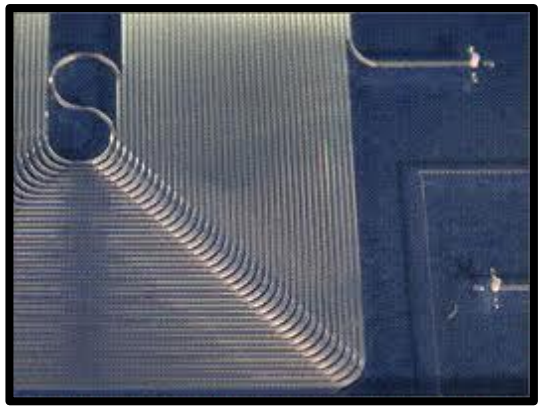
Health Criteria Values

& the Toxicological Implications of eLiquid Ingredients

Dr Jaydene Halliday
Chief Scientific Officer

A Little Bit About Me…

- **Dr Jaydene Halliday BSc (Hons) MRSC**, *Chief Scientific Officer*
  - **BSc (Hons) in Pharmaceutical and Forensic Analysis, Limerick Institute of Technology**
  - **PhD in "Technologies toward a Lab-On-A-Chip GCxGC", University of York**
  - **Toxicology Lab Analyst, The Irish State Laboratory**
  - **Lead GC Technical Specialist, Phenomenex**
  - **Science Director, CSI Drug & Alcohol Testing**



EL-SCIENCE
LEADING E-LIQUID
QUALITY

- Dedicated solely to improving the quality and safety of eLiquids
- Highly technical and experienced team
  - **Analytical, Flavour, Organic, Toxicology & Production Chemists**
- State-of-the-art laboratories
  - **Analytical Laboratories**
    - **7 GC-MS, with EI/CI, FID, ECD and TD capabilities**
    - **2 HPLC, with UV and RI detection**
    - **AAS**
    - **ICP-MS**

eLiquid Manufacture

EL-SCIENCE
LEADING E-LIQUID
QUALITY

+

+

=

eLiquid Manufacture

EL-SCIENCE
LEADING E-LIQUID
QUALITY

Recipe?

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eLiquid Manufacture

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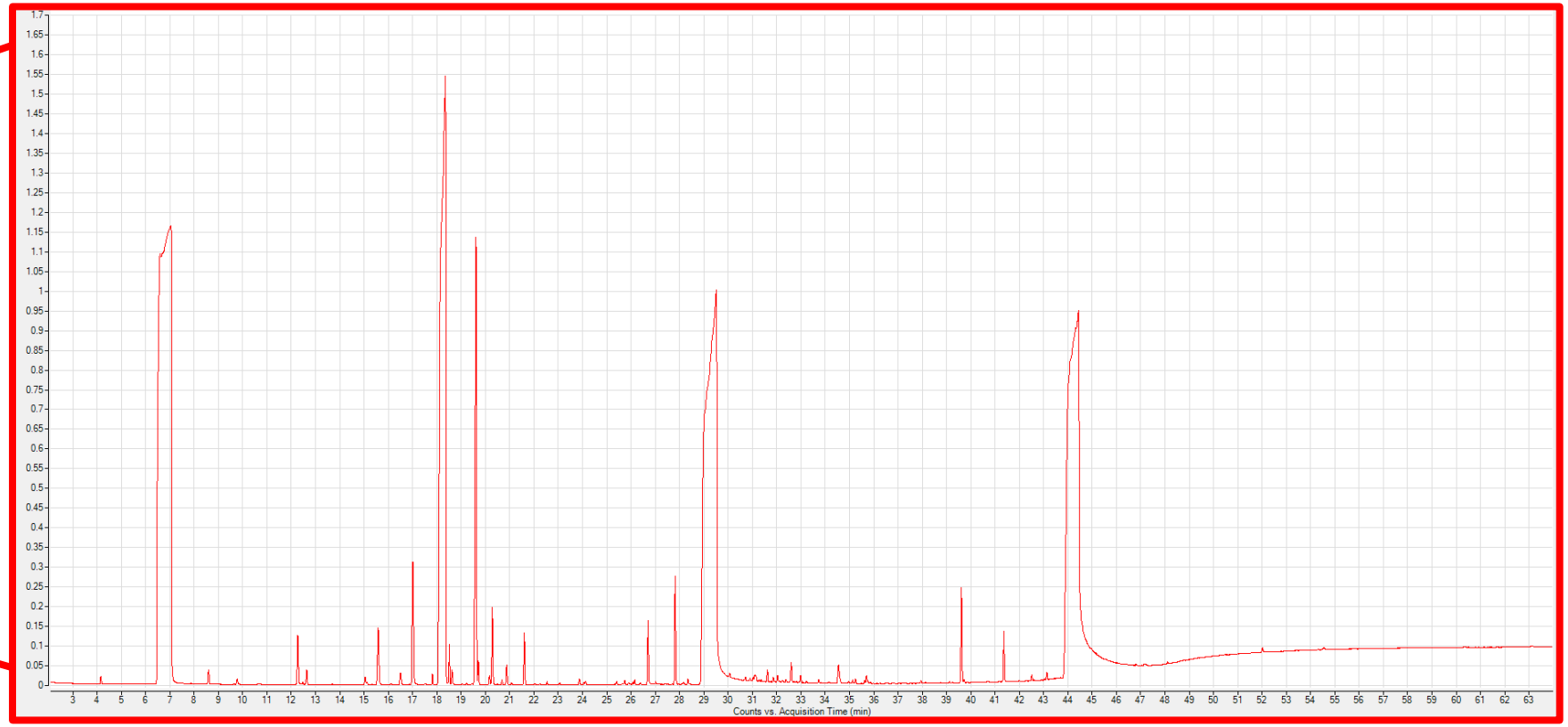
QUALITY



eLiquid Manufacture

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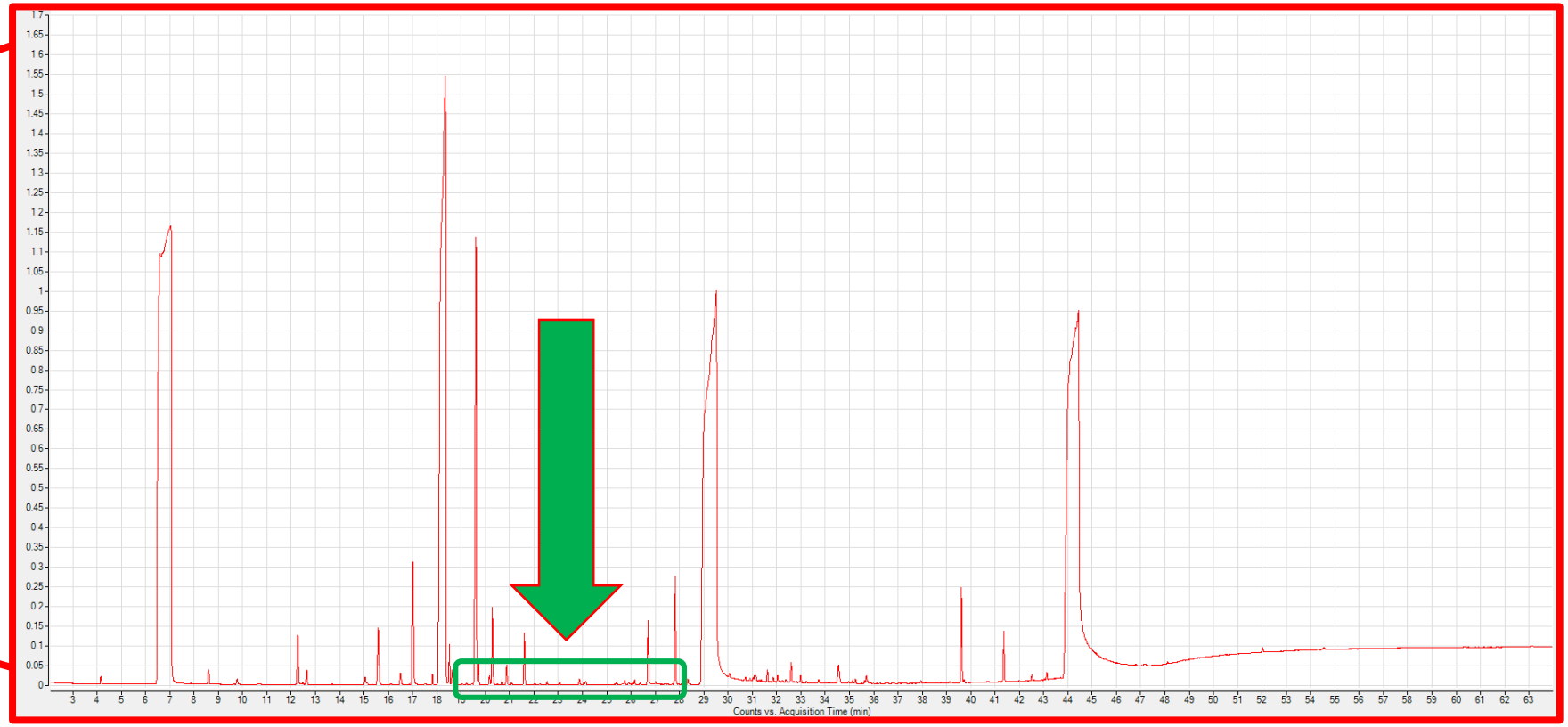
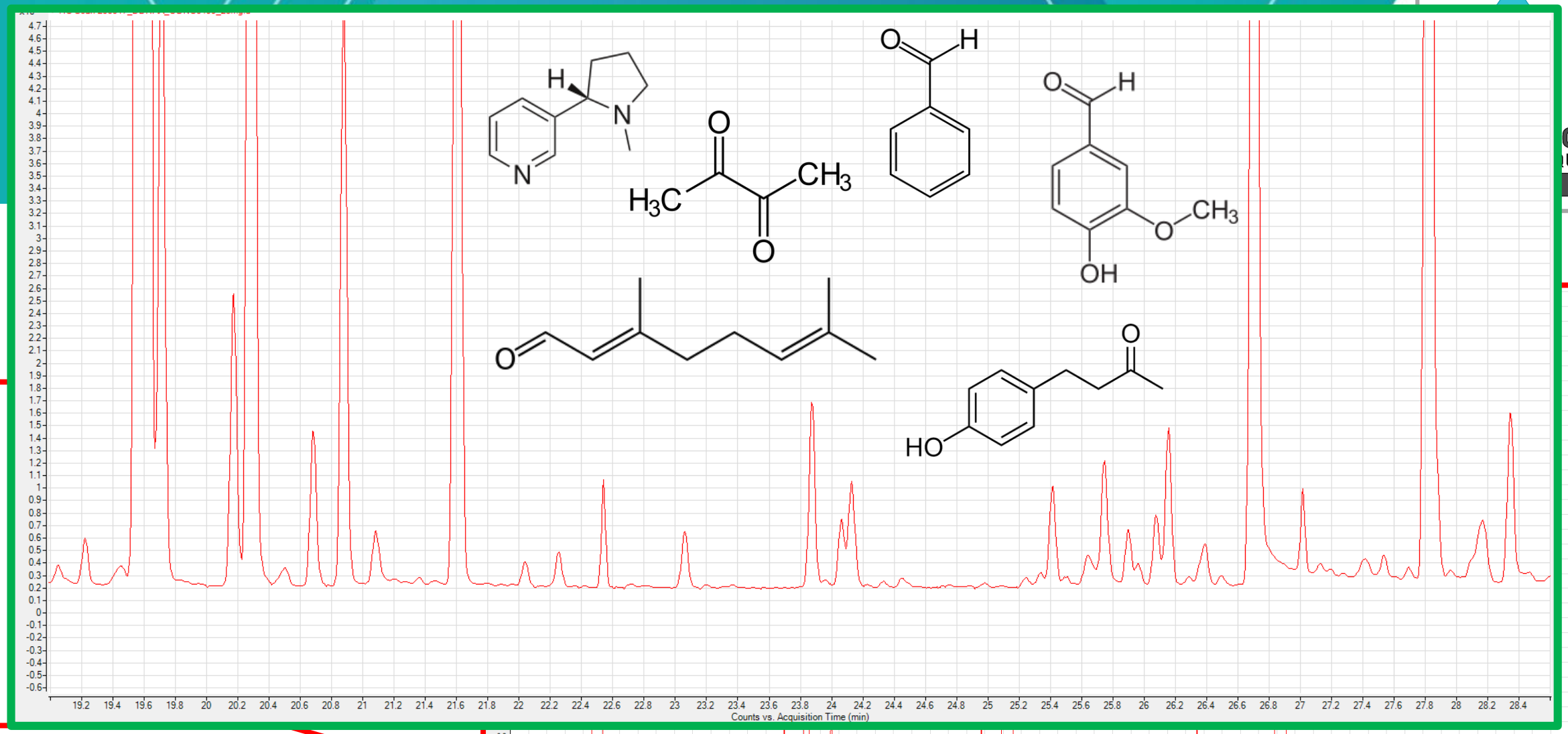
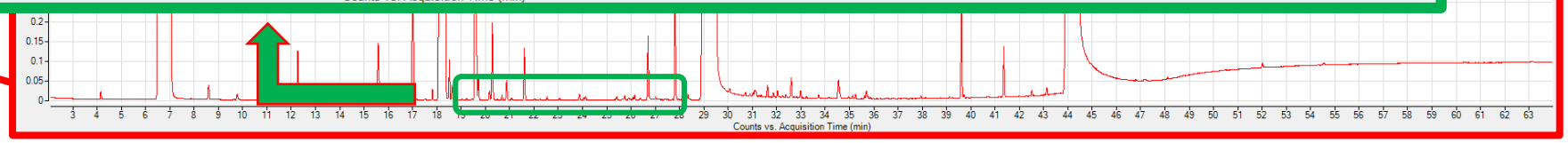
QUALITY

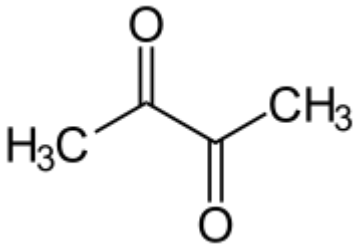



Counts vs. Acquisition Time (min)

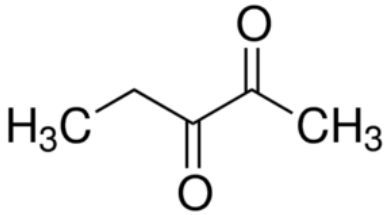

Counts vs. Acquisition Time (min)

The Diketones

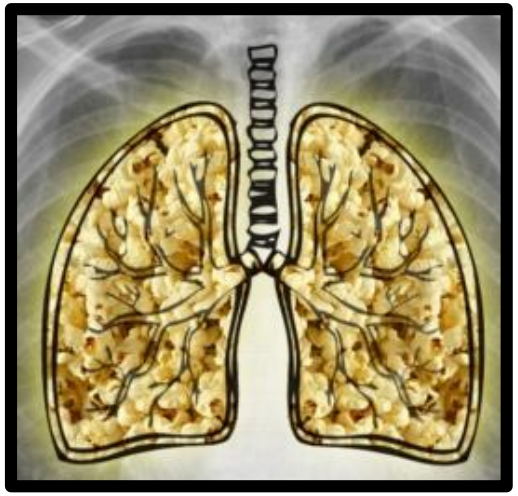
Diacetyl
(2, 3-butanedione)



Acetyl Propionyl
(2,3-pentanedione)



Bronchiolitis Obliterans
"Popcorn Worker's Lung"



Custard

Butterscotch

Caramel

Maple syrup

Brown sugar

Coffee

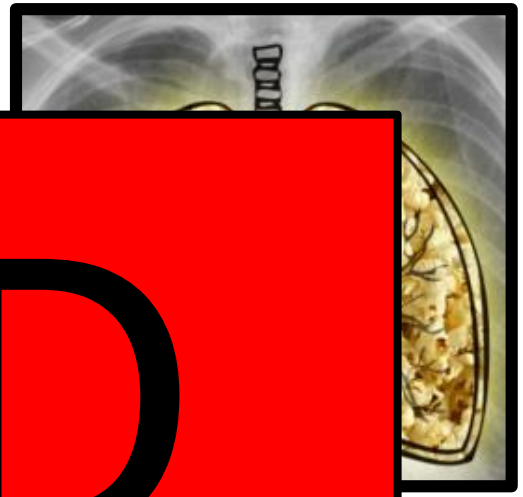
Some fruit flavours

The Diketones

Diacetyl
(2, 3-butanedione)

Acetyl Propionyl
(2,3-pentanedione)

Bronchiolitis Obliterans
"Popcorn Worker's Lung"



H_3C

BANNED

Custa

ruit flavours

The Diketones

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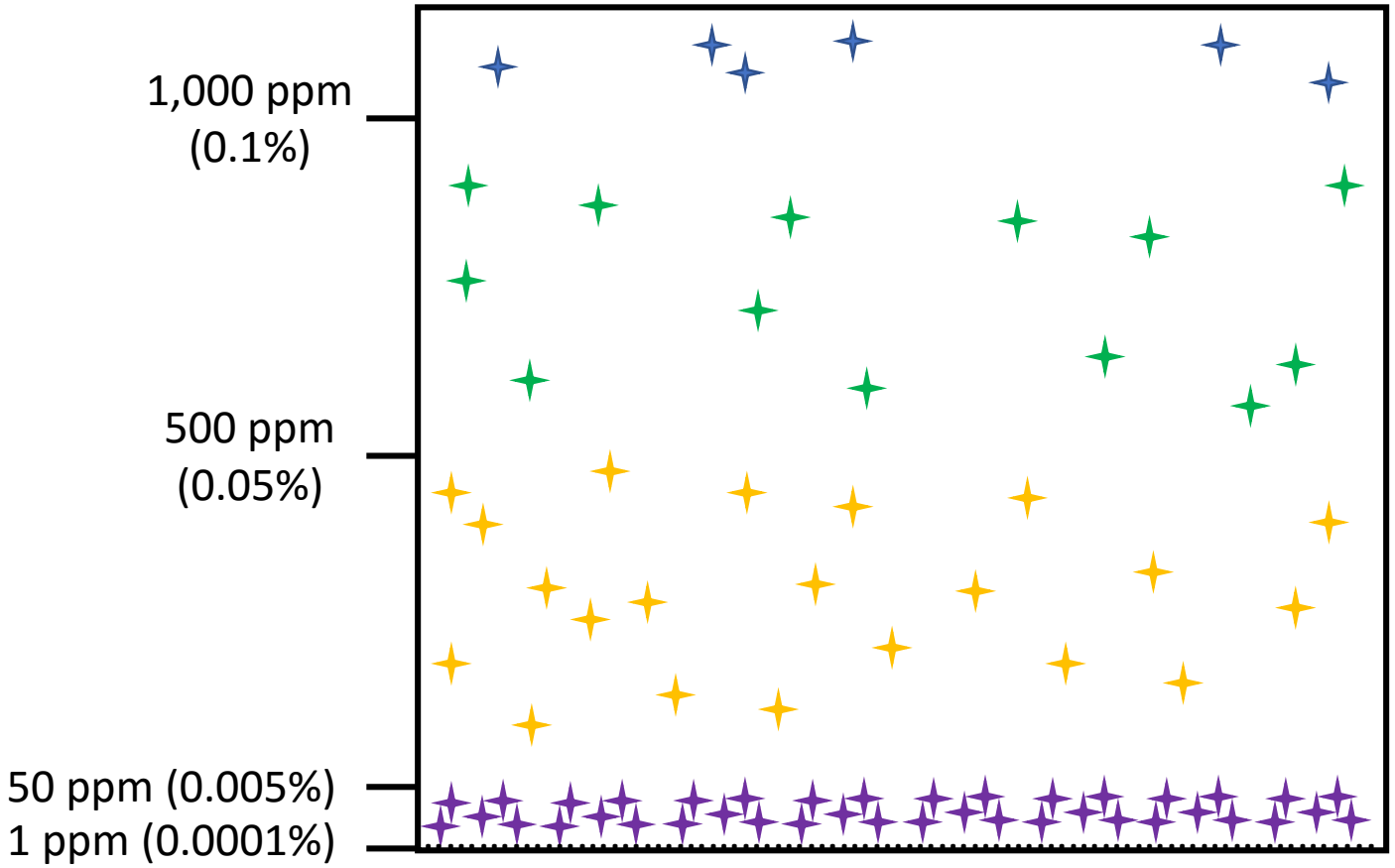
QUALITY

- **Limit of Detection:** In analytical chemistry, the **limit of detection** or **LOD** is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit



The Diketones

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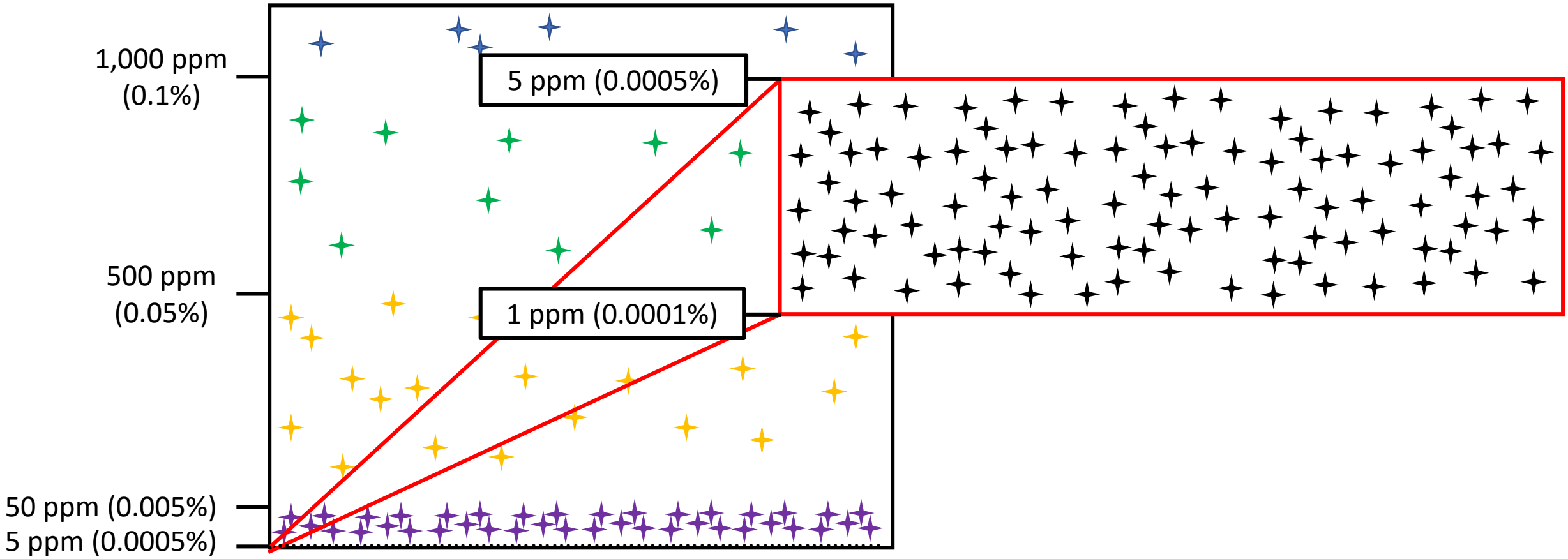

The Diketones

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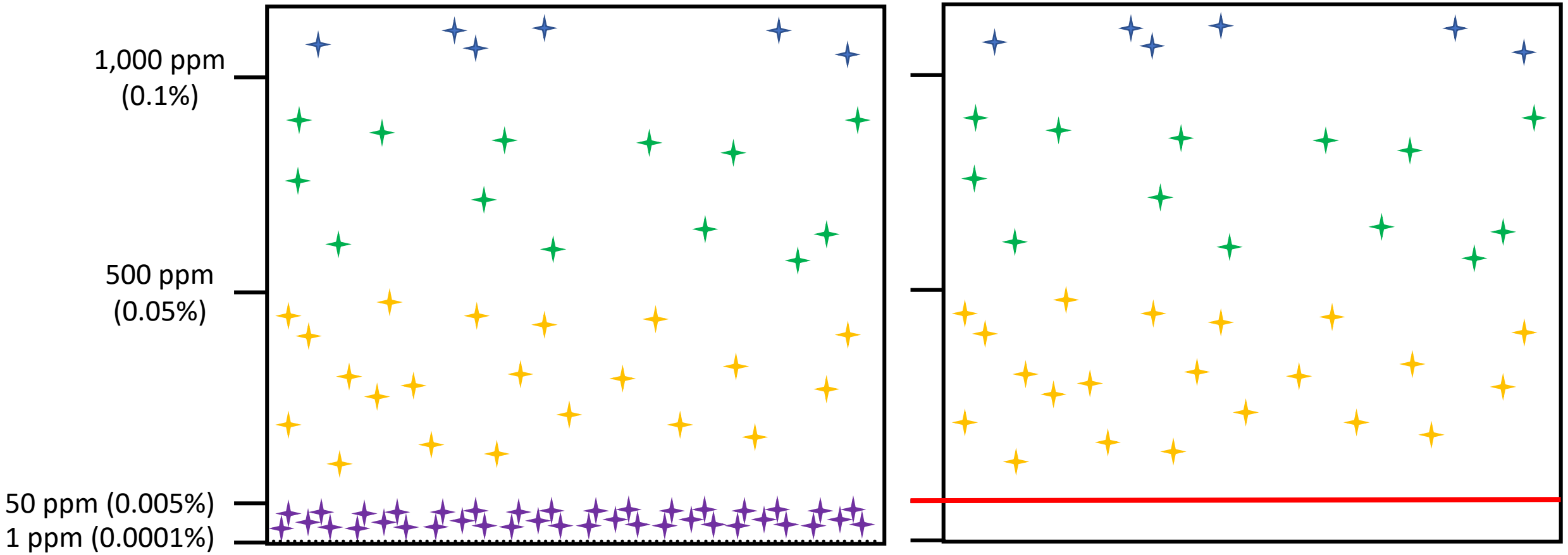

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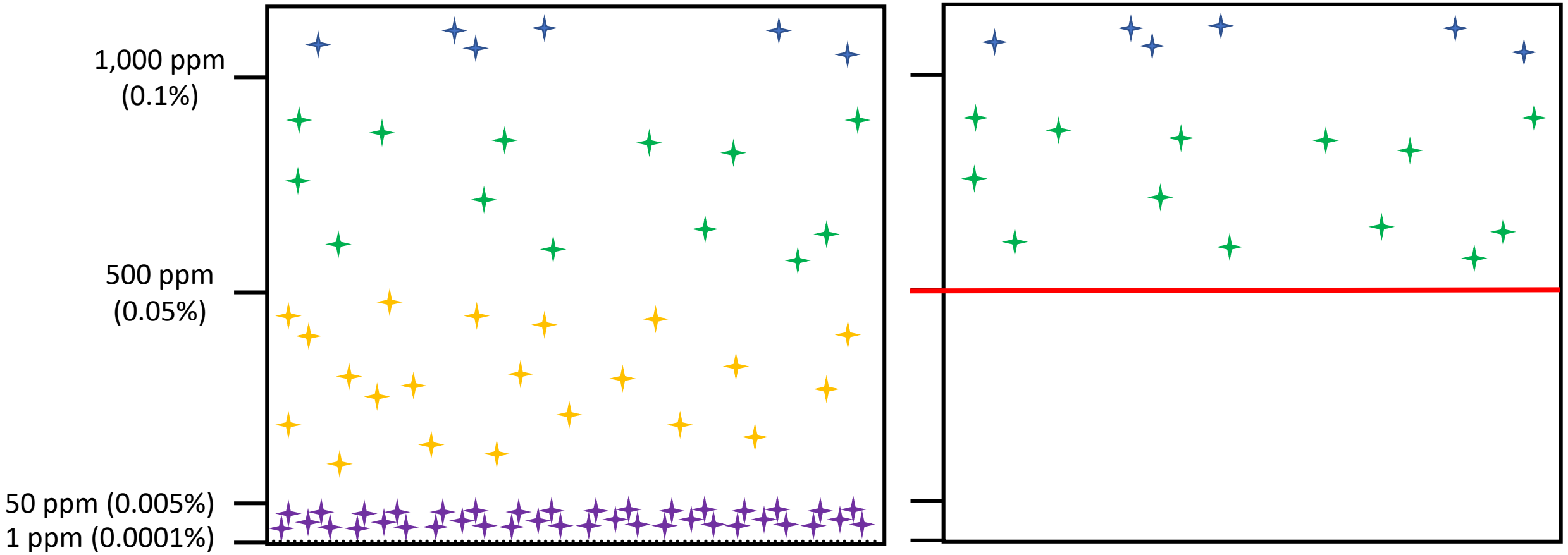

The Diketones

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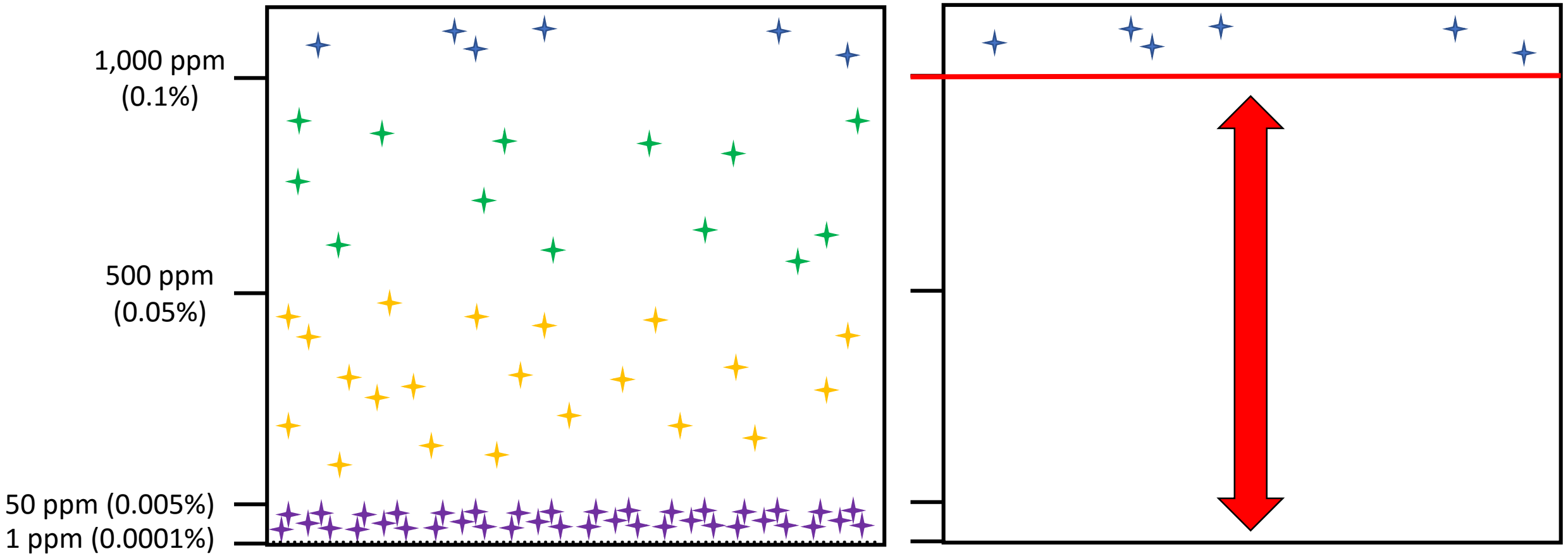


Paracelsus

1493 - 1541

Founded the discipline of **Toxicology**

Sola dosis facit venenum

“The dose makes the poison”

Health Criteria Values
HCVs

- Guidance levels set by expert groups
- Assessors use HCVs combined with estimates of exposure to determine risk to human health
- Helps determine whether further investigation, assessment and/or remediation is required
- Established by reviewing evidence from:
  - **Occupational studies**
  - **Epidemiological studies**
  - **Laboratory animal studies**
  - **Understanding of chemical's ADME & mechanism/mode of action**

The Diketones

- **NIOSH REL-TWA – Threshold Concentration (continuous exposure):**
  - Inhaled intake of 60 µg/day (3 µg/m^3 as continuous exposure for an adult inhaling 20 m^3 of air per day)

- **E-Cigarette Exposure:**
  - Series of peaks followed by periods of essentially negligible background exposure

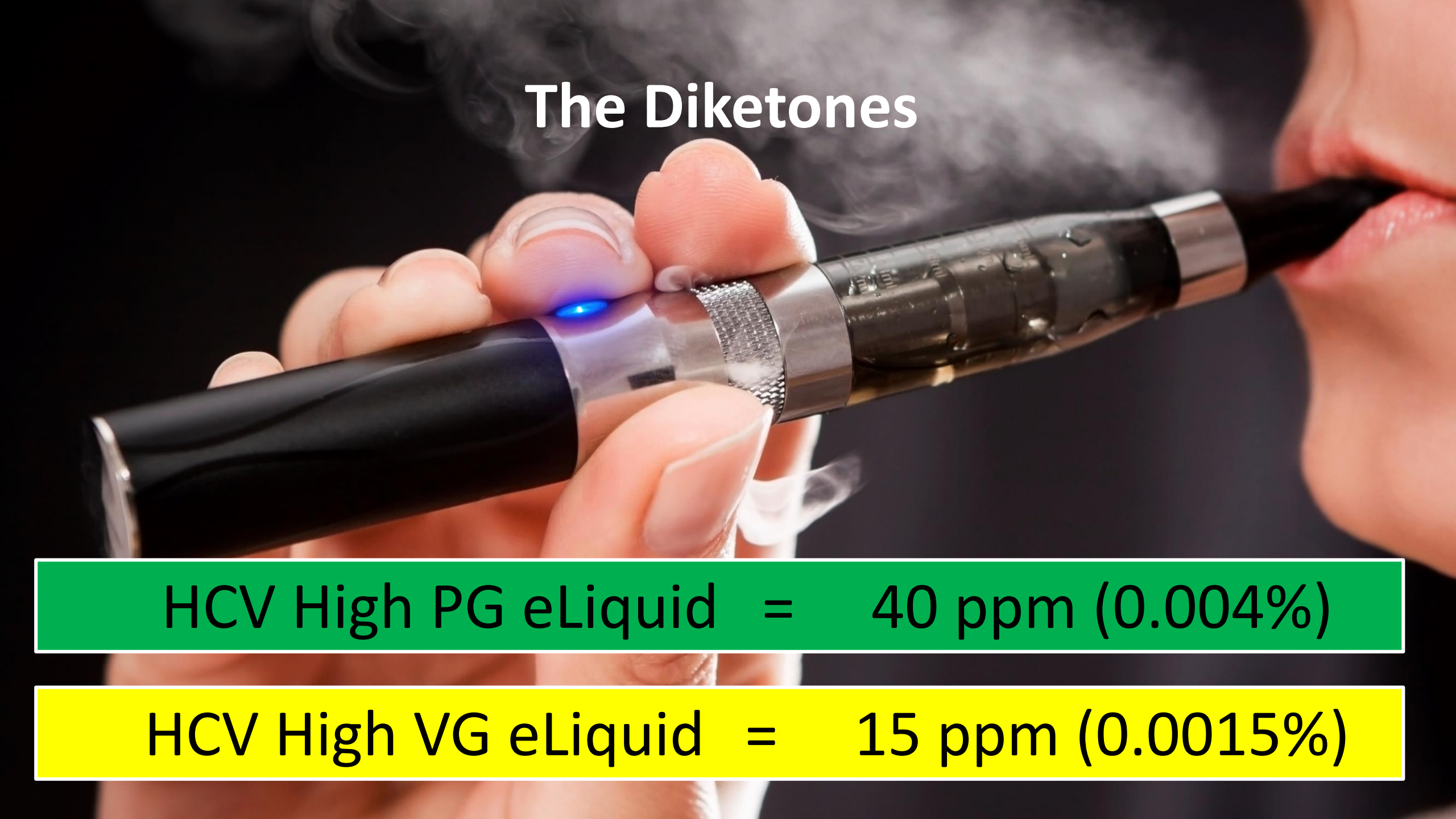
  - Average puffs/day = **300**
  - Mean puff volume = **55 mL**

  - Total inhaled volume = **16.5 L/day (0.0165 m^3/day)**

  - High PG User = **1.5 mL/day**
  - High VG User = **4 mL/day**

HCV per puff

$$\frac{\text{Proposed daily intake}}{\text{Total daily puff volume}} = 3.6\ \text{mg/m}^3$$



The Diketones

HCV High PG eLiquid = 40 ppm (0.004%)

HCV High VG eLiquid = 15 ppm (0.0015%)

eLiquid Ingredients

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1. eLiquids contain tens to hundreds of ingredients
2. The dose makes the poison

eLiquid Ingredients

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Amount in product

eLiquid Ingredients

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LEADING E-LIQUID
QUALITY

1. eLiquids contain tens to hundreds of ingredients
2. The dose makes the poison

Amount in product

Amount of product consumed

eLiquid Ingredients

EL-SCIENCE
LEADING E-LIQUID
QUALITY

1. eLiquids contain tens to hundreds of ingredients
2. The dose makes the poison

Amount in product

Amount of product consumed

Applies to ALL ingredients in eLiquid product

eLiquid Ingredients

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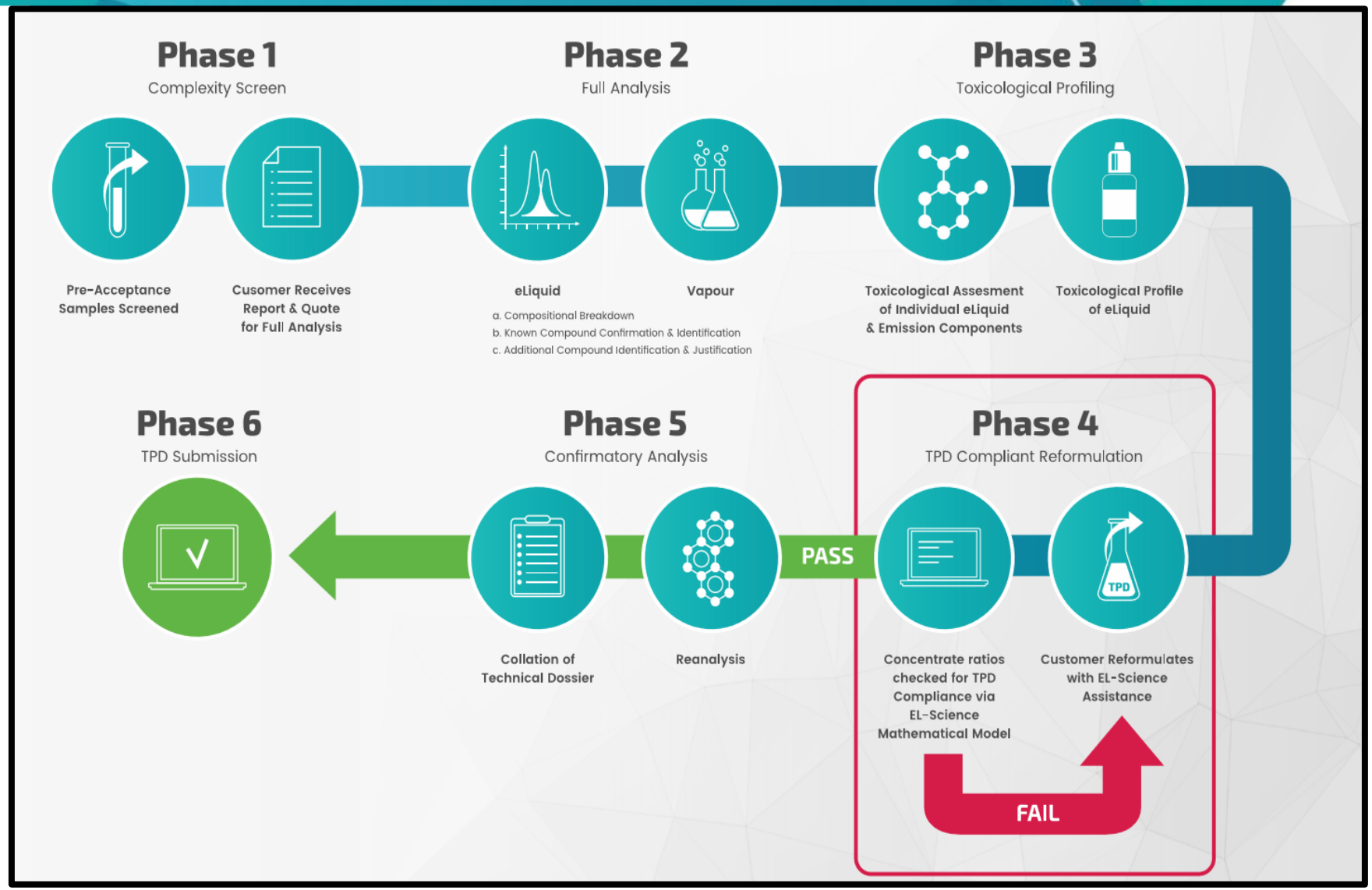

Toxicological Hazard Assessments

- Identify and quantify individual ingredients
- Consider the **product as a whole**:
  - **Ingredients**
  - **HCVs**
  - **Quantities of chemicals present**

Toxicological Hazard Assessments

- Identify and quantify individual ingredients
- Consider the **product as a whole**:
  - **Ingredients**
  - **HCVs**
  - **Quantities of chemicals present**

24% > HCV

Other Diketones

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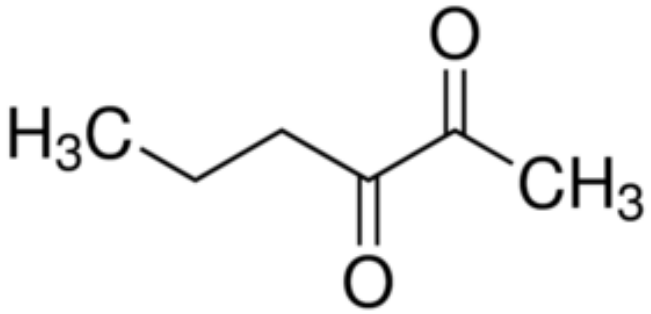
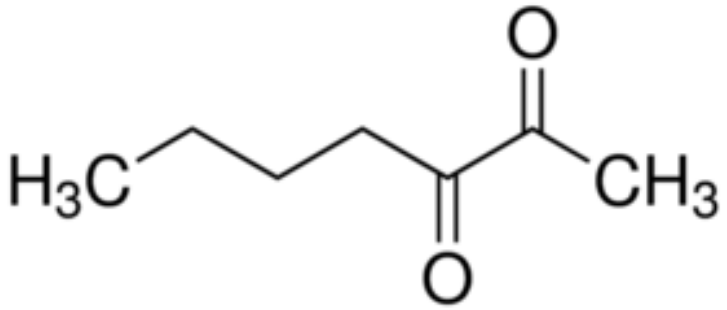
QUALITY

2,3-hexanedione



2,3-heptanedione



Odour description: sweet, creamy, caramellic, buttery with a fruity jammy nuance

Taste description: creamy, fruity, toasted caramellic notes

Same toxicological effects as diacetyl & acetylpropionyl

Other Diketones

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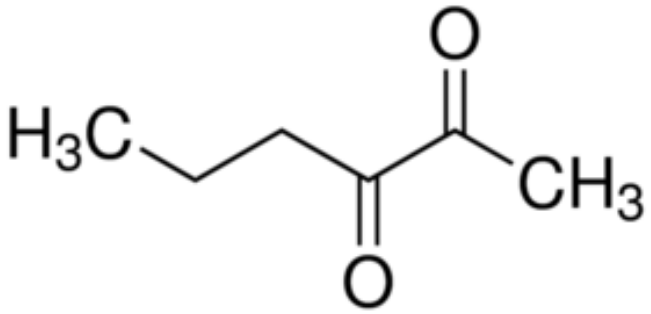
LEADING E-LIQUID
QUALITY

2,3-hexanedione



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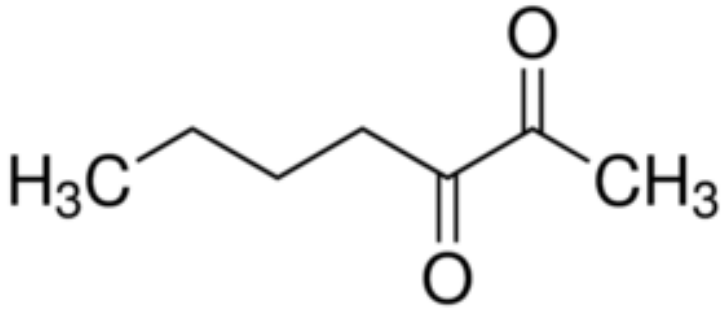


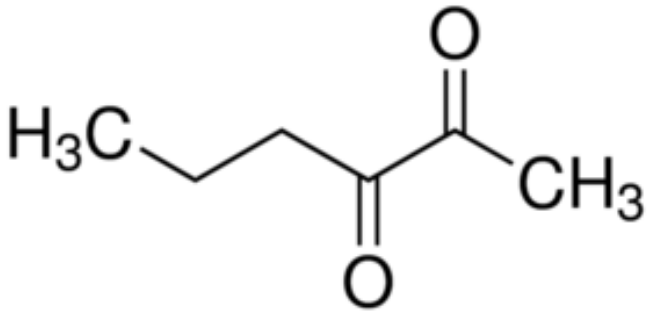
Cardiopulmonary Effects: Local toxin to the respiratory tract *(Morgan et al., 2016)*

BfR has recommended a **total ban** for 2,3-hexanedione and 2,3-heptanedione in eLiquids and ecigarettes based on their potential to cause severe respiratory inflammation following inhalation *(BfR, 2015)*

Other Diketones

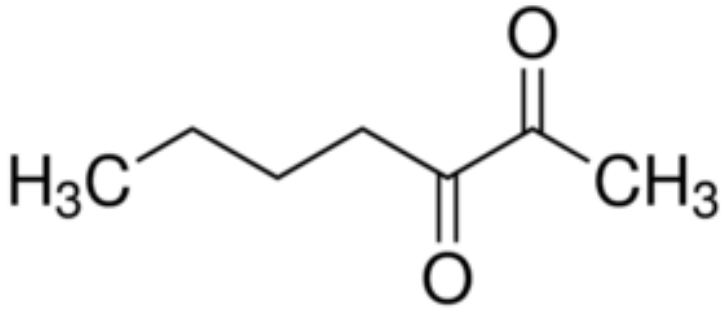
EL-SCIENCE
LEADING E-LIQUID
QUALITY

2,3-hexanedione



2 %

2,3-heptanedione



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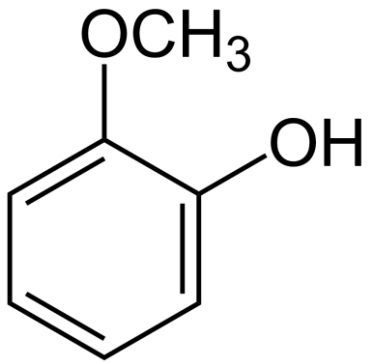
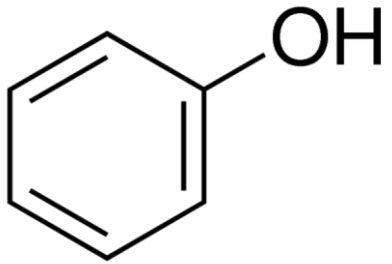
Guaiacol and Phenol

EL-SCIENCE
LEADING E-LIQUID
QUALITY

Guaiacol



Phenol



Odour description: Phenolic, smoky, spicy, medicinal, vanilla and savoury meaty with a woody nuance

Taste description: Woody, phenolic, bacon, savoury, smoky and medicinal

Starting materials for >85% of the world's vanillin

Guaiacol and Phenol

EL-SCIENCE
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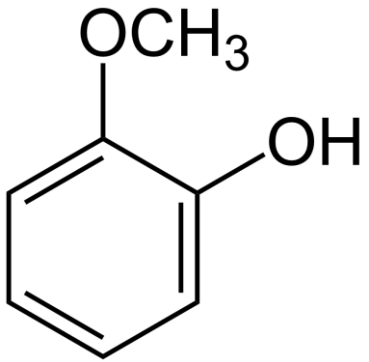
QUALITY

Guaiacol

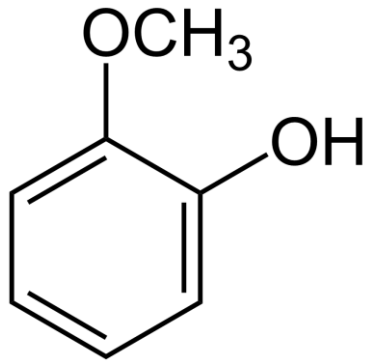


Addictiveness: Included in SCHEER's priority list of tobacco additives. Has a use as a local anaesthetic which can enhance smoke inhalation, thus potentially contributing to addictiveness *(SCHEER, 2016)*

Can cause neurological, hemodynamic (shock), respiratory, metabolic (metabolic acidosis), renal (acute tubular necrosis), digestive and hematologic adverse effects" *(SCENIHR, 2016)*

Guaiacol and Phenol

Guaiacol



Cardiopulmonary Effects: Respiratory tract irritant *(SCHEER, 2016; SCENIHR, 2016)*

"Subcutaneous administration (6.25-400 µL/40 g) of guaiacol to male Swiss Webster mice (30-45 g) produced tachycardia... Necropsy showed pulmonary oedema" *(Martinez Enriquez et al., 2009)*

Genotoxicity: Conflicting studies

Guaiacol and Phenol

EL-SCIENCE
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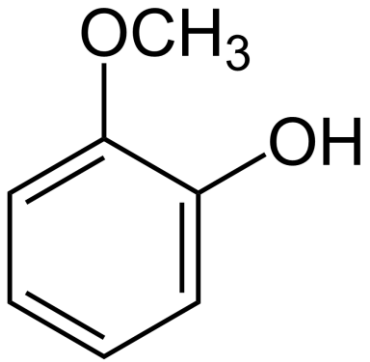
QUALITY

Guaiacol



HCV
High PG: 0.018%
High VG: 0.007%

Carcinogenicity: "Repeated exposure via the oral route of a structurally-related compound (4-methoxyphenol) produced hyperplasia of the forestomach in rats..., suggesting an effect at the site of contact, likely associated with the irritating properties of the compound on the gastrointestinal mucosa. A NOAEL of 0.25% corresponding to 125 mg/kg bw/day was derived *(Wada et al., 1990)*

Administration for 104 weeks caused an increase in atypical hyperplasia (male, 67%, female, 37%) followed by the presence of papillomas (50%, 23%) and squamous cell carcinomas (77%, 20%) *(Asakawa et al., 1994; SCENIHR, 2016)*

Guaiacol and Phenol

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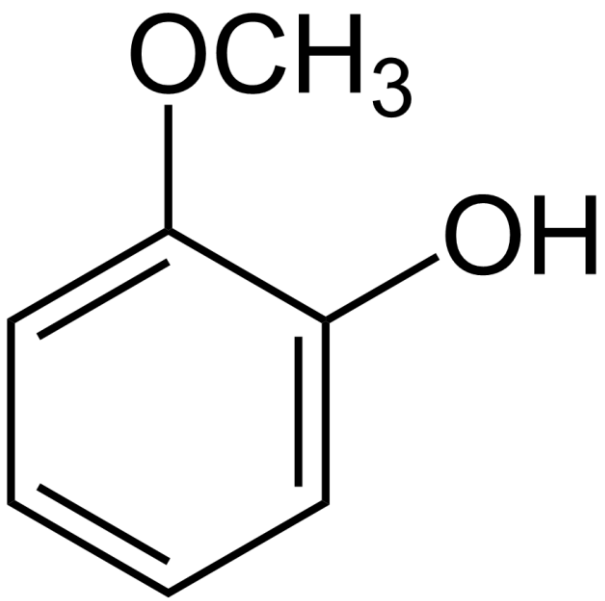
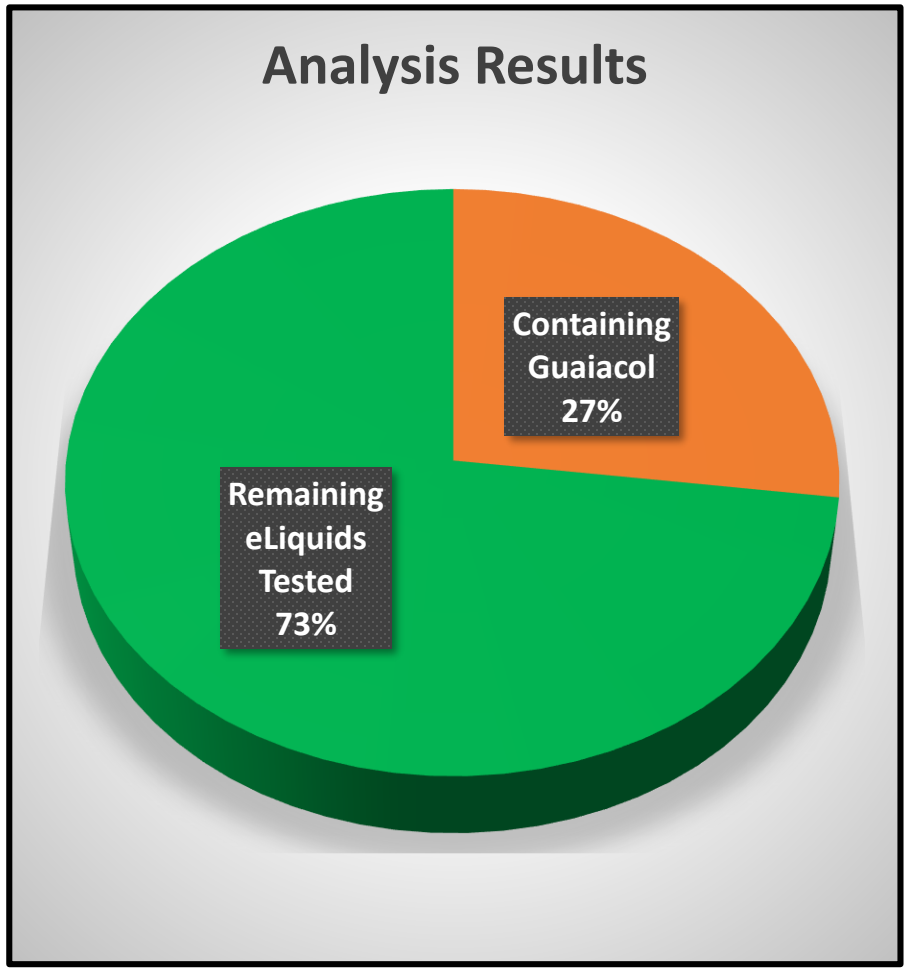
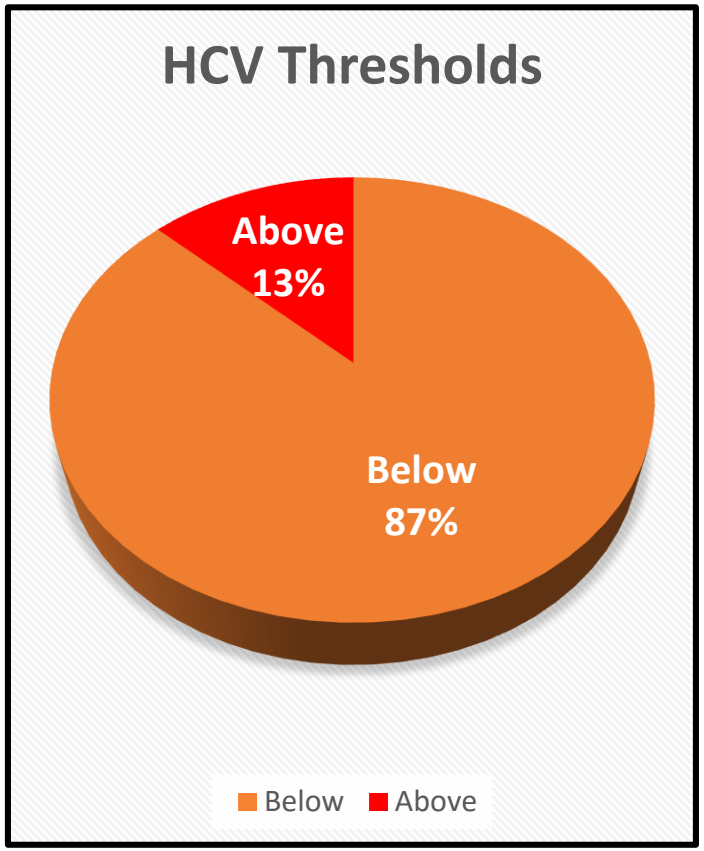
Guaiacol







Guaiacol and Phenol

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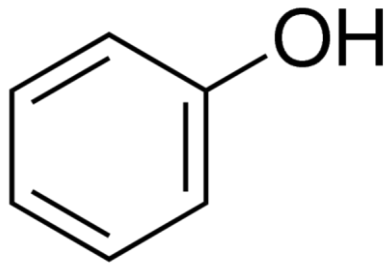
Phenol

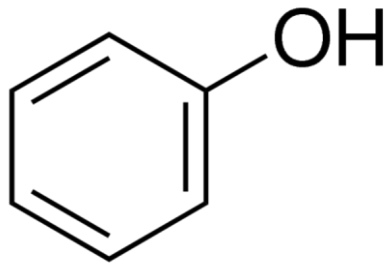


Addictiveness: Anaesthetic properties. Can destroy nerve endings. As such, the local toxicity it causes can be painless, possibly leading to less awareness and a higher degree of damage *(NICNAS, 2016; PHE, 2016)*

Genotoxicity: Expert Group Opinion - Phenol is subject to harmonised classification and labelling as a mutagen *(ECHA, 2016)*

Guaiacol and Phenol

Phenol



Cardiopulmonary Effects: Inhaled phenol causes local toxicity to the respiratory tract. Linked to effects on heart rate and blood pressure *(Danish EPA, 2014; NICNAS, 2016; PHE, 2016)*

Other: Skin irritant (causes dermatitis)
Eye irritant

Guaiacol and Phenol

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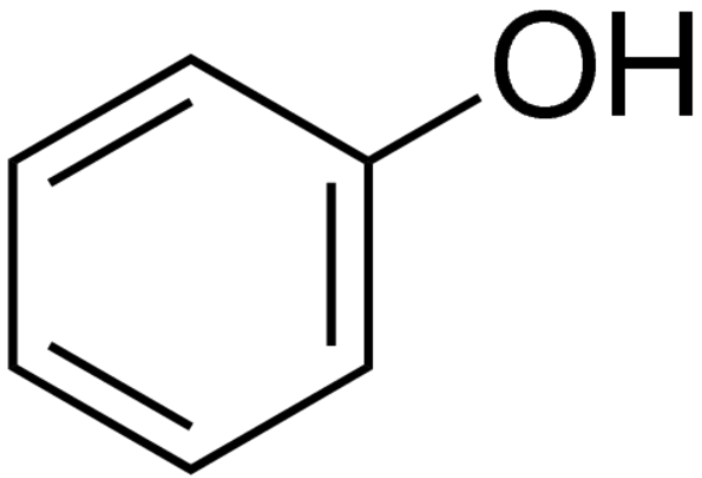
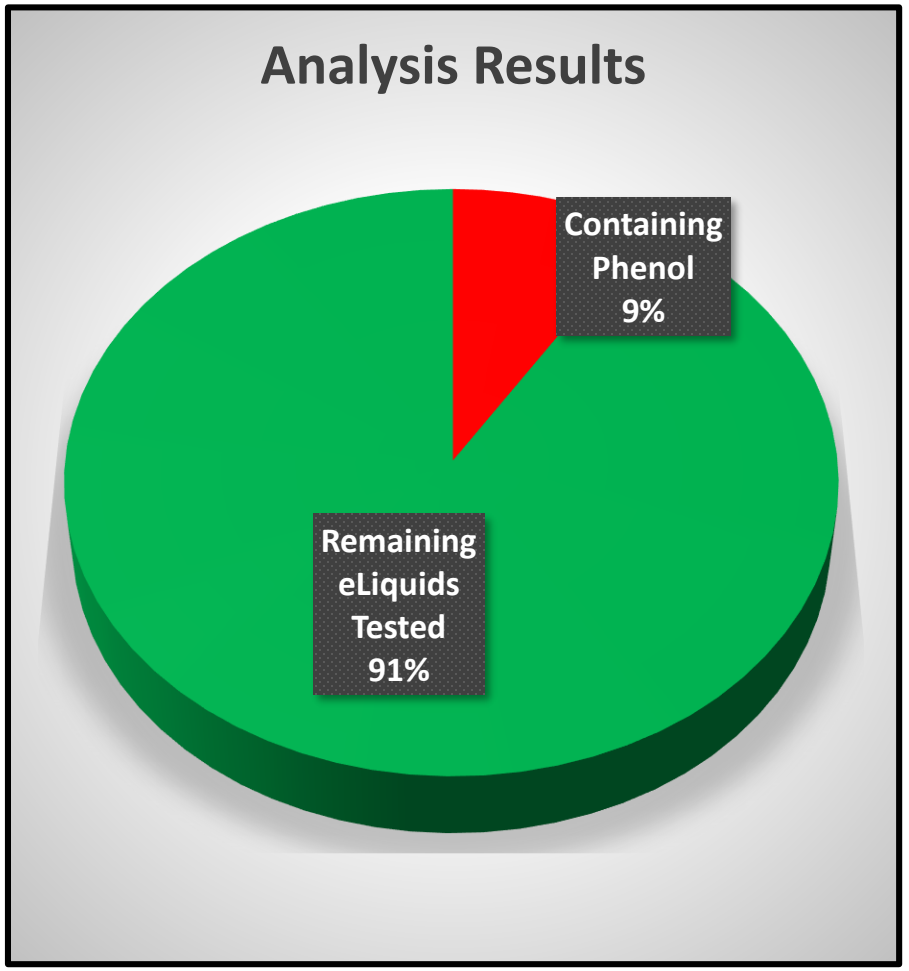
Phenol



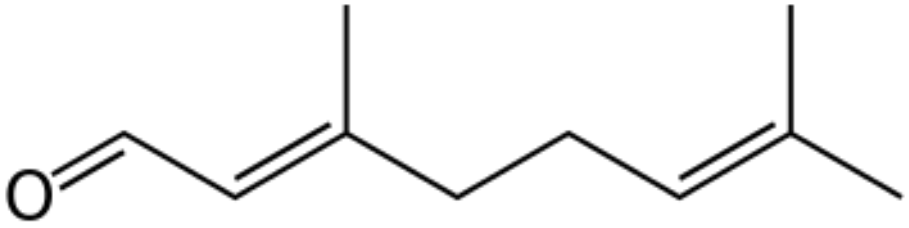


Inclusion of Phenol in eLiquids is NOT recommended

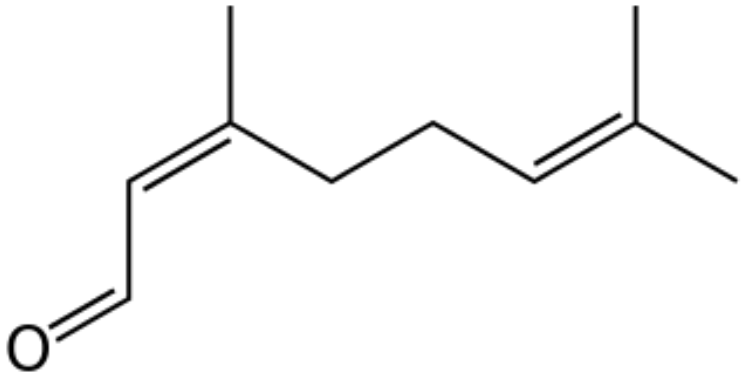
Citral

- Citral is either a pair, or a mixture of terpenoids
  - **The two compounds are double bond isomers**

The *E-isomer* is known as:
Geranial or **Citral A**



The *Z-isomer* is known as:
Neral or **Citral B**

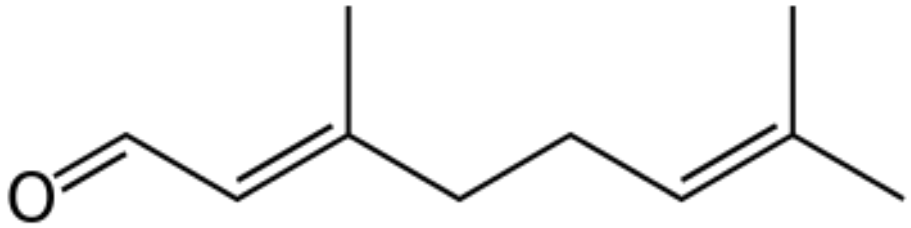


Odour description: Fresh, juicy, lemon peel, with a sweet tangy green nuance

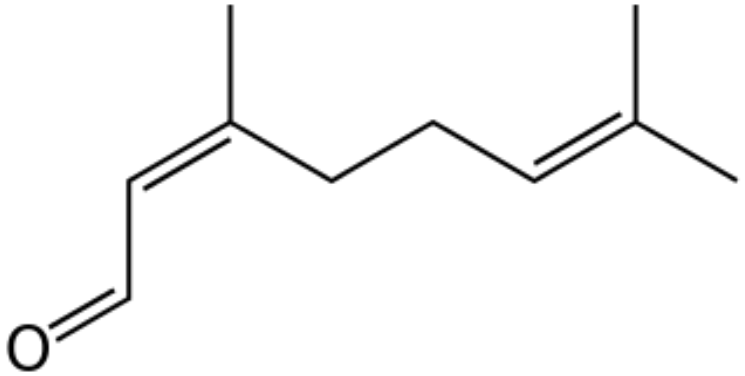
Taste description: Lemon peel, citrus, juicy, green, lime, woody and herbal

Citral

Geranial



Neral



Addictiveness: Linked to painkilling effects following injury/inflammation *(Nishijima et al., 2014; Ortiz et al., 2010)*

Cardiopulmonary Effects: Respiratory irritant

HCV
High PG: 0.31%
High VG: 0.12%

Citral

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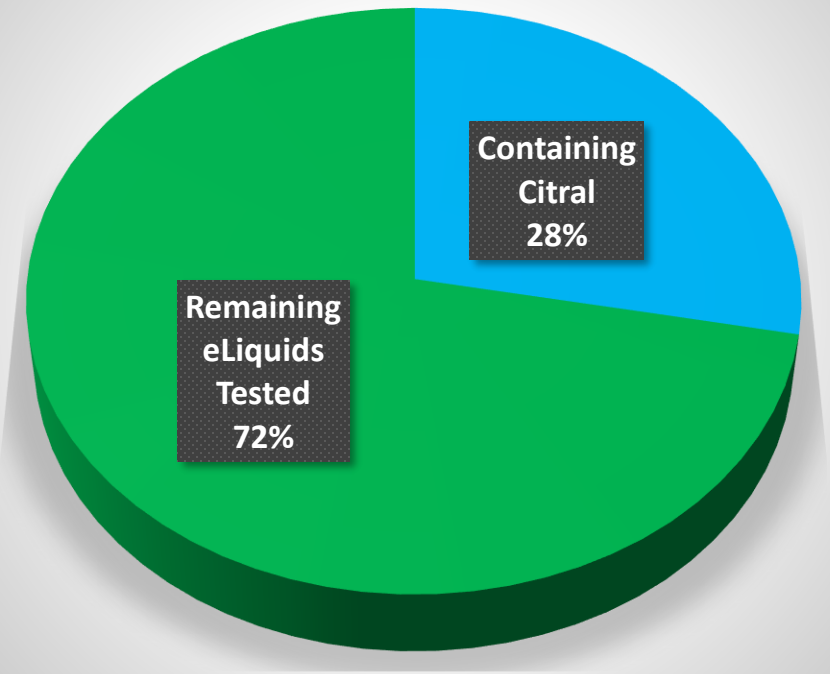
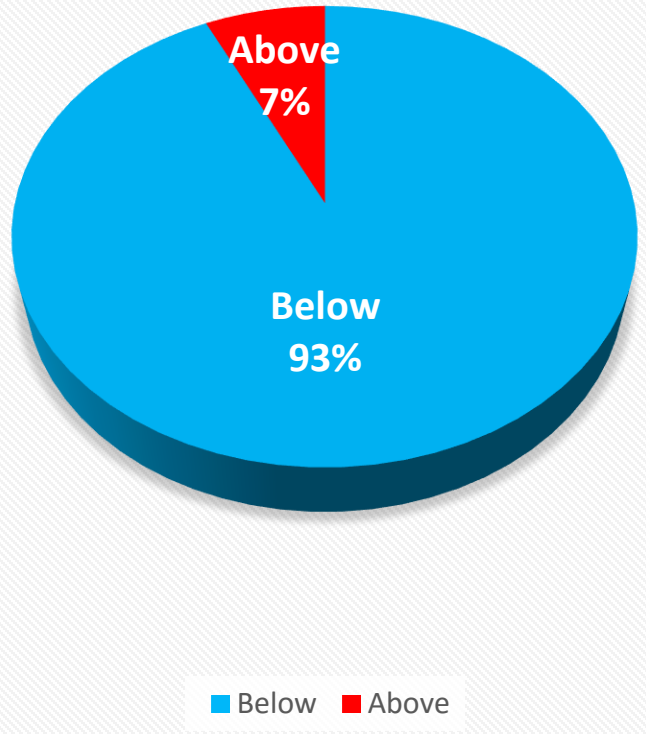
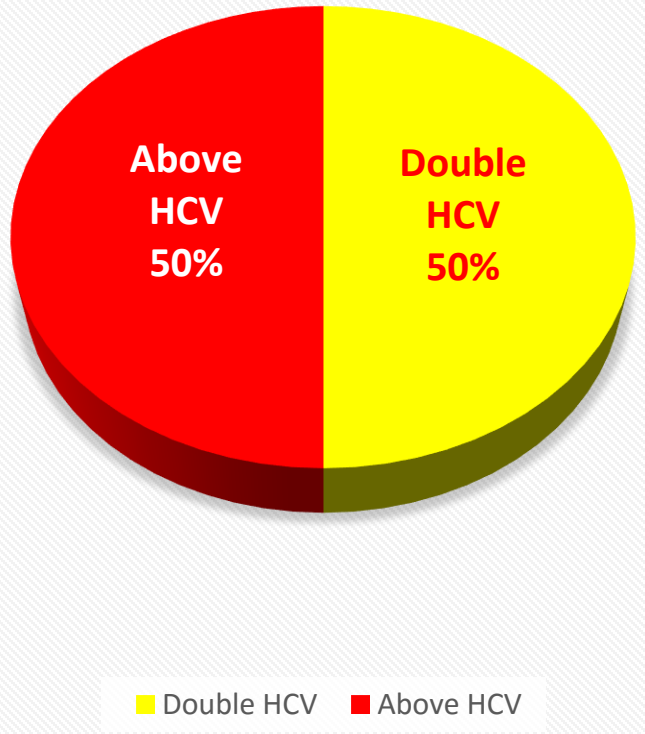
Analysis Results



HCV Thresholds



HCV Breakdown



Raspberry Ketone

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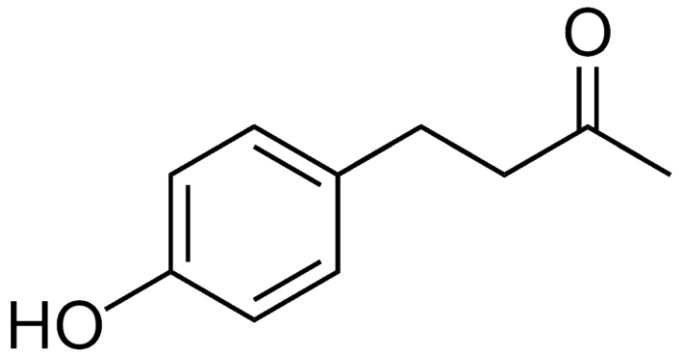
4-(p-Hydroxyphenyl)-2-butanone



Odour description: Berry, sweet, woody and raspberry with a ripe, jammy, seedy character

Taste description: Fruity, jammy, berry, raspberry and blueberry with seedy, cotton candy nuances

Raspberry Ketone

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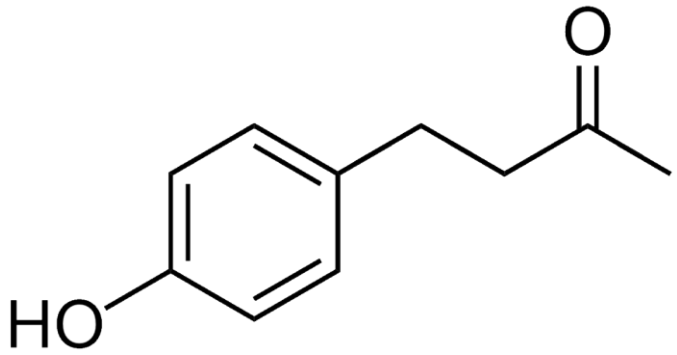
4-(p-Hydroxyphenyl)-2-butanone



Cardiopulmonary Effects: Reliable 90-day studies on rats found no evidence of heart or lung toxicity at dietary doses of up to 700 mg/kg bw/day *(Gaunt et al., 1970; Hoffman, 2004)*

Genotoxicity: In (Q)SAR analyses using Toxtree and Leadscope, there were no indications that raspberry ketone would be genotoxic

Carcinogenicity: A (Q)SAR analysis using Toxtree and Leadscope did not predict carcinogenic potential for raspberry ketone *(Bredsdorff et al., 2015)*

Raspberry Ketone

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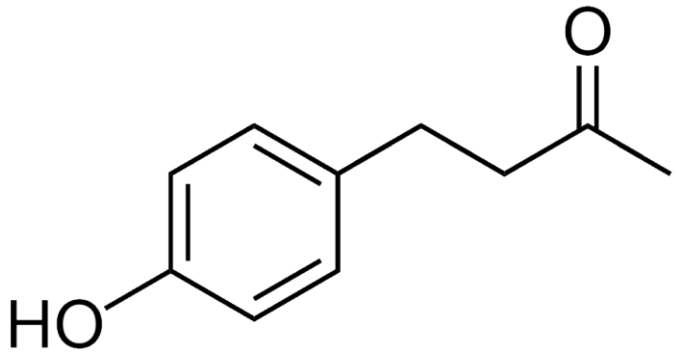
4-(p-Hydroxyphenyl)-2-butanone



Moderate acute oral toxicity (that would warrant classification)

<u>Significant data gaps, including:</u>

- Sensitisation
- Inhalation
- Genotoxicity
- Carcinogenicity
- Repro/developmental toxicity studies

Raspberry Ketone

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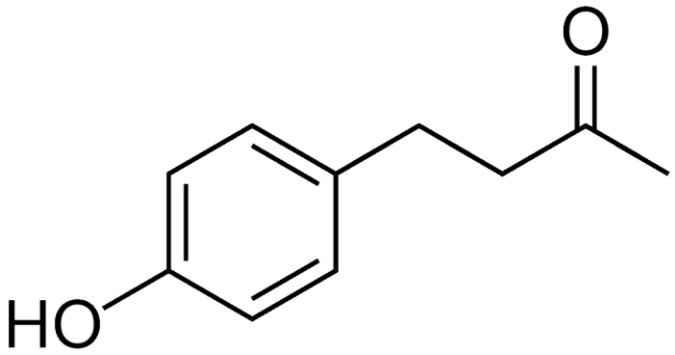
4-(p-Hydroxyphenyl)-2-butanone



At high concentrations can produce similar effects to over-exposure to phenol compounds

HCV

High PG: 0.5%

High VG: 0.19%

Raspberry Ketone

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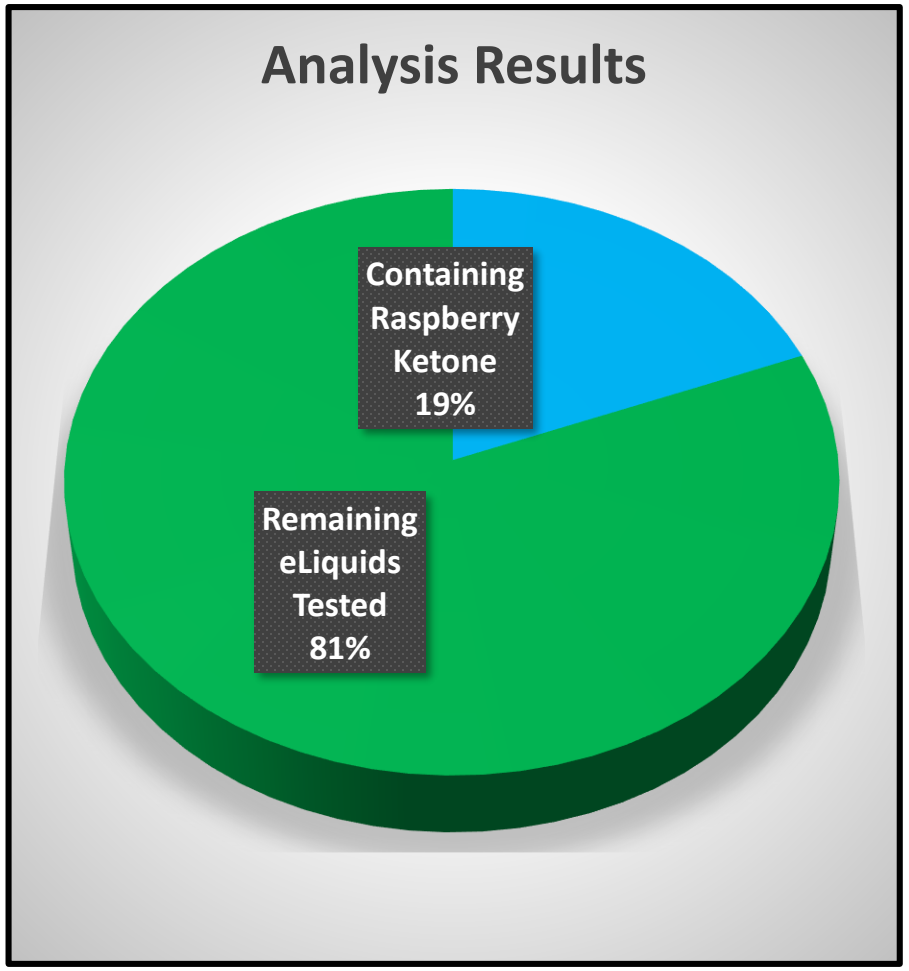
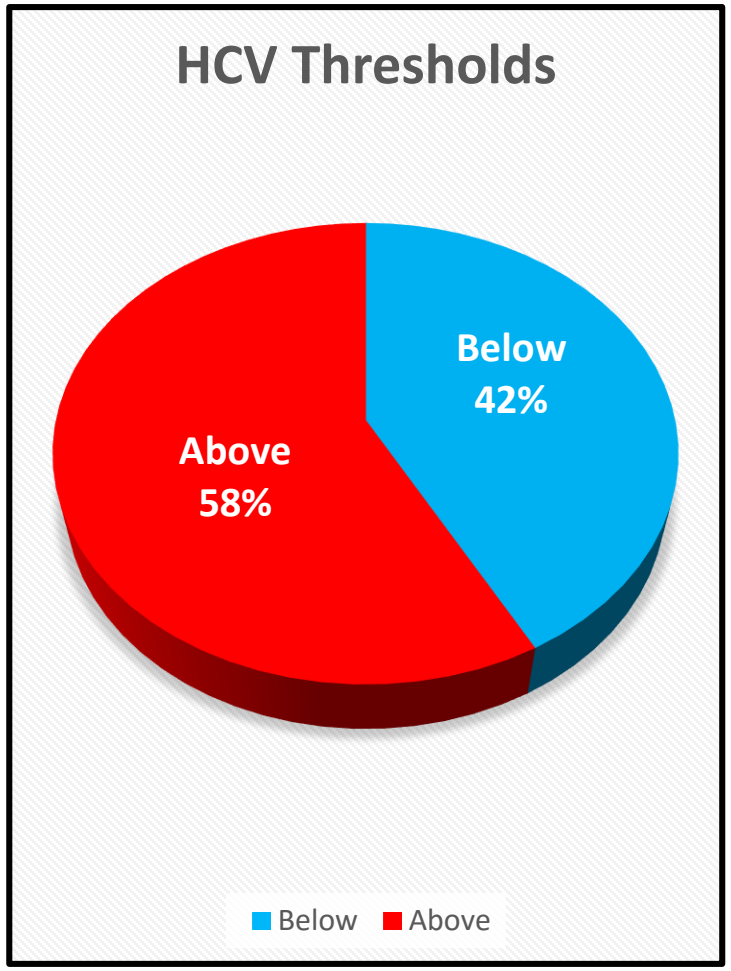
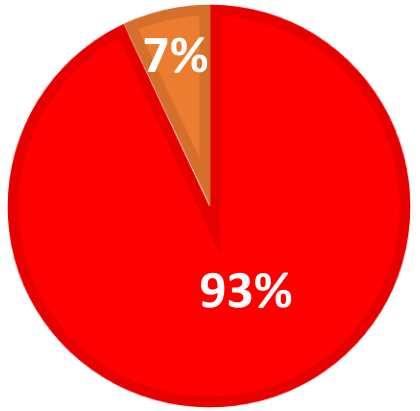
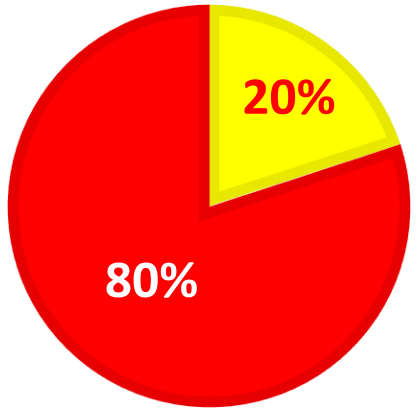






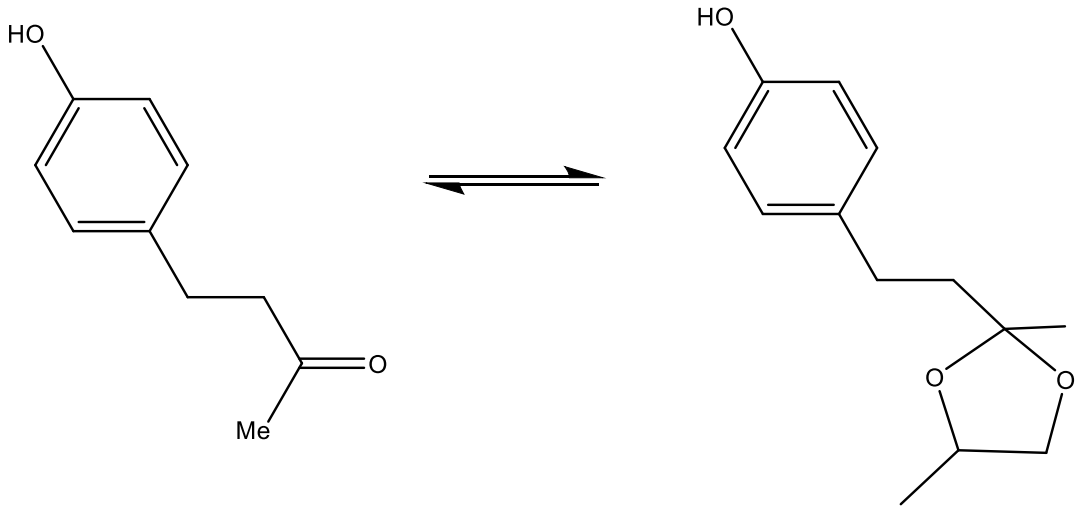
93% more than <u>double</u> HCV



20% more than <u>triple</u> HCV

Acetal and Ketal Formation

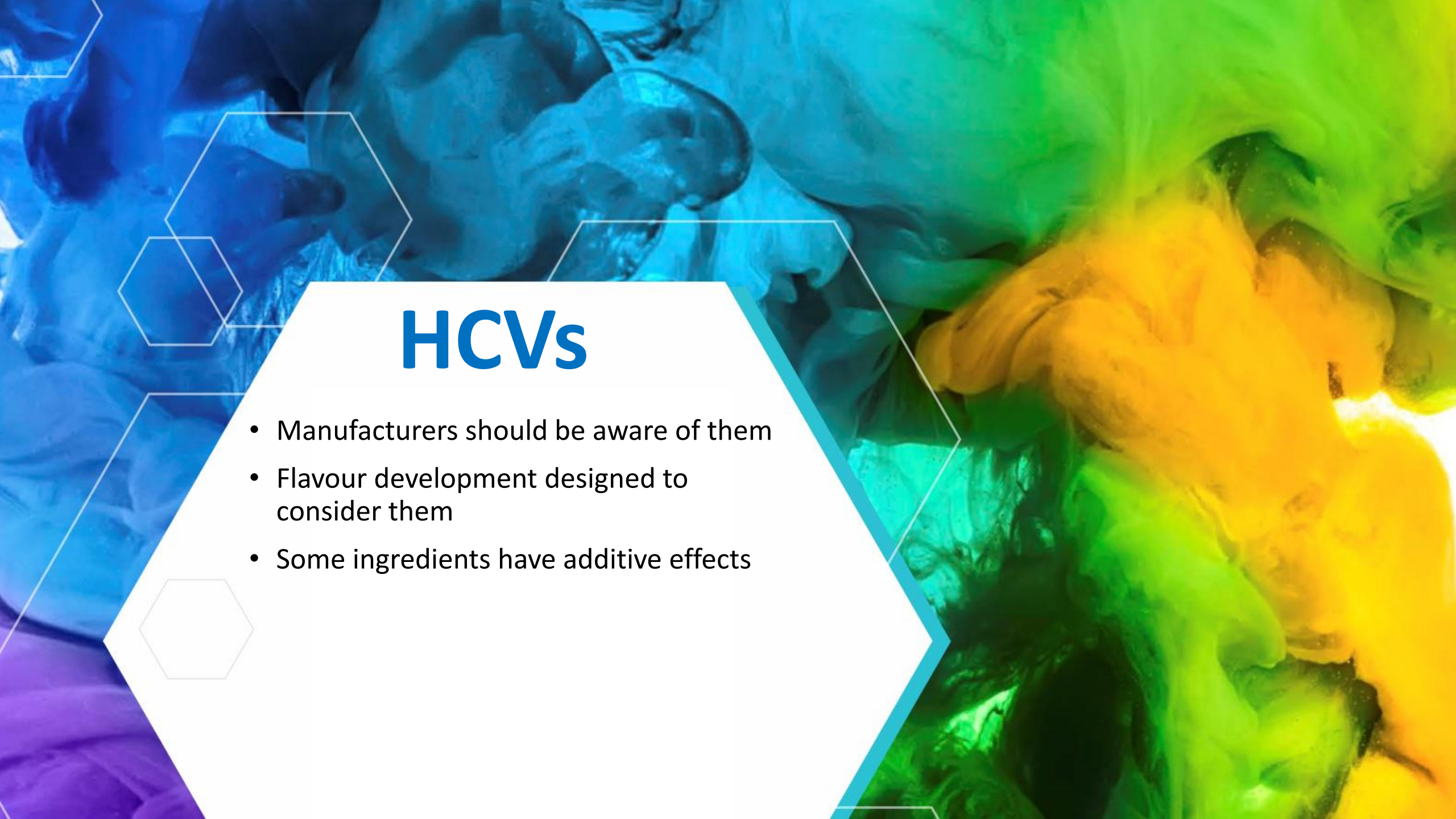
- Characteristic reaction of aldehydes and ketones
- In eliquids, this occurs in conjunction with propylene and other glycol carriers



Upon standing in propylene glycol for long periods, an equilibrium between raspberry ketone and it's propylene glycol acetal will occur

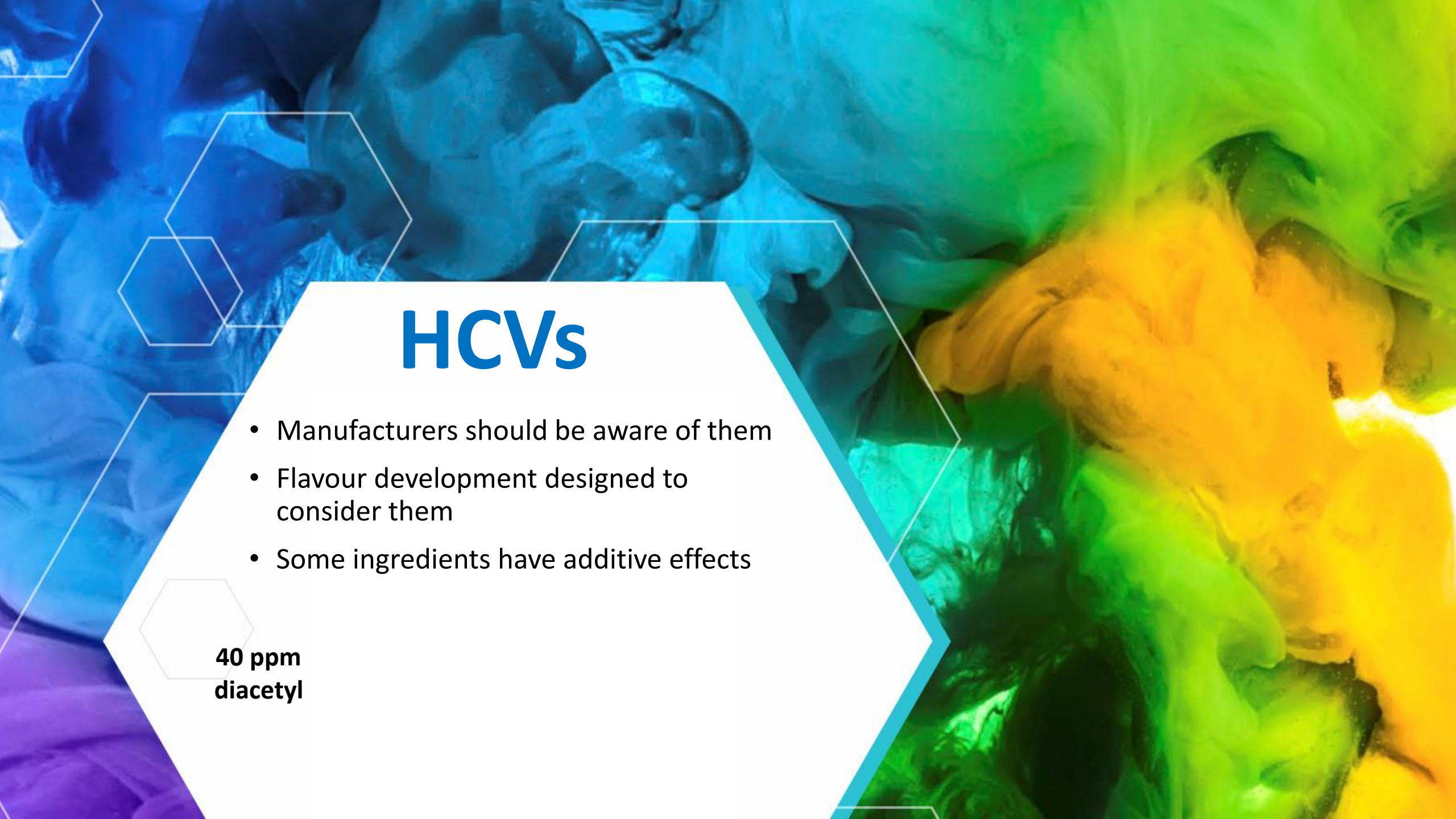
- Due to the presence of numerous stereocentres and E/Z isomerism, several acetal forms can be found during analysis

Each of these contribute to the total percentage of the ingredient in the eLiquid



HCVs

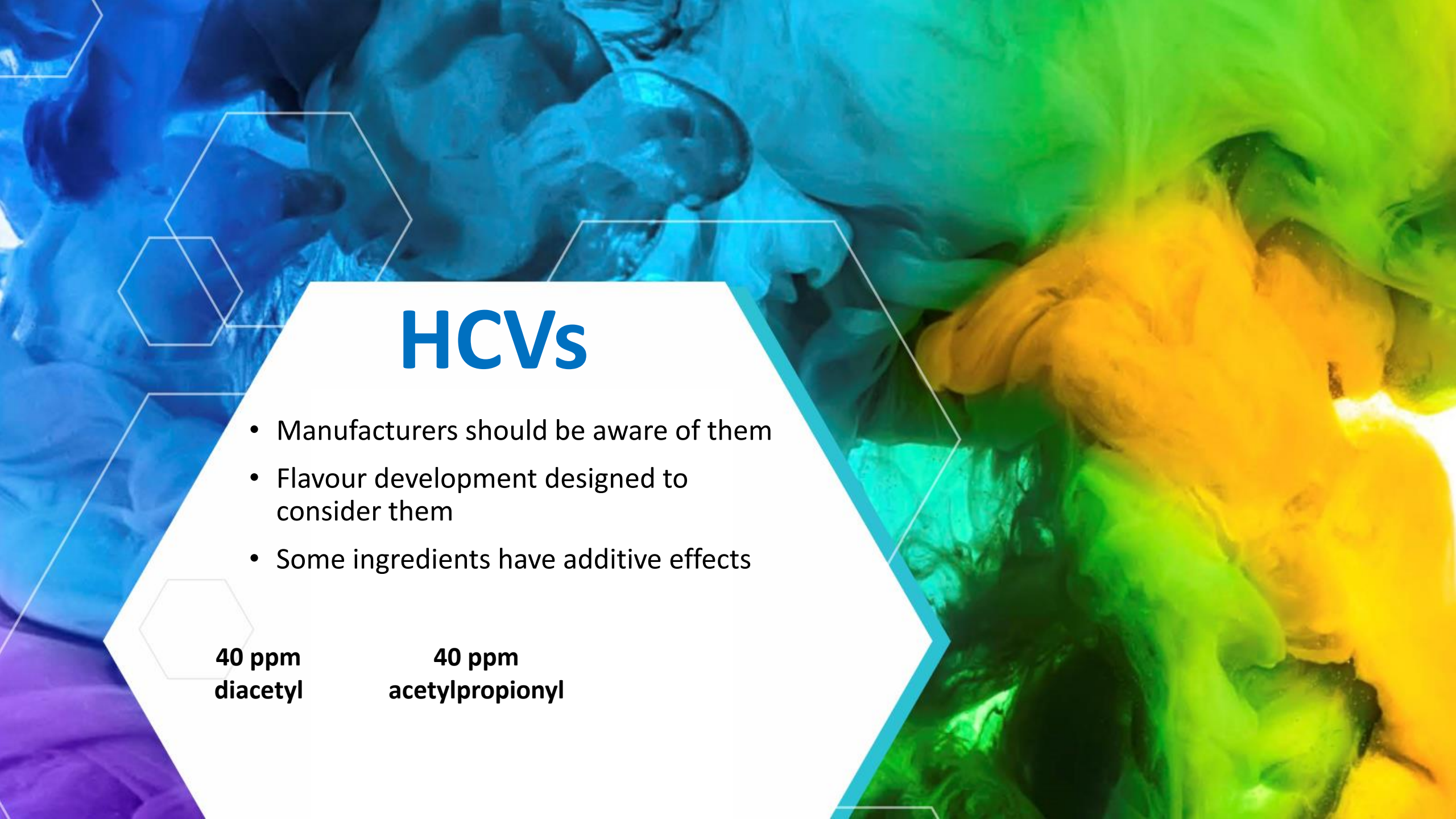
- Manufacturers should be aware of them
- Flavour development designed to consider them
- Some ingredients have additive effects



HCVs

- Manufacturers should be aware of them
- Flavour development designed to consider them
- Some ingredients have additive effects

40 ppm diacetyl



HCVs

- Manufacturers should be aware of them
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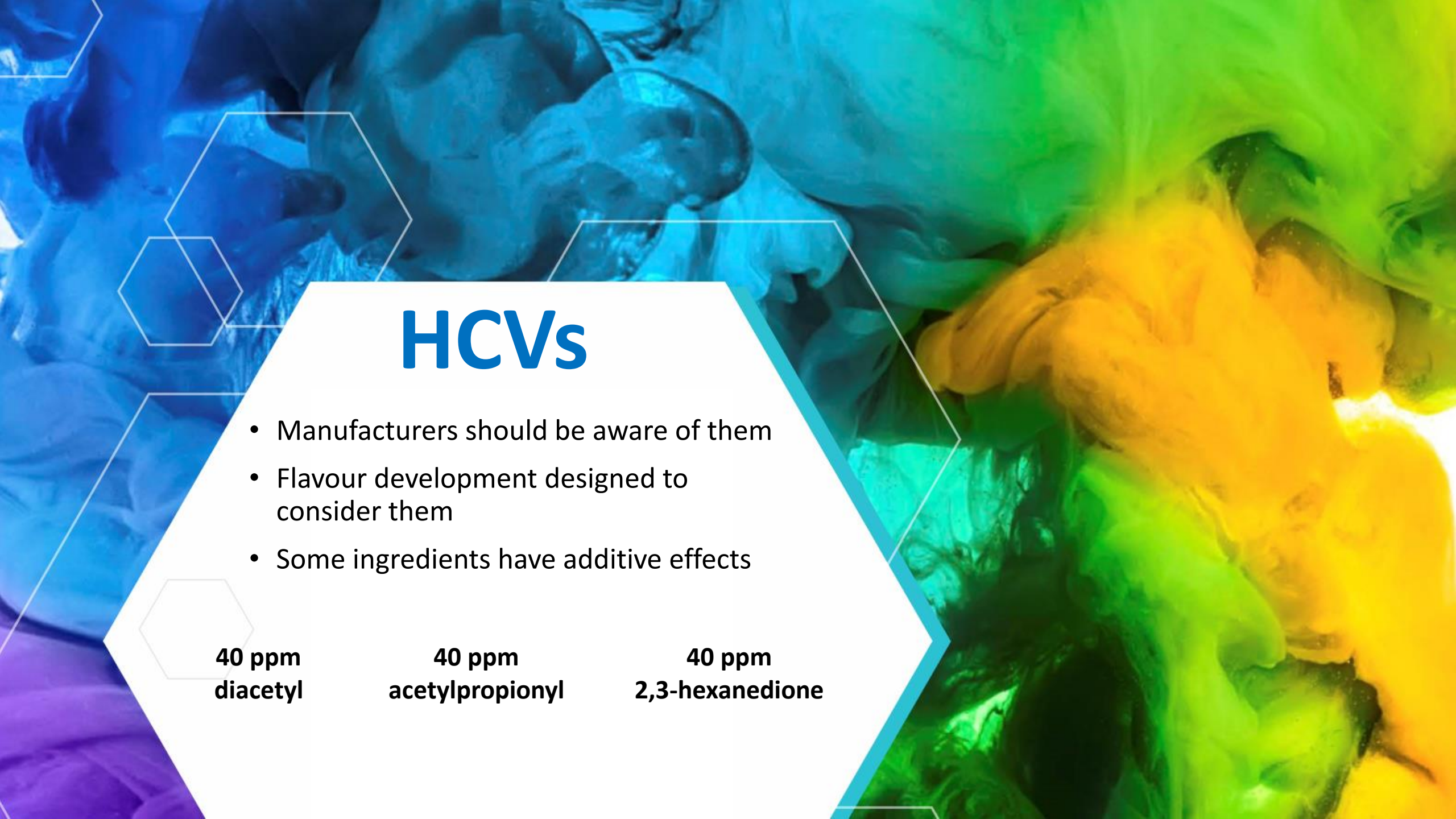
- Some ingredients have additive effects

40 ppm diacetyl

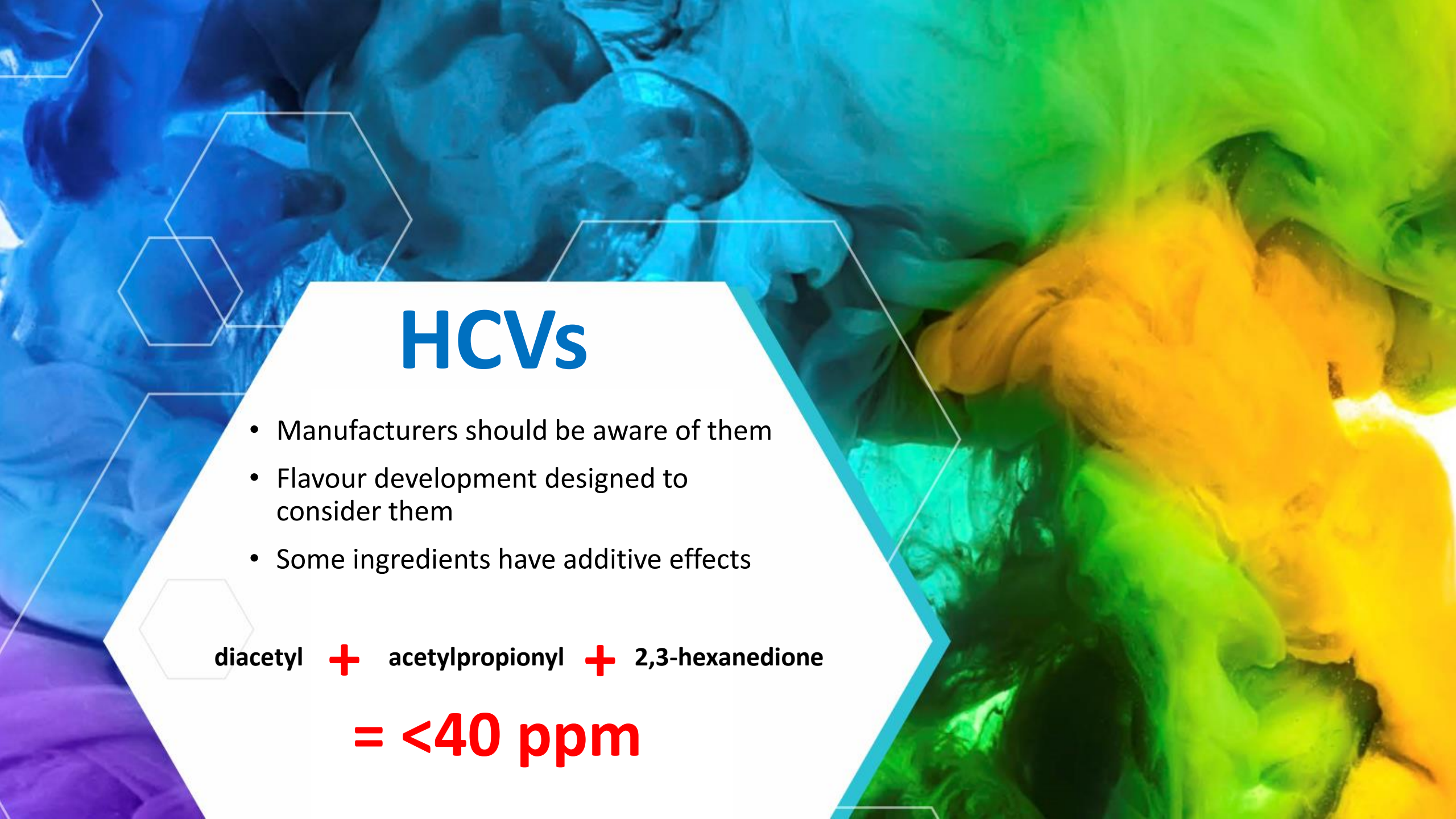
40 ppm acetylpropionyl



HCVs

- Manufacturers should be aware of them
- Flavour development designed to consider them
- Some ingredients have additive effects

40 ppm	40 ppm	40 ppm
diacetyl	**acetylpropionyl**	**2,3-hexanedione**



HCVs

- Manufacturers should be aware of them
- Flavour development designed to consider them
- Some ingredients have additive effects

diacetyl + acetylpropionyl + 2,3-hexanedione

= <40 ppm

The Future of the Vape Industry

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The Demon Vaper

Lemon Lush

THE VAPER EXPO UK

Winner!
Best Dessert

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Conclusion

- Raise awareness of HCVs amongst:
  - Consumers
  - Retailers
  - Manufacturers
  - Public health bodies
- HCVs are relevant to all eLiquid ingredients, not just diacetyl and acetylpropionyl
- Important for TPD and General Consumer Safety

Questions?

Let's Work Together

We believe that working together as an industry will help set the standard with regard to TPD compliance, and will set our partners and customers aside as being responsible and ethical eliquid manufacturers.

For more information please call **+44 (0) 1733 352 553,**

email **contact@elscience.co.uk**

or visit our website **www.elscience.co.uk.**