IDEA

International Dialogue for the Evaluation of Allergens

State of knowledge on abiotic hapten formation (hydrolysis) using examples of fragrance ingredients and state of the art on the technical management of those transformations

1. Chemistry and Theory

Hypothesis: "Levels of the 26 fragrance allergens can increase in consumer products due to conversion from other ingredients in the fragrance"

Hydrolysis is the most likely route, where three chemical classes of Fragrance ingredients can be identified with this potential:

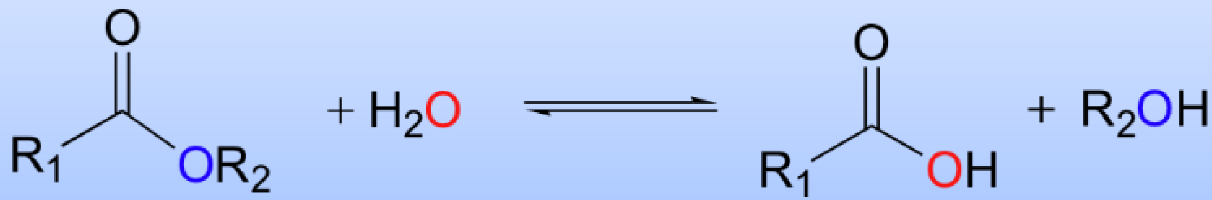
- Esters
- Acetals
- Schiff bases

} Aldehydes and alcohols (potential haptens)

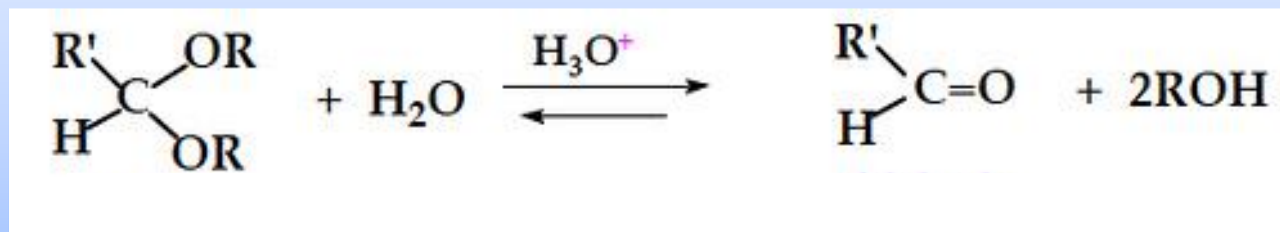
Opportunities for Hydrolysis:

- In fragrance (abiotic)
- In consumer product (abiotic)
- On Skin (abiotic/biotic)

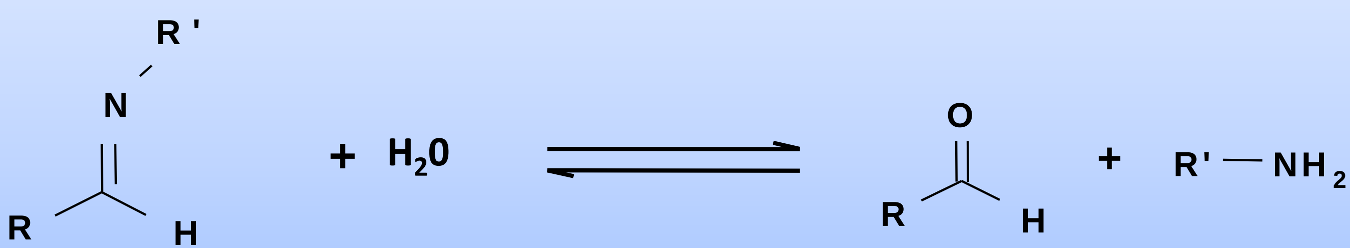
Ester Hydrolysis:



Acetal Hydrolysis:



Schiff Base Hydrolysis:



Chemical Complexity

Based on a chemical appraisal of the perfumers palette, 124 chemicals were identified as having potential to form one or more of the original "list of 26" as a result of abiotic hydrolysis

124 CHEMICALS

Rate of Hydrolysis will depend on chemistry of Product Matrix, e.g:

pH
Water activity
Catalysts
etc...

…and physical conditions of storage, e.g:

Temperature

…. as a function of time

Higher levels of the 26

2. Analytical Evidence for Hydrolysis in Real Systems

Rationalisation of Product matrices

10 commercial Cosmetic product bases were selected as a representative cross section

Un-fragranced product bases were manufactured at pilot-scale

- Soap based Toilet Bar
- Non soap based Toilet Bar
- Shampoo
- Hair conditioner
- Antiperspirant deodorant
- Emulsion roll-on deodorant
- Body spray
- Aftershave
- Calcium Carbonate based Toothpaste
- Silica based Toothpaste

Rationalisation of Precursors

Four "probes" were designed (including 18 of the 124) to ensure:

- An example from most common chemical groups was included (to allow read across for those not included)
- None of the precursors in each "probe" would give rise to the same "allergen"

PROBE 1	PROBE 2
Benzyl acetate Citronellyl acetate Geranyl acetate Eugenyl acetate	alpha-amyl cinnamic aldehyde dma Benzyl salicylate Citronellyl formate Linalyl acetate Farnesyl acetate
PROBE 3	**PROBE 4**
Cinnamyl acetate Iso-eugenyl acetate Benzyl benzoate Citronellyl isovalerate Aurantiol	Citral dea Iso-eugenyl benzyl ether Terpineol extra Cinnamyl cinnamate

Table 5. Probe Compositions

Study Design

- 10 un-fragranced Cosmetic product matrices
- 4 “probes” (containing 4-5 precursors)
- Probes dosed to deliver 0.2% of each precursor
- Samples stored at 20°C and 37°C (in triplicate)
- Samples analysed at T = 0, T = 4wks and T = 12 wks
- Precursor and product (i.e., allergens) extracted and measured at each time point

The amount of allergen produced for each potential precursor in each product matrix was assigned into quartiles:

(red)	over 75% conversion to allergen measure
(orange)	50-75% conversion to allergen measured
(peach)	25-50% conversion to allergen measured
(yellow)	0-25% conversion to allergen measured

Study Results Summary

Summary of results after 12 wks @ 37C *(i.e., worst case)*

CHEMICAL GROUP	PRECURSOR	Antiperspirant pH 3.8	Emulsion roll-on pH3.8	Hair conditioner pH 3.9	Shampoo pH 4	Aftershave pH 6.1	Si toothpaste pH 6.3	Non-Soap Bar pH 7	Bodyspray pH 7.8	CaCO toothpaste pH 8.1	Soap Bar pH 10.4
ACETATES	Benzyl acetate	<5%				<5%		<5%			
	Citronellyl acetate	<5%	<5%	<5%		<5%	<5%	<5%	<5%	<5%	
	Geranyl acetate	<5%	<5%	<5%		<5%	<5%	<5%	<5%	<5%	
	Eugenyl acetate	<5%		<5%		<5%					
	Farnesyl acetate	<5%		<5%			<5%	<5%	<5%	<5%	
	Linalyl acetate	<5%						<5%	<5%		<5%
	Cinnamyl acetate	<5%	<5%	<5%		<5%			<5%		
	Iso-eugenyl acetate	<5%	<5%	<5%							
OTHER ESTERS	Citronellyl formate										
	Benzyl salicylate	<5%	<5%	<5%	<5%	<5%	<5%	<5%			
	Benzyl benzoate	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	
	Cinnamyl cinnamate	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	
	Citronellyl iso valerate	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%
ACETALS	Citral dea			<5%							
	Alpha-amyl cinnamic ald. dma										
SCHIFF BASE	Aurantiol	<5%		<5%							
ETHER	Iso eugenol Benzyl ether	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%
	Terpineol extra	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%	<5%

over 75% conversion to allergen measured
50-75% conversion to allergen measured
25-50% conversion to allergen measured
0-25% conversion to allergen measured

3. Model System Work and generation of Kinetic Constants

Experimental Design

- Hydrolysis studied in buffered Ethanol/water (70/30) media
- 5 temperature points (10°C to 60°C)
- 5 pH points (3 to 10)
- Analysis by HPLC or GC/MS

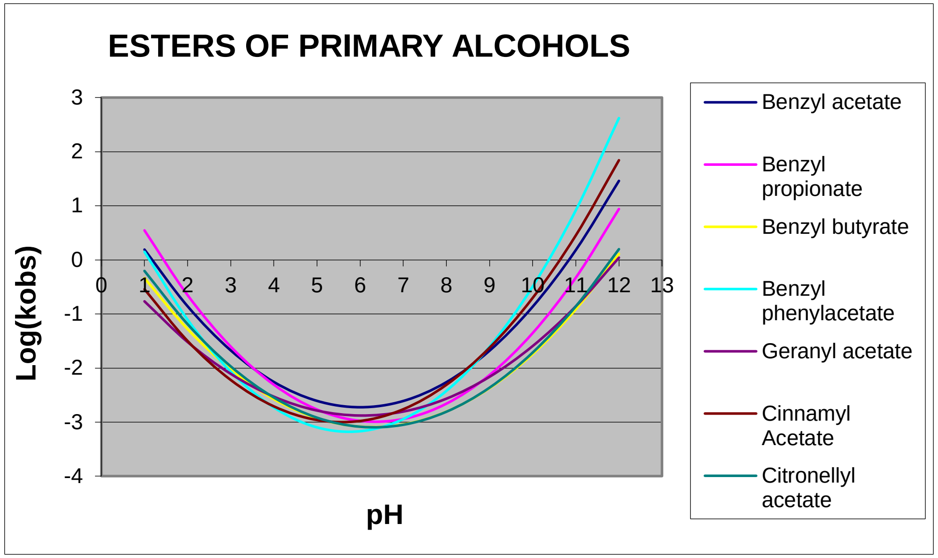
Results Summary: Half life of esters @ 50°C (days)

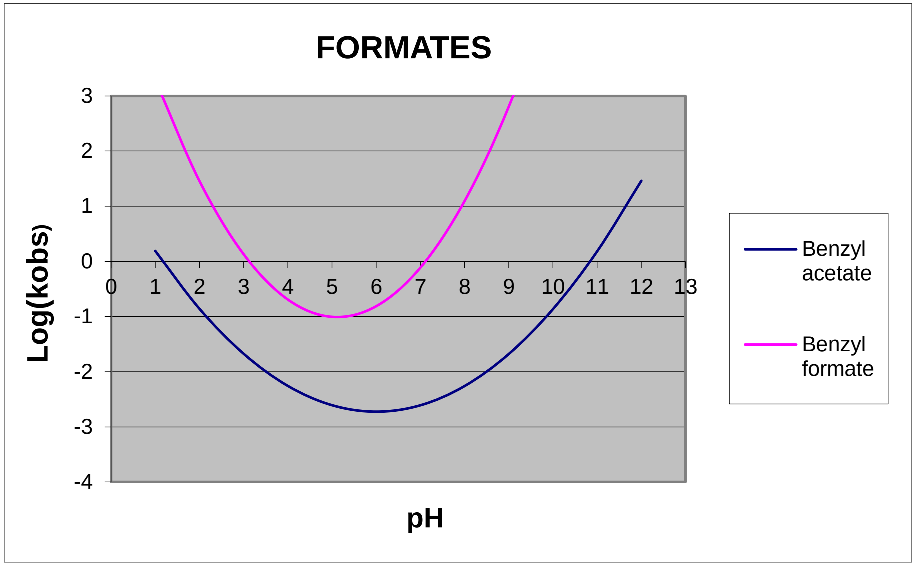
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
BENZYL FORMATE	1	3	7	4	1	0	0	0
BENZYL ACETATE	22	120	322	427	281	92	15	1
BENZYL PROPIONATE	30	143	391	620	569	303	93	17
BENZYL BUTYRATE	73	260	586	839	764	442	163	38
BENZYL ISOBUTYRATE	403	752	1090	1226	1071	727	383	157
BENZYL SALICYLATE	>>365	>>365	2975	630	133	28	6	1
BENZYL BENZOATE	> 365	> 365	> 365	> 365	> 365	736	288	113
BENZYL PHENYL ACETATE	83	389	916	1092	659	201	31	2
BENZYL TIGLATE	> 365	> 365	> 365	> 365	> 365	> 365	> 365	> 365
EUGENYL ACETATE	239	476	493	266	74	11	1	0
EUGENYL PHENYL ACETATE	727	774	529	232	65	12	1	0
ANISYL ACETATE	27	112	264	363	290	135	36	6
CINNAMYL ACETATE	115	354	632	659	400	141	29	3
CINNAMYL CINNAMATE	9377	7232	4482	2232	893	287	74	15
CITRONELLYL ACETATE	65	243	573	842	774	445	160	36
LINALYL ACETATE	8	13	18	23	26	27	25	21
GERANYL ACETATE	89	234	423	523	442	255	101	27

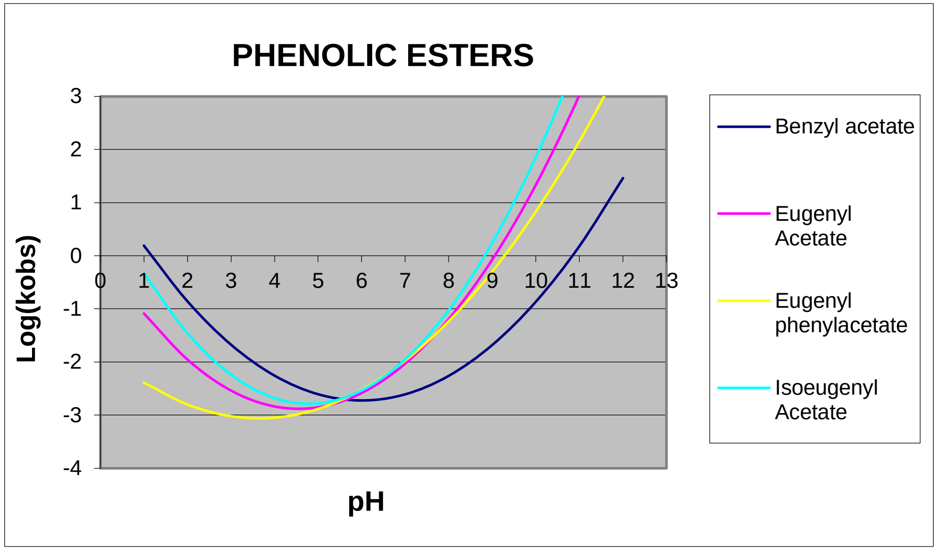
= Half life > 365 days

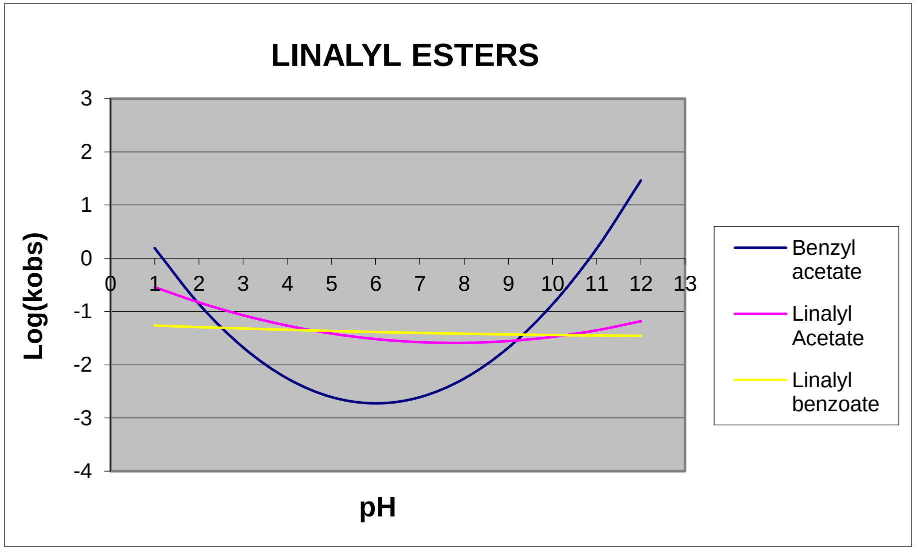
= Half life < 365 days

Extrapolated Trend Lines (Esters)









NOTE: Dark Blue line = Benzyl Acetate (reproduced on all four graphs)

Results Summary: Effect of Temperature on Ester Half life

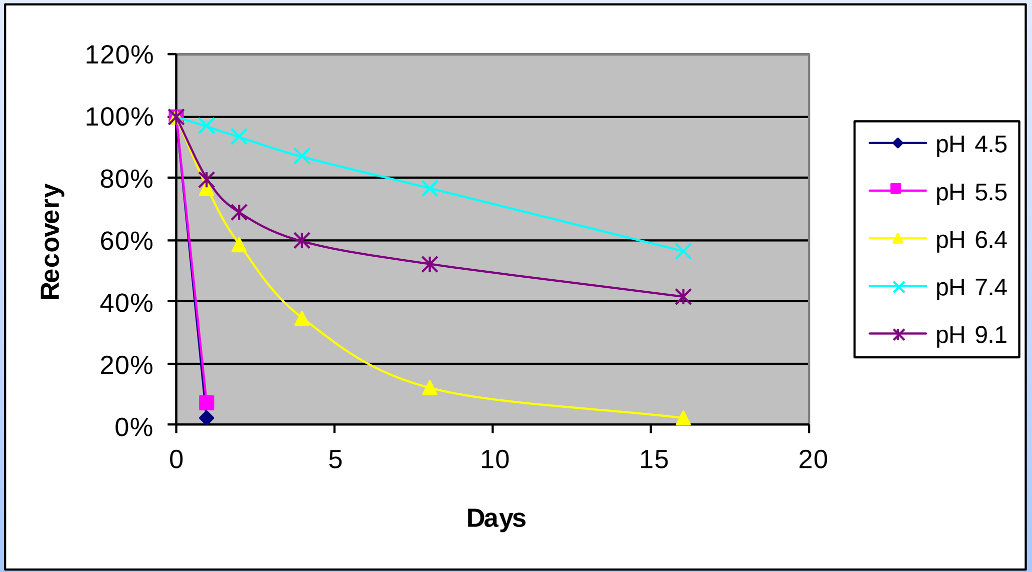
BENZYL ACETATE	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
10°C	1038	2257	3404	3560	2582	1299	453	110
20°C	602	1467	2360	2509	1761	817	250	51
30°C	265	779	1410	1568	1071	450	116	18
40°C	88	339	726	869	581	217	45	5
50°C	22	120	322	427	281	92	15	1
60°C	4	35	123	186	121	34	4	0

LINALYL ACETATE	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
10°C	2180	4143	6924	10173	13144	14930	14912	13096
20°C	349	736	1312	1982	2537	2751	2527	1967
30°C	75	158	279	416	520	546	482	357
40°C	21	41	67	94	113	117	103	79
50°C	8	13	18	23	26	27	25	21
60°C	4	5	5	6	6	7	7	7

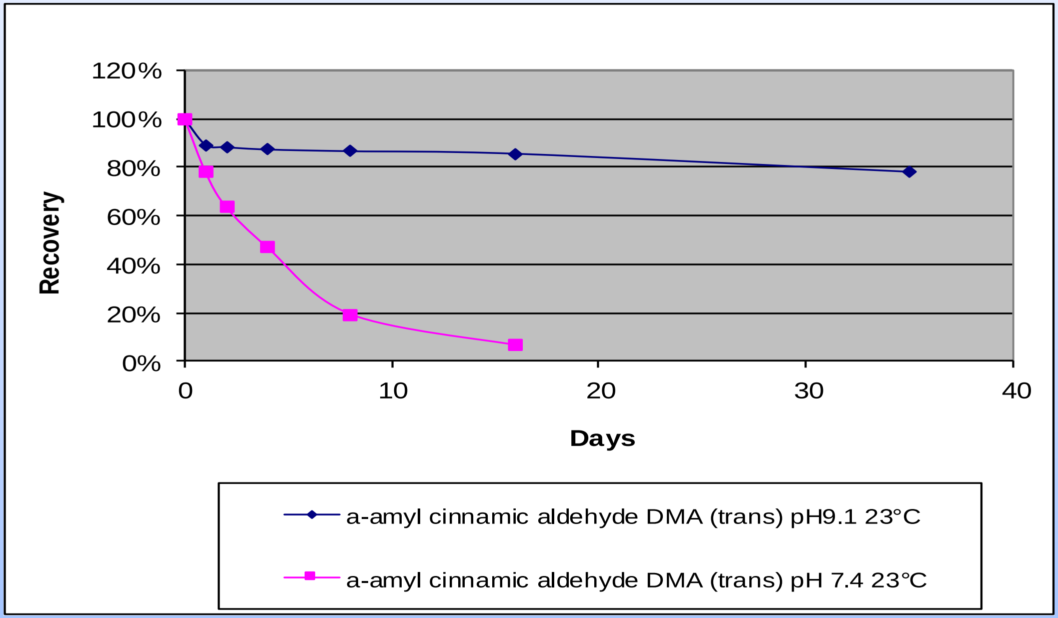
(green) = Half life > 365 days

(orange) = Half life < 365 days

Data on Schiff Bases:
(@23°C)



Data on Acetals:
(@23°C)



4. Conclusions

- Ester hydrolysis does occur in many product matrices
- Rate of Ester Hydrolysis is hugely variable and dependent upon many factors:
  - *Chemistry of the acid "leaving group" (e.g., formate > acetate > cinnamate)*
  - *Chemistry of the alcohol group (e.g., (iso)eugenol > citronellol)*
  - *Product matrix (e.g., pH, solubility)*
  - *Storage temperature*
- Acetals are unstable structures that readily hydrolyse in many product matrices
- Schiff bases are stable in some product matrices but are prone to hydrolysis in others
- Many of the products of hydrolysis (aldehydes and alcohols) also exhibit varying degrees of instability *(i.e., maximum theoretical yield was rarely observed and in some cases the level peaked at an earlier time point and then declined)*
- No evidence for instability of Ether or Terpineol

5. Key Messages

- Sensitisers (aldehydes and alcohols) can be generated in product, over time, as a result of abiotic hydrolysis of precursors

- Rate of conversion is dependant upon:

  - *The chemistry of the precursor*
  - *The chemistry of the product matrix*
  - *The physical conditions of storage*

- Sensitisers can also be lost from a product due to their intrinsic instability

- The levels of most sensitisers are in a state of flux in most (all?) product types

5. Final Thoughts

- Which are the important (higher risk) precursor materials?
  - *Frequency and level of use*
  - *Probability/rate of hydrolysis*
  - *Potency of chemical produced*

- Which are the important (higher risk) product matrices?
  - *Is it possible to predict hydrolysis rates, in specific product matrices, from kinetic constants?*
  - *Are there additional parameters that may be influential?*

- What additional understanding is required to support the development of proportional, evidence based Risk Management measures?
  - *e.g., Precursor restrictions in specific product types?*
  - *e.g., Consumer awareness for the "potential" of allergen presence ?*