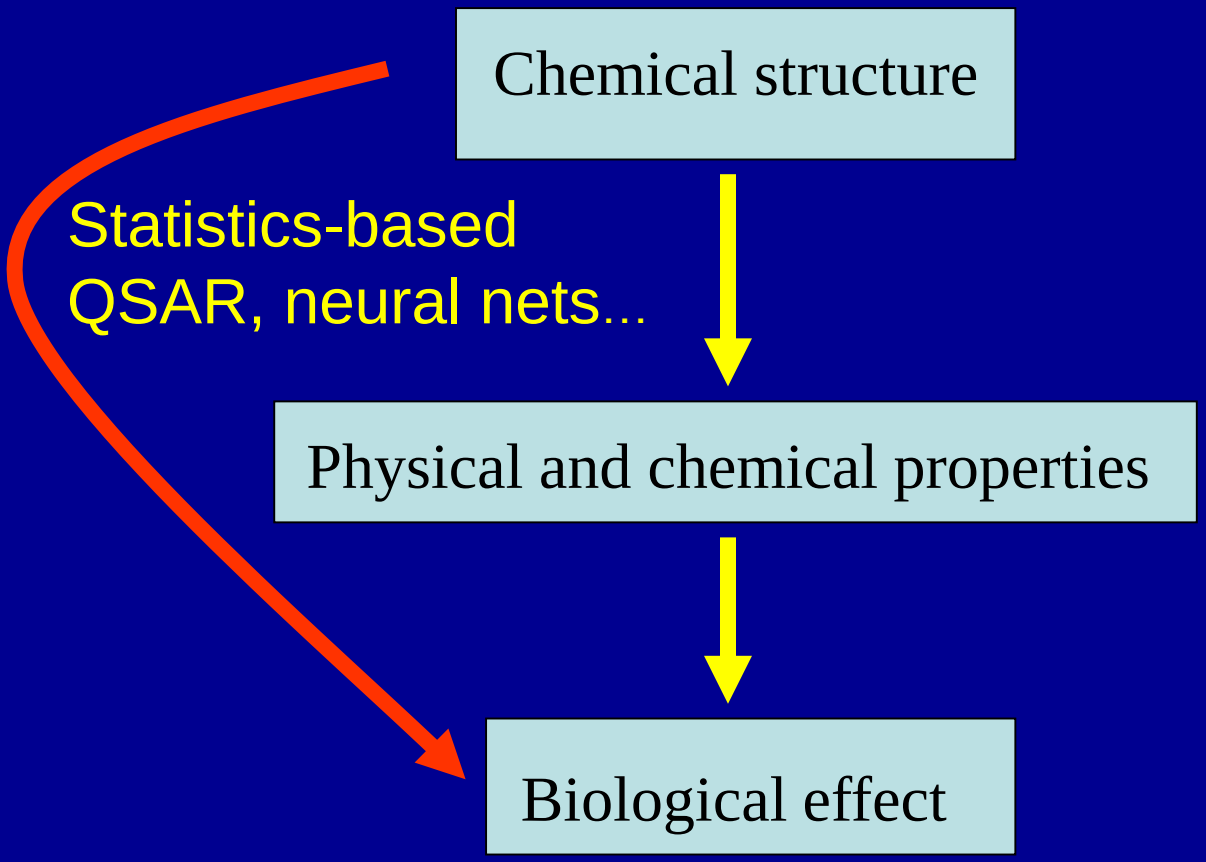
How can we use chemistry to decide if a chemical:

- is a sensitizer or not
- how potent it is, if at all

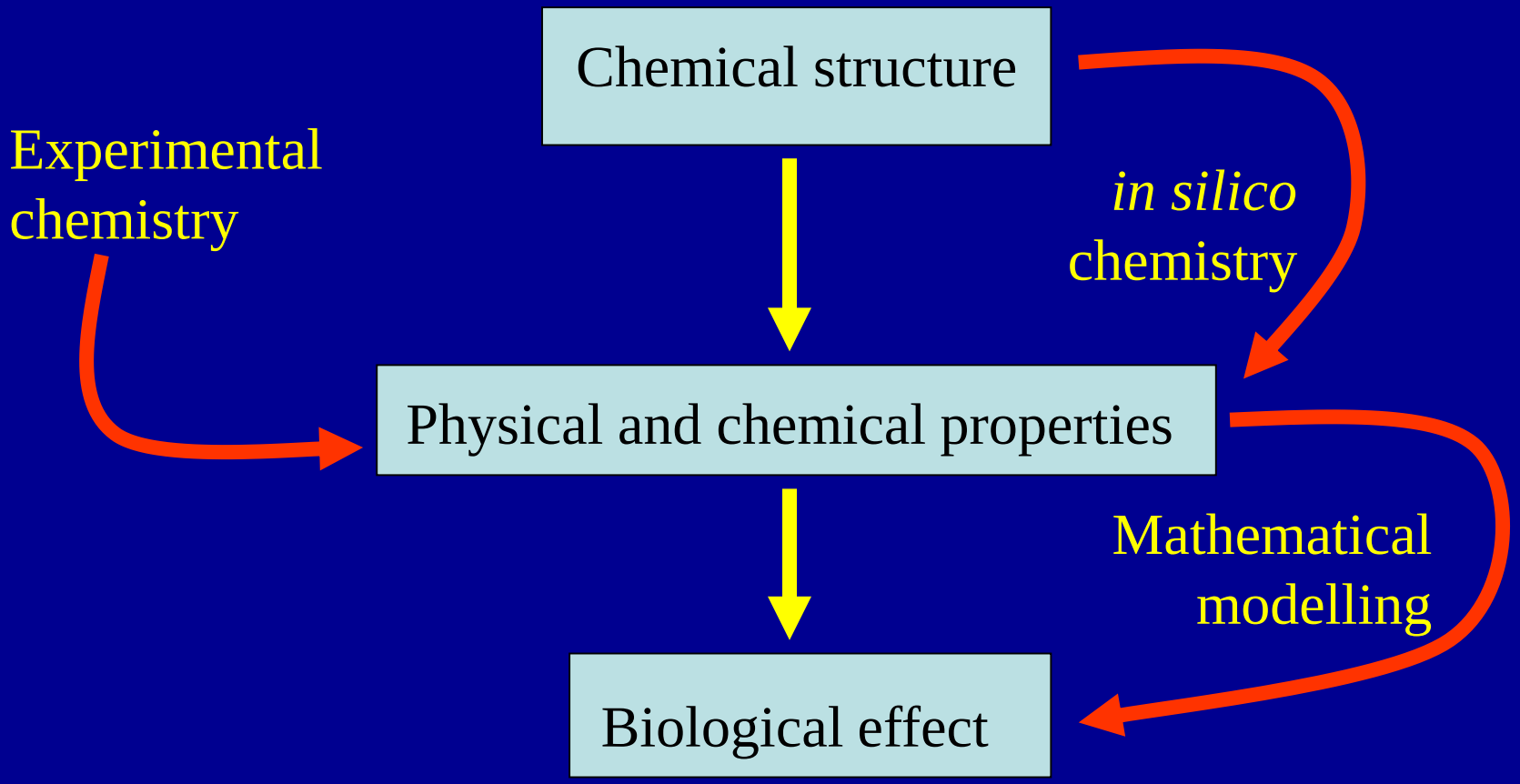
Predictive Modelling



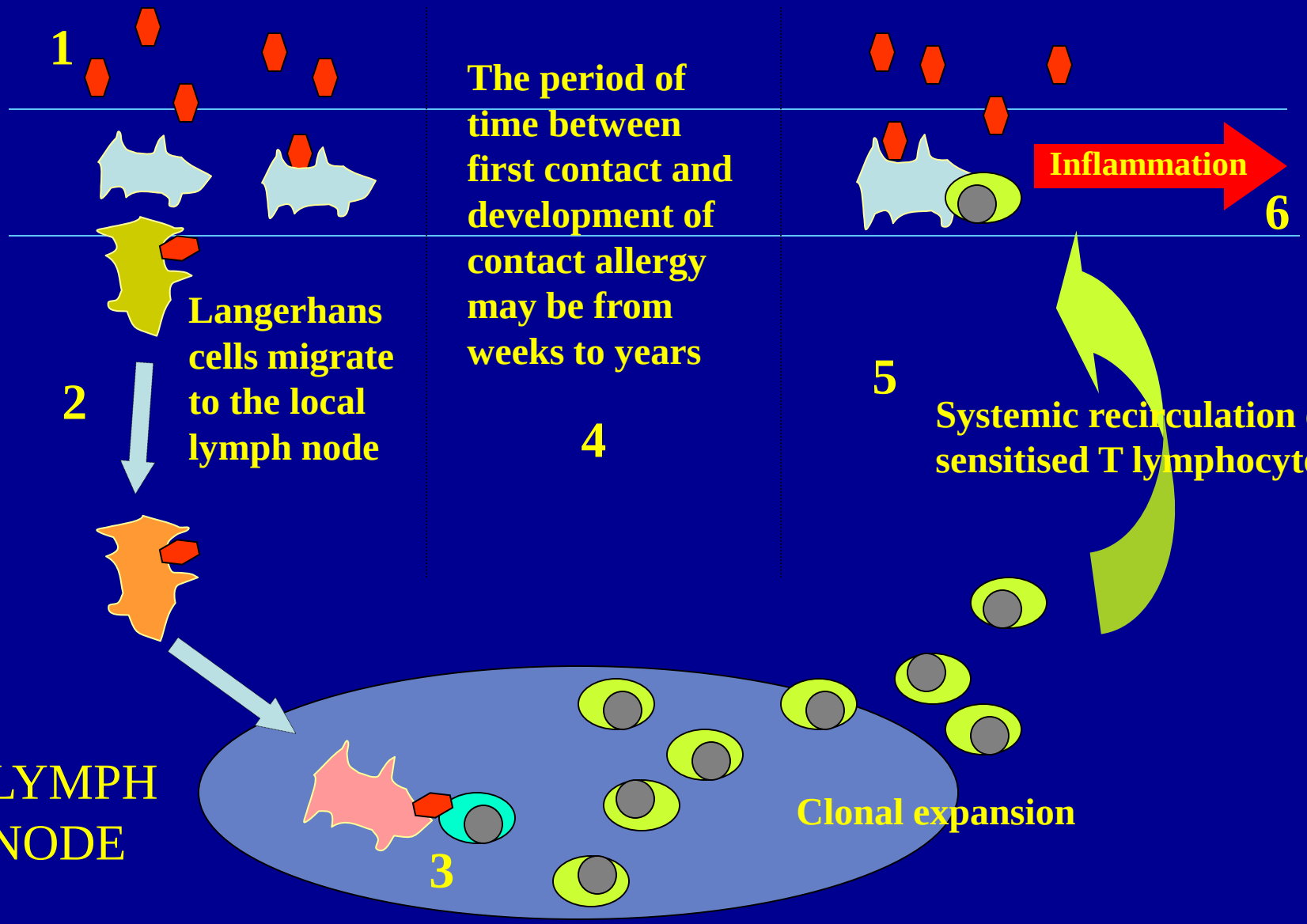
Predictive Modelling – no mechanistic insight



Predictive Modelling – Mechanism-based



SKIN SENSITIZATION - what do we know?



Current preferred animal test

Mouse local lymph node assay – LLNA

- Apply test chemical, in vehicle, to skin (ear)
- Inject tritiated thymidine (in tail)
- Excise local lymph node, measure Th uptake
- Potency quantified by EC3:
  - Concentration giving 3x increase in Th uptake compared to controls

Binding to carrier protein

Extensive evidence dating back to 1930s

For compounds in the same reaction mechanistic domain QMMs based on reactivity and hydrophobicity can be developed

Bio-activation and abiotic-activation can be important

Reaction Mechanistic Applicability Domains

Michael acceptor

S_NAr

S_N2

Schiff base

Acyl transfer

S_N1

Free radical

} Contain sensitizers and non-sensitizers

Non-reactive, non-proreactive — non-sensitizers only

What protein or peptide?

- At least 2 types

1. Cytosolic, modelled by cysteine peptide

2. Membrane-bound, modelled by lysine peptide

- 1. modelled by reactivity alone

- 2. also depends on hydrophobicity

Why we can get by without knowing the identity of the in cutaneo carrier proteins

LFER principles apply

Swain-Scott relationship: $k_{rel} = ns$

n = nucleophilicity; s = susceptibility to change in n

So one nucleophile can model another…

…but only if it is the same for all the electrophiles

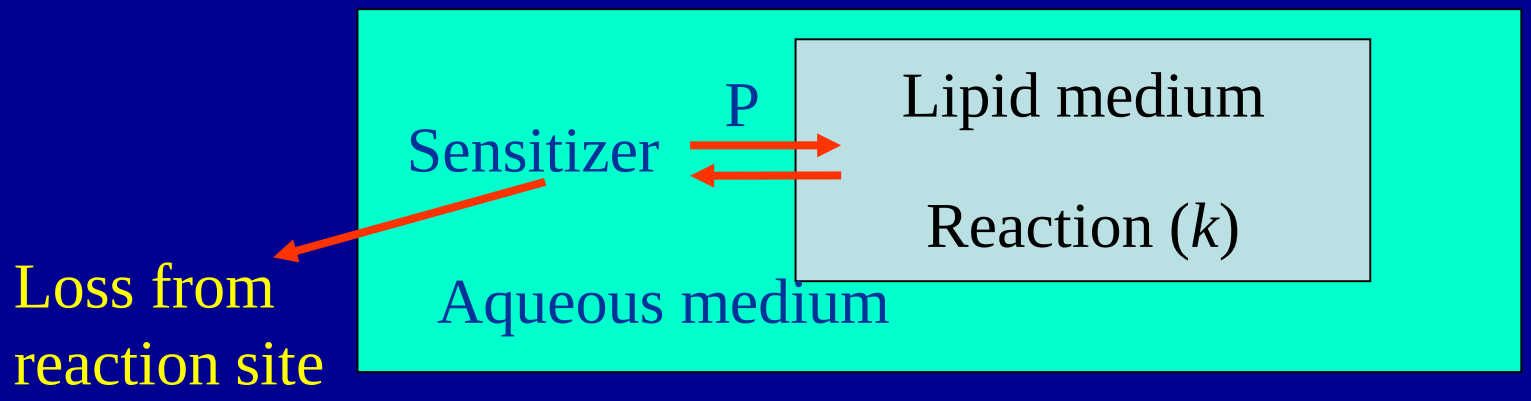
Why we have to keep within one mechanism

s varies between different mechanisms

HISTORY

Making it quantitative

The RAI Model



Reaction (protein alkylation) depends on dose D, on P, on *k*

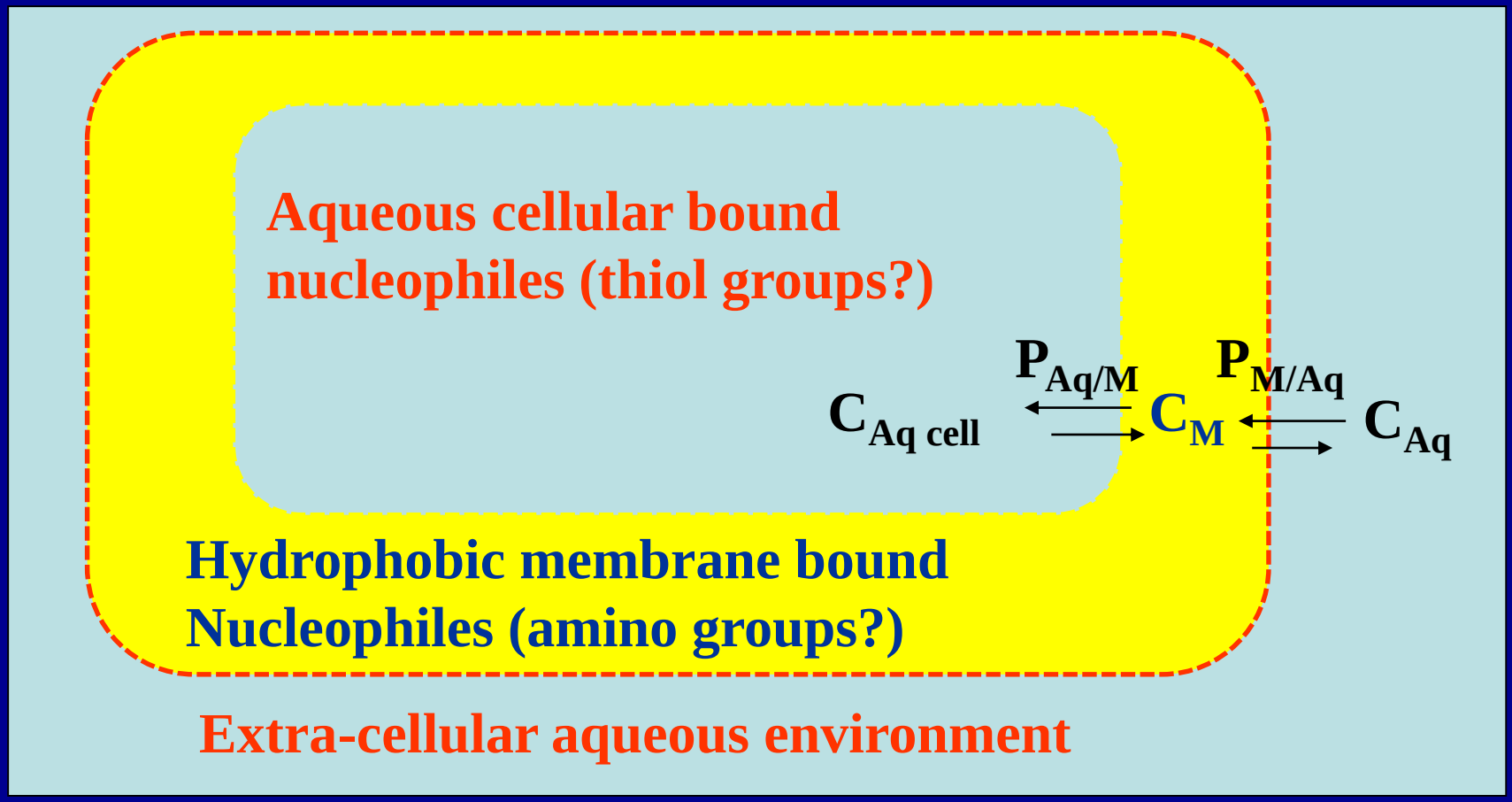
Relative alkylation index = RAI = $\log[Dk_{rel}/(P+P^2)]$

Model for P: (MeOH +H_2O)/hexane Model for *k*: $BuNH_2$ kinetics

Good DR/QSARs for GP data.

Gives general potency model: potency = a log k + b $\log P_{o/w}$ + c

Double reaction site basis for new RAI model

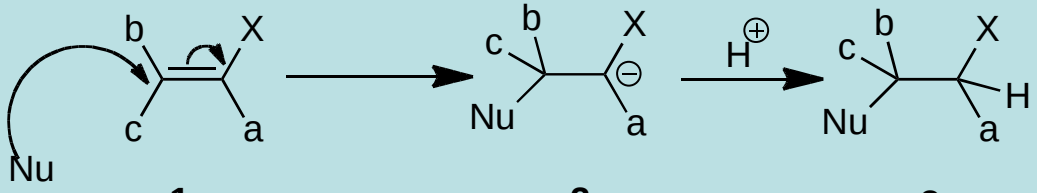


Thiol: $C_{Aq\ cell} \approx C_{Aq}$ $\therefore$ sensitisation ← reactivity only

Amino: $C_M = C_{Aq} \times P_{M/Aq}$ $\therefore$ sensitisation ← reactivity + hydrophobicity

Michael acceptor domain

Michael acceptors



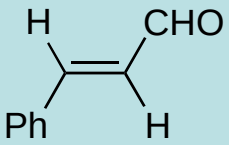
1 Michael acceptor

2 Intermediate

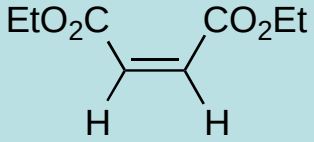
3 Adduct

X = electronegative group capable of stabilising negative charge in intermediate **2**. Reactivity depends on X and on effects of substituents a, b and c on stability of **2**.

Examples



Cinnamic aldehyde
EC3 = 3.1%

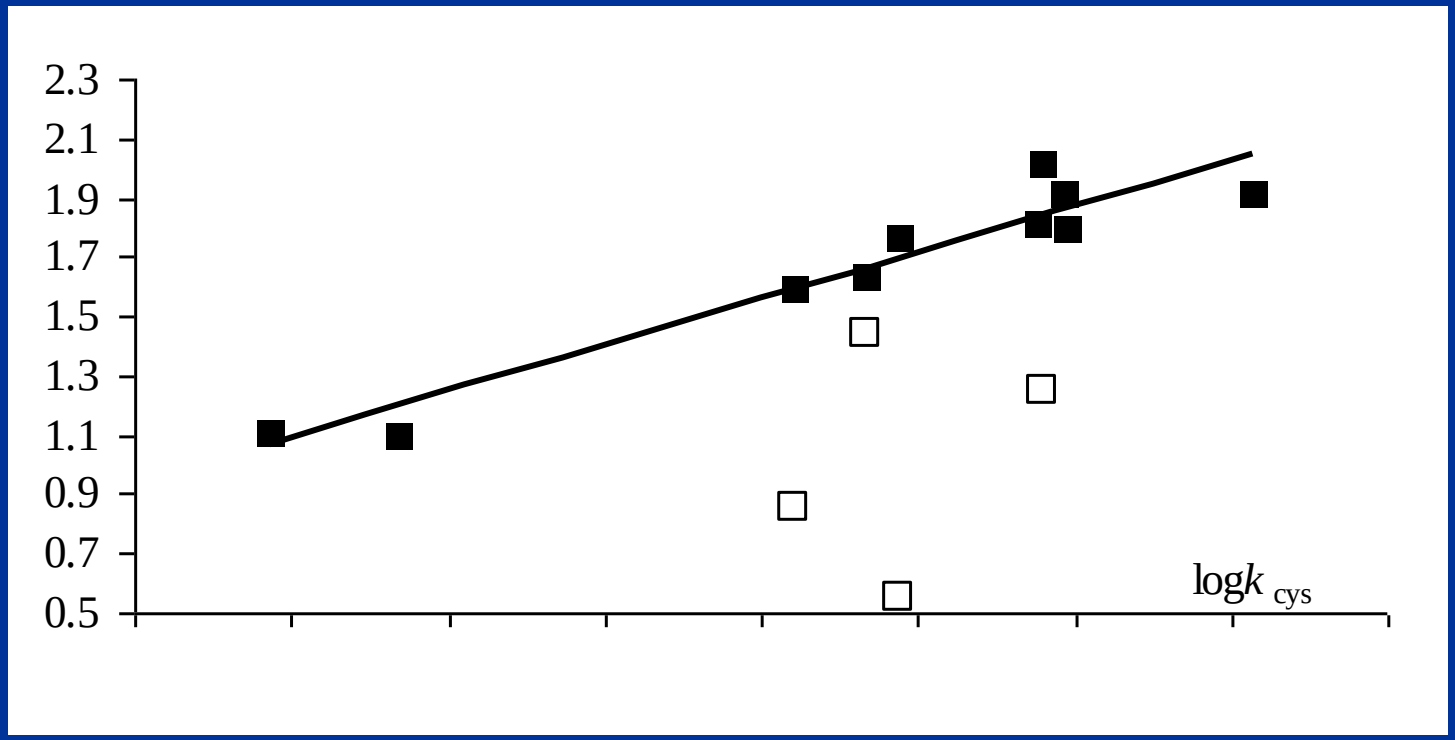


Diethyl maleate
EC3 = 5.8%

Benzoquinone
EC3 = 0.01%

QSAR for Michael Acceptor domain

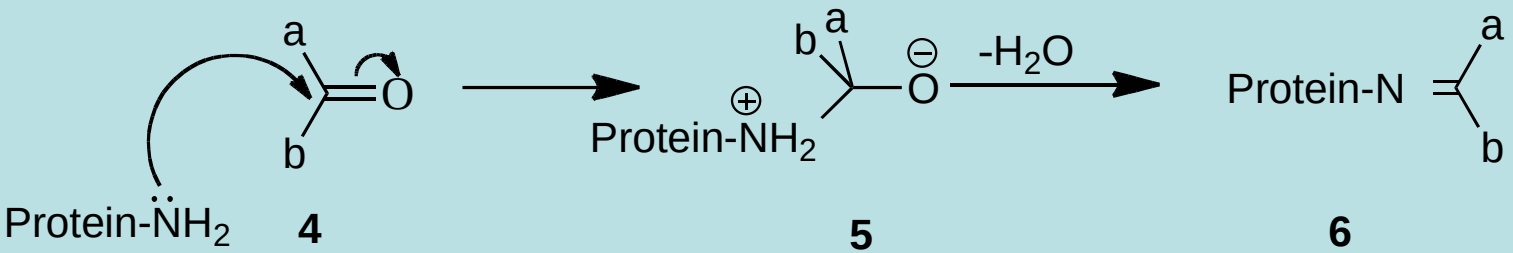
pEC3 vs log*k* for reaction with cysteine-based peptide



pEC3 = 0.24 log*k* + 2.11 n = 10, R^2 = 0.836, s = 0.11, F = 40.8

Schiff base domain

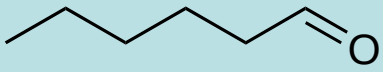
Schiff base electrophiles



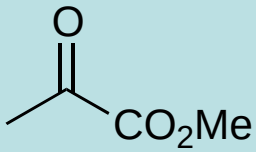
SB Electrophile — Intermediate — Schiff base

Reactivity depends on inductive effects of groups a and b: electronegative groups stabilise the negative charge in intermediate **5**.

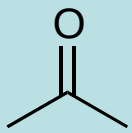
Examples



Hexanal,
EC3 = 45%

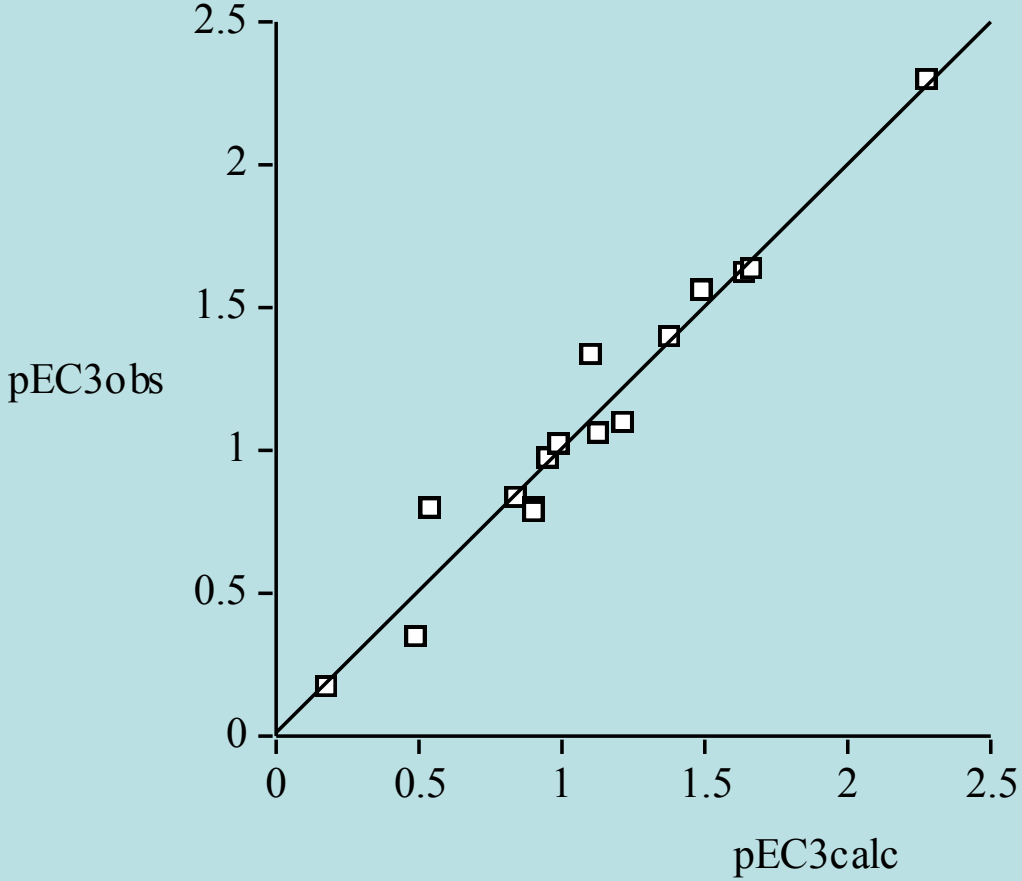


Methyl pyruvate,
EC3 = 2.4%



Acetone,
non-sensitizer

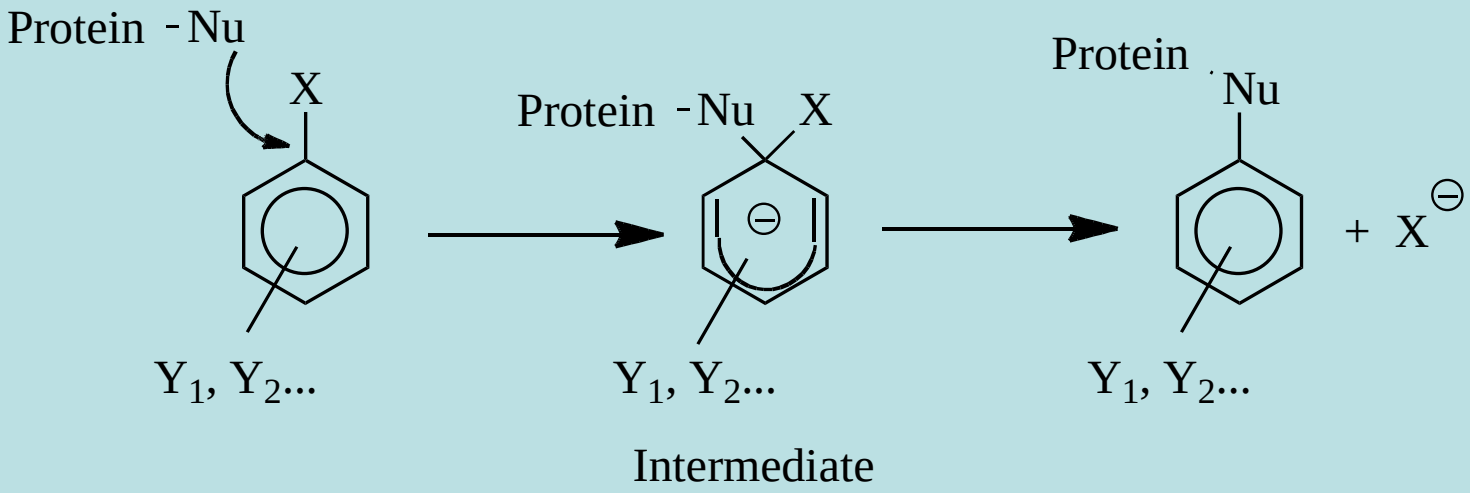
Schiff base mechanistic domain



$pEC3 = 1.12(\pm 0.07)\ \Sigma\sigma^* + 0.42(\pm 0.04)\ \log P - 0.62(\pm 0.13)$

$n = 16\ R^2 = 0.952\ R^2_{adj} = 0.945\ s = 0.12\ F = 129.6$

S_NAr domain



Reactivity depends on stabilization of negative charge in the intermediate:

By the X group – inductive effect, modelled by σ^*

By the Y groups –resonance + inductive effect, modelled by $\Sigma\sigma^-$

HISTORY

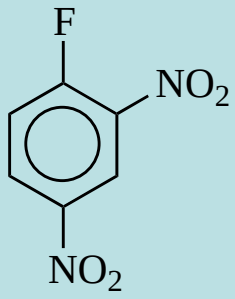
Lansdsteiner and Jacobs 1930s

20 Aromatic NO_2/Hal compounds

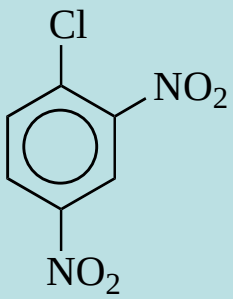
		Sensitizer (GP)	
Aniline reaction		Yes	No
	Yes	10	0
	No	0	10

Covalent modification of proteins model

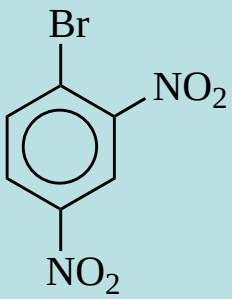
S_NAr domain



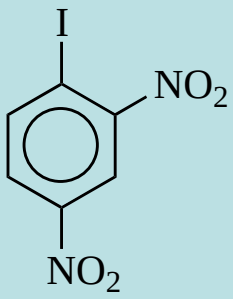
1, DNFB



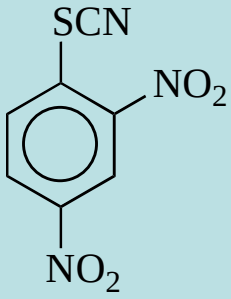
2, DNCB



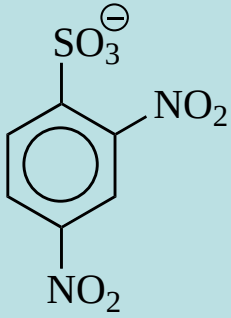
3, DNBB



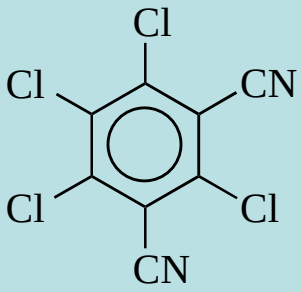
4, DNIB



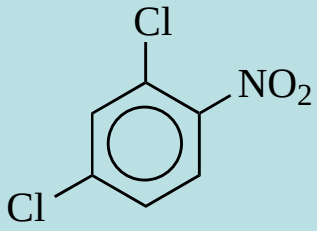
5, DNTB



6, DNBS

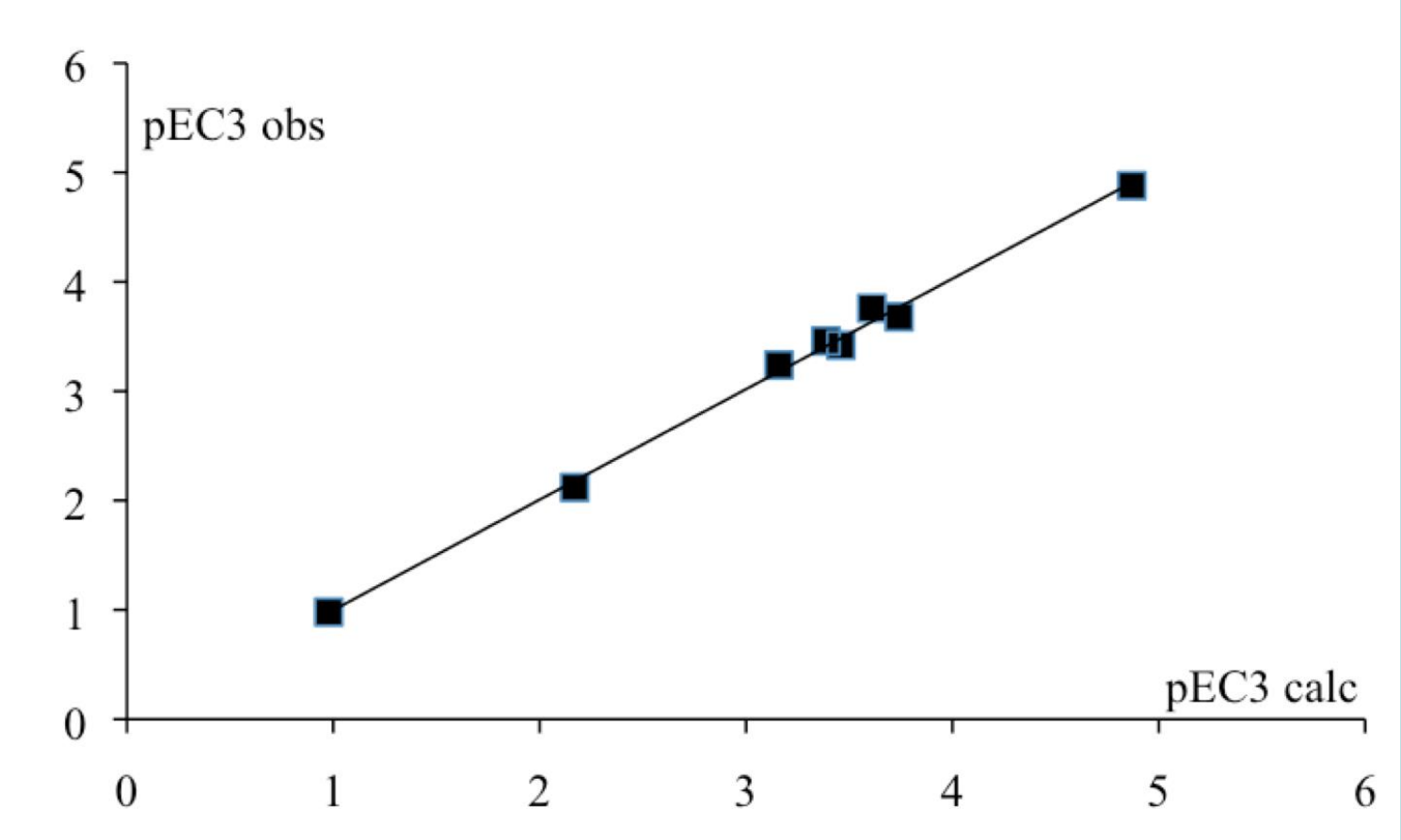


7, TCPN



8, DCNB

S_NAr domain QMM



pEC3 = 2.50 $\Sigma\sigma^-$ + 0.57 σ^* – 4.52, n = 8, R^2 = 0.984, s = 0.16, F = 365

The *in chemico* approach

If we know enough about the chemistry we can predict the sensitisation potency (or lack of)

What we need to know:

- How (if) it reacts – reaction mechanistic domain
- How reactive it is – rate constant or equivalent
- How hydrophobic it is – partition coefficient

Reaction Mechanistic Applicability Domains

Michael acceptor	Reactivity only (LLNA)
S_NAr	Reactivity only (LLNA)
S_N2	Reactivity + hydrophobicity (LLNA)
Schiff base	Reactivity + hydrophobicity (LLNA)
Acyl transfer	
S_N1	
Free radical	
Non-reactive, non-proreactive	non-sensitizers only

Testing Without Animals

Presented with a new chemical:

1. Classify it into its reaction mechanistic domain
2. Quantify its reactivity/hydrophobicity relative to known sensitizers in the same domain

3. Use mechanism-based QSAR to predict potency

1 and 2 can sometimes be done form inspection of structure. If not, experimental chemistry data needs to be generated (no animals are harmed)

3 can only be done if a QSAR exists for the new chemical's mechanistic domain

What can already be done

Presented with a new chemical:

Using SAR

- likely to be a sensitizer (identification of reactivity alerts), or…
- likely to be a non-sensitizer (absence of reactivity alerts), or…
- can't predict (unfamiliar features) by inspection – experimental chemistry needed

Using QSAR

- can predict LLNA potency from structure, or…
- need experimental chemistry parameters (eg rate constants), or…
- can't predict (no QSAR for this type of chemical) – but SAR-based read-across may sometimes be able to give a semi-quantitative estimate

What we still need

Kinetics for SB domain

Better predictive capability for pro-electrophiles

Some difficult types:

aliphatic amino groups

multifunctional aromatics

epoxidisable (or not?) olefins and aromatics

Cell based assays

THE VISION
Testing Without Animals

Presented with a new chemical:

1. Classify it into its reaction mechanistic domain
2. Quantify its reactivity/hydrophobicity relative to known sensitizers in the same domain
3. Use mechanism-based QSAR or mechanistic read-across to predict potency

Apply in tandem with *in vitro* assays when available