Toxicokinetic insights into the formation and inactivation of haptens

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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)

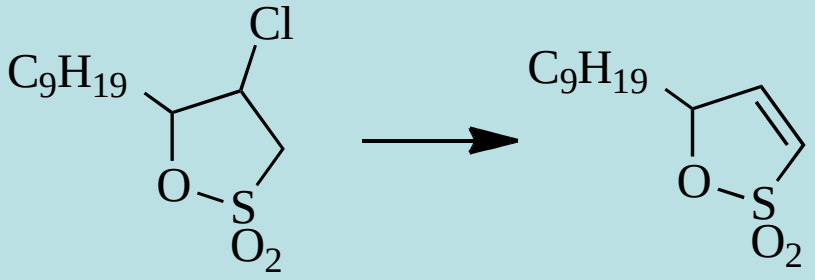
Activation reactions

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

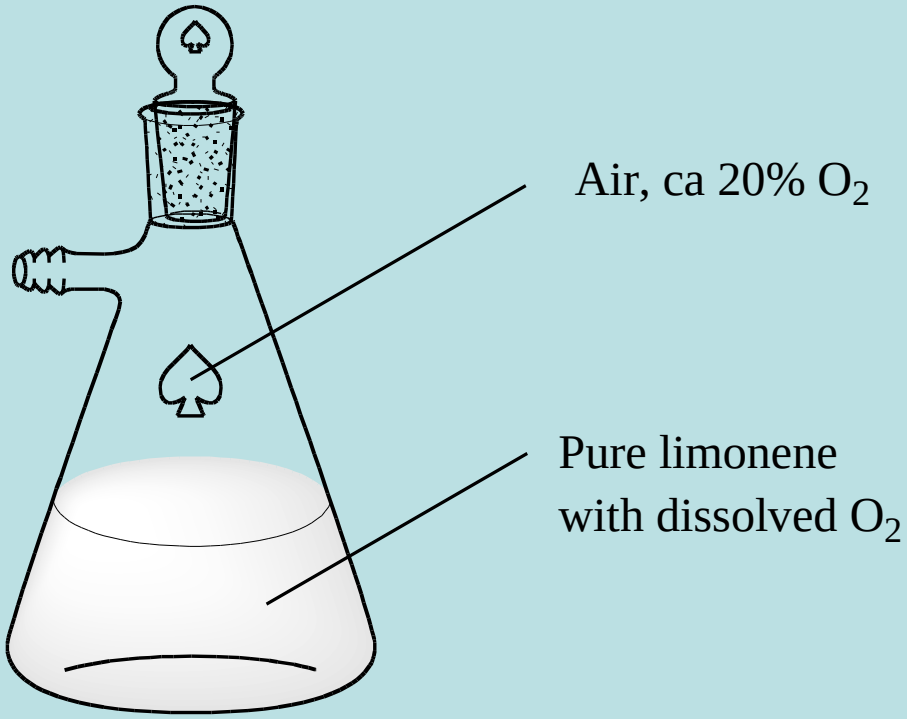
Limited by stability of allergenic autoxidation products

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%

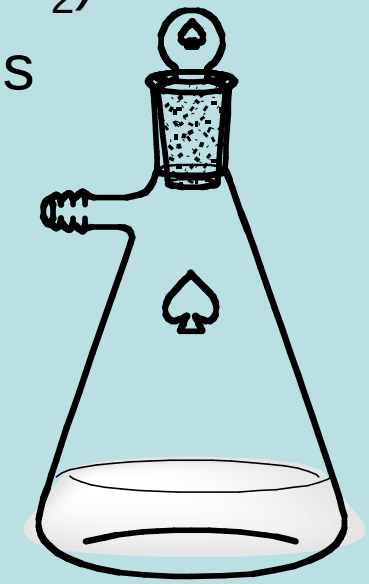
Total maximum hydroperoxide level, 0.39%

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



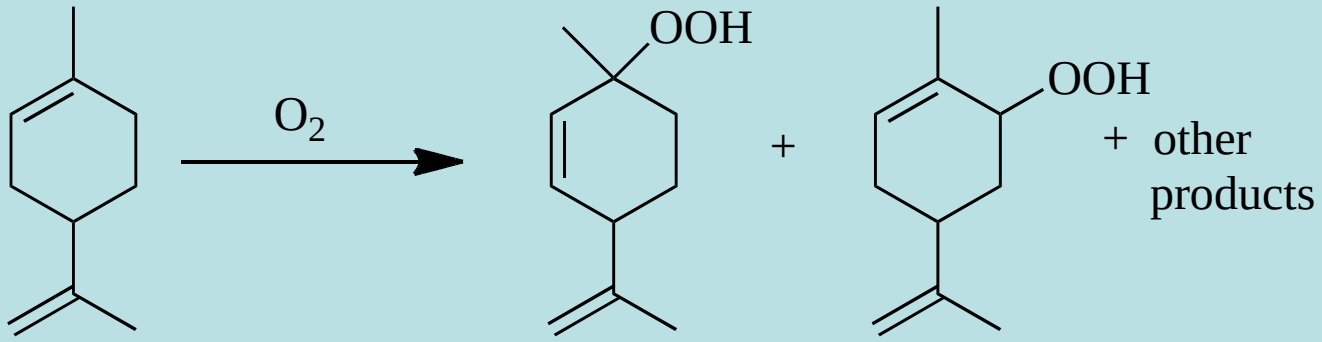
Total maximum hydroperoxide level now 0.53%

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%

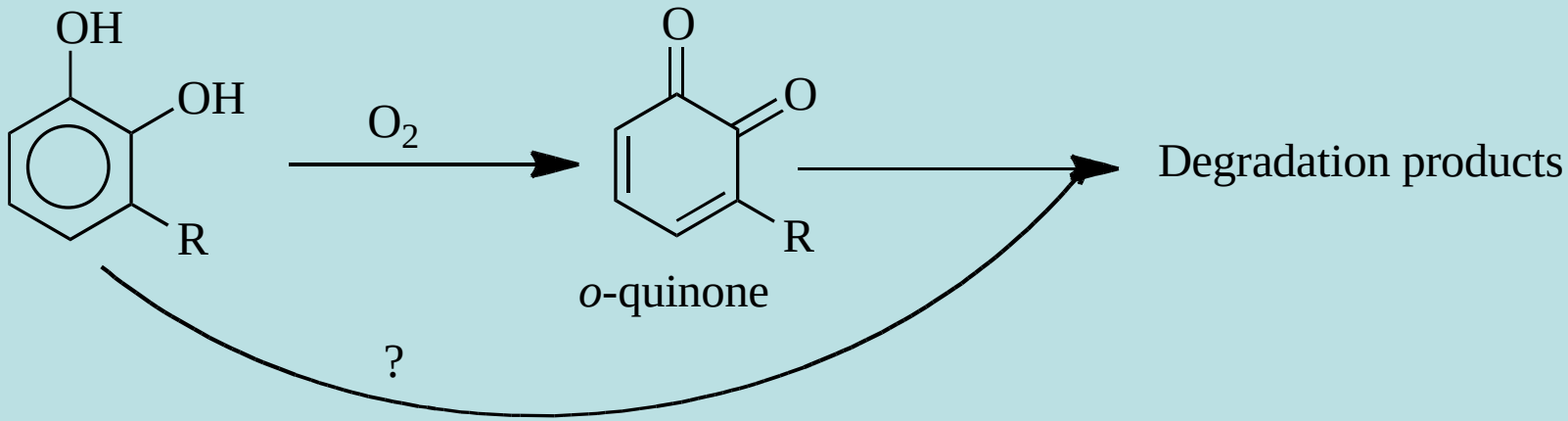
Prolonged storage, occasional removal of liquid

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

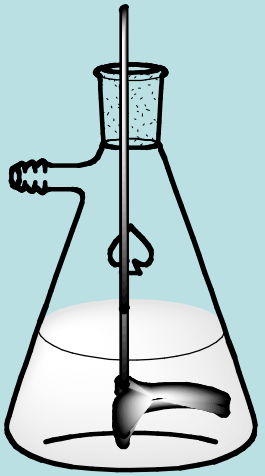
AESA = air exposed surface area; V = volume

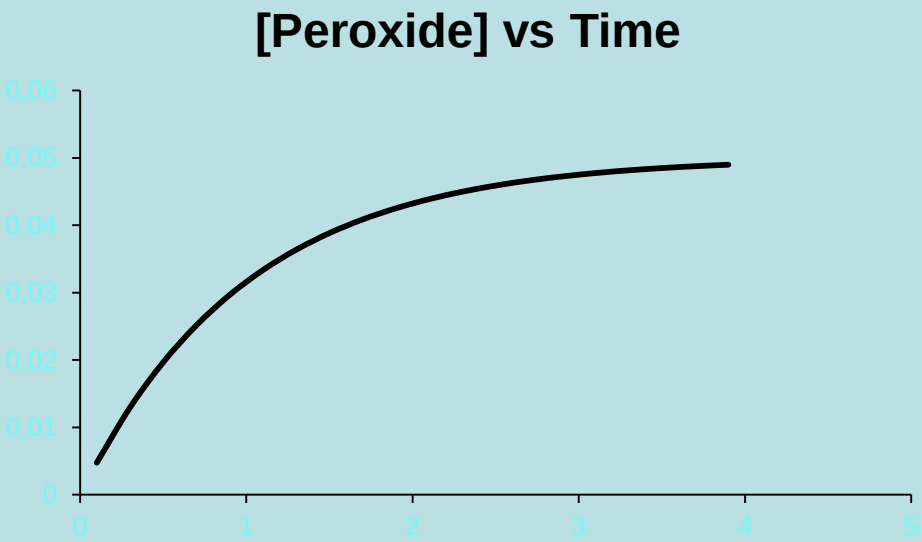
Steady state concentration of quinone = (k_1/k_2) $[O_2]_{air}$[AESA/V]/[poison ivy]

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

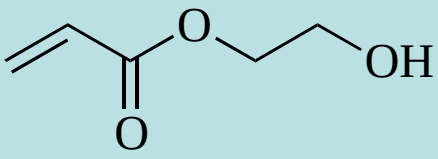
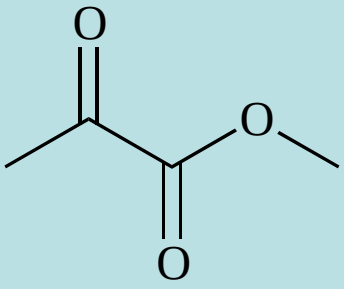
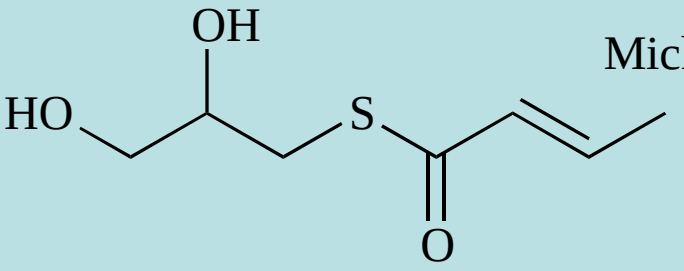
Be involved in reaction in R^2 (S_N2 leaving group)

Get hydrolysed:

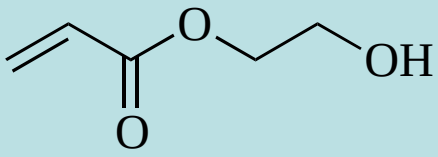
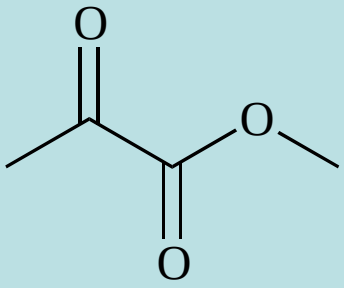
Releasing an allergenic R^2OH, or…

Losing reactivity in R^1, losing acyl transfer reactivity

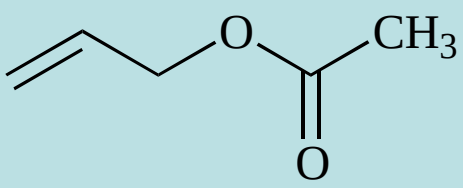
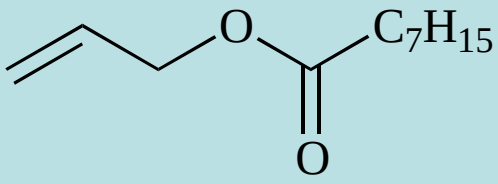
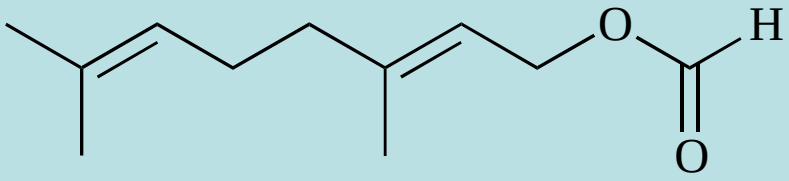
Some esters

Ester	Mechanistic domain
	Michael acceptor
	Schiff base
	Michael acceptor, acyl

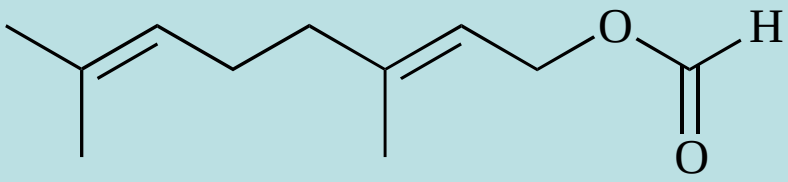
Some esters

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

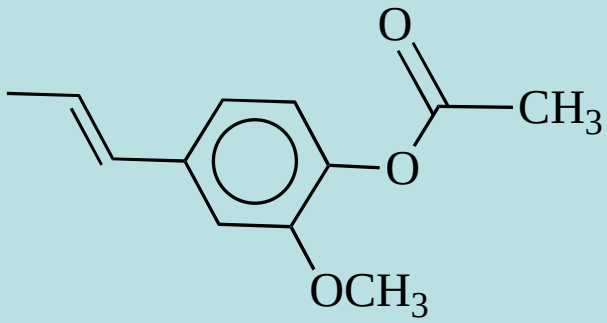
Some more esters

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

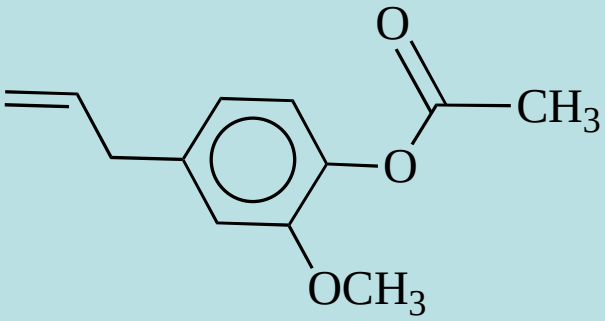
And two more



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



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



Mechanistic domain	EC3 (%)
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.