Nicoventures – manufacturer of e-cigarettes

Aim: To provide adult smokers wanting to reduce, replace or stop smoking with the best range of quality alternative products, delivering much of the experience they expect from a cigarette without the serious health risk of smoking.

An autonomous business within the British American Tobacco Group
Outline

• Introduction
• Ingredients
  • Ingredient screening
  • Inhalation data gap
    1. In vitro method
    2. TTCs for ingredients
  • Consumer exposure data
• Aerosol – Potential thermal breakdown and reaction products
  • Analytical
  • TTC for aerosol contaminants
• Summary
Composition of e-cigarette aerosols

Many components impact e-cigarette aerosols

Main formulation ingredients:
- Glycerol
- PG
- Nicotine
- Water
- Flavourings
## Product Areas that need Toxicological Assessment

<table>
<thead>
<tr>
<th>Ingredients / device materials</th>
<th>Thermal degradation products</th>
<th>Degradation products over time</th>
</tr>
</thead>
<tbody>
<tr>
<td>All formulation ingredients</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Contaminants from formulation ingredients</td>
<td>√</td>
<td>Stability and leachables feeds back into tox risk assessment</td>
</tr>
<tr>
<td>Device materials functionality</td>
<td>√</td>
<td>(Thermal resistance)</td>
</tr>
<tr>
<td>Device materials migrated into formulation</td>
<td>√</td>
<td>Includes metals: Ni, Cr, Fe, etc.</td>
</tr>
</tbody>
</table>

- Includes carbonyls: formaldehyde, acetaldehyde, acrolein
Risk Assessment Paradigm?

- **Supportable** level of risk?

- Potential comparators:
  - Harm reduction opportunity for smoking -> cigarettes
  - Smoking cessation product -> Nicotine Replacement Therapy
  - General consumer product -> household products, cosmetics, foods, environmental background exposures

- Our philosophy is based on:
  - Avoidable exposures should not add any health risk over and above that from the base product
    - E.g. flavour choices, device material choices
  - Exposures from base product should be as low as reasonably practicable* and tolerable, lower than cigarettes where applicable

* ALARP changes constantly with state of the art. Also needs to take into account effectiveness of product, making them acceptable to smokers

Includes glycerol/PG, carbonyls and metals
E-liquid safety assessment process

Ingredients

- E-liquid formulation
  - Supplier confirmed food/pharma grade? Full disclosure?
    - No
      - Full quantitative disclosure
        - Any ingredients classified CMR* or respiratory sensitizers?
          - Yes
            - Review toxicological data on each ingredient.
          - No
            - Risk assess proposed levels of use for systemic and local toxicity
            - Supportable?
              - Yes
                - Supportable?
                  - Yes
                    - Ingredient not supported
                  - No
                    - Formulation/ingredient supported at proposed level of use
              - No
                - Overall risk assessment

- Thermal breakdown & reaction products

GC/MS of aerosol

- Semi-quantify. Identify peaks resulting in estimated exposure above 1.5 ug/day.

- Identify peaks not due toingoing ingredients or nicotine-related.

- Risk assess compounds identified based on semi-quantified levels

- Supportable?
  - Yes
  - No

*CMR – Carcinogenic, Mutagenic, Toxic for Reproduction

Purity requirements – Pharma and food grade

- Require pharma grade for nicotine and humectants, food grade for flavour ingredients
  - Limits potential contaminants
  - Provides some qualitative reassurance on systemic toxicity
  - Provides some reassurance on quality assurance in the supply chain

- Full ingredient disclosure
  - CAS and FEMA#s
  - For naturals: botanical and geographical origin, extraction processes
Hazard exclusion screening – 1. No CMRs

Exclude CMR:
- IARC* group 1, 2A or 2B carcinogens
- FDA or harmonized EU classification
- ECHA SVHC
- FDA HPHC
- If not evaluated for classification: Weight of Evidence approach

*IARC – International Agency for Research on Cancer

*CMR – Carcinogenic, Mutagenic, Toxic for Reproduction
Hazard exclusion screening – 2. No respiratory sensitizers

Why?
- Relevant route of exposure
- Potential severity of symptoms
- Potentially very low derived tolerable levels
  - E.g. occupational exposure guidelines for isocyanates and anhydrides in μg/m³, in ng/m³ for several enzymes

Hazard identification is weight of evidence approach:
- Occupational exposure
- Literature data
- Regulatory classifications
- Compendiums

Note: contact sensitisation approach published in Eurotox 2014 poster, available at www.BAT-science.com

*CMR – Carcinogenic, Mutagenic, Toxic for Reproduction
Toxicological risk assessment – Review of existing data

- Review existing toxicological data, including occupational experience
- Local responses via other routes may help inform respiratory toxicity, e.g. irritation
- Use international scientific opinions where available (viz. JECFA, IARC)
- If no data on naturals – break down into separate constituents

This should identify inhalation specific issues, e.g. diacetyl potential for bronchiolitis obliterans
Toxicological risk assessment – Review of existing data

- Common finding: lack of inhalation data, especially on flavours
  - Most common adverse event reported in relation to vaping is respiratory irritation
    → Developed in vitro cytotoxicity method to assess
  - Developed TTC approach to be able to support low level ingredients/contaminants

- Risk assessment requires informed consumer exposure estimates as well
Respiratory irritation – developed In vitro cytotoxicity model*

- Appropriate cells/tissue – EpiAirway™
  - From species of interest: reconstruct from primary human cells
  - Relevant cell types: tracheobronchial epithelium
  - Realistic complexity: 3D fully differentiated, metabolically active. Includes variety of cell types, including mucus producing goblet cells, columnar cells, ciliated cells

- Exposure to the appropriate entity – the vaping aerosol

- Based on same principles as OECD 439 – Skin irritation cytotoxicity testing
  - Irritant criterium in OECD 439: 50% reduction in cell viability versus control

Test set up
Use in risk assessment – 1. Hazard identification

- No decrease in tissue viability over 6 hours continuous exposure
  - N=3 for e-cig samples, N=6 for air control

This e-cigarette aerosol did not act as an irritant to the physiologically relevant respiratory epithelium
Use in risk assessment – 2. Comparison against a relevant benchmark

At whole product level
- E-cigarette aerosol (80/3/30) versus 3R4F smoke (35/2/60)
- E-cigarette aerosol significantly less irritating than cigarette smoke

At flavour ingredient level
- Demonstrate no difference in effect of aerosol from flavoured formulation versus unflavoured base liquid
- Support for menthol level in Vype
The TTC concept

Threshold of (no) Toxicological Concern
• In 2012 reviewed by AMGP, EFSA and jointly by 3 non-food European safety committees SCCS, SCHER and SCENIHR

Globally in use/proposed for use, e.g.
• FDA – food additives
• ICH – M7: genotoxic impurities in human medicines
• JECFA & EFSA – food flavourings
• WHO proposal – food chemicals

Exclusion criteria
• Biological end points: ‘allergy’ (contact and respiratory sensitisation, intolerance), radiation effects, hormonal, enzymatic and potent pharmacological effects
• Certain types of compounds:
  • Aflatoxin-like, azoxy-, N-nitroso-compounds, benzidines and hydrazines, metals and polyhalogenated dibenzo-p-dioxins, polyhalogenated dibenzofurans and polyhalogenated biphenyls, or other compounds known to accumulate in the body, e.g. Ochratoxin A.
  • High molecular weight chemicals, such as polymers, because such structures are not covered by the databases.
  • Insoluble particles and nanomaterials,
  • Complex or highly unique chemical structures having several structural elements are not adequately represented in the available databases.

All ingredients that have reached this stage in the process are:
- Food or pharma grade purity
- Not CMR, i.e. also not genotoxic
- Not known respiratory sensitisers

Main consumer exposure route will be inhalation

Contact sensitisation not addressed by TTC. Assessment approach proposed elsewhere
<table>
<thead>
<tr>
<th>Source</th>
<th>Area of use</th>
<th>TTC [ug/day]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM</td>
<td></td>
<td>0.15</td>
<td>For genotoxins and mutagens</td>
</tr>
<tr>
<td>FDA</td>
<td>Food additives</td>
<td>1.5 or 0.5ppb in food</td>
<td>Regardless of toxicity profile, assuming 10% of chance of being carcinogenic. Based on oral data on 709 carcinogens</td>
</tr>
<tr>
<td>EMEA &amp; ICH</td>
<td>Medicines</td>
<td>1.5</td>
<td>For non-threshold, genotoxic contaminants. Risk benefit, therefore $10^{-5}$ life time cancer risk considered acceptable</td>
</tr>
<tr>
<td>ILSI</td>
<td>Food</td>
<td>0.15</td>
<td>Where structural alerts for genotoxicity</td>
</tr>
<tr>
<td></td>
<td>Food &amp; flavourings (JECFA)</td>
<td>1.5</td>
<td>For non-genotoxins. Based on analysis of carcinogenicity databases with genotoxic and non-genotoxic compounds</td>
</tr>
<tr>
<td></td>
<td>Food (JECFA &amp; EFSA)</td>
<td>90</td>
<td>Cramer Class 3</td>
</tr>
<tr>
<td></td>
<td>Food (JECFA &amp; EFSA)</td>
<td>540</td>
<td>Cramer Class 2</td>
</tr>
<tr>
<td></td>
<td>Food (JECFA &amp; EFSA)</td>
<td>1800</td>
<td>Cramer Class 1</td>
</tr>
</tbody>
</table>
## Inhalation TTCs

<table>
<thead>
<tr>
<th>Area of proposed use</th>
<th>TTC [ug/day]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic air toxins</td>
<td>0.7</td>
<td>Recalculating oral 1.5ug/day TTC to continuous, environmental inhalation exposure. Validated by comparing against 1857 air guideline values.</td>
</tr>
<tr>
<td>Cigarettes</td>
<td>1.5</td>
<td>PMI threshold for evaluating ingredients and contaminants, based on oral TTC.</td>
</tr>
<tr>
<td>Industrial chemicals - Cramer Class 3</td>
<td>2.7</td>
<td>Excl. genotoxins and organophosphates. Based on inhalation data on 250 compounds in RepDose database [Escher et al, 2013]</td>
</tr>
<tr>
<td>Excluding genotoxins and organophosphates. Based on inhalation data on 122 compounds in RepDose database [Escher et al, 2010]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial chemicals - Cramer Class 1</td>
<td>3979</td>
<td>Based on inhalation data on 250 compounds in RepDose database [Escher et al, 2013]</td>
</tr>
<tr>
<td>Excluding genotoxins and organophosphates. Based on inhalation data on 122 compounds in RepDose database [Escher et al, 2010]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumer aerosol products - Cramer Class 3</td>
<td>170</td>
<td>Excl. genotoxic carcinogens. Based on 92 rat inhalation studies.</td>
</tr>
<tr>
<td>Consumer aerosol products – Cramer Class 1</td>
<td>980</td>
<td>Excluding genotoxic carcinogens. Based on 92 rat inhalation studies.</td>
</tr>
</tbody>
</table>
Consumer Exposure Data

- Exposure estimate requires quantitative data on how consumers use the product
  - Can vary between vaping products
  - Vype consumption and topography study
  - To protect majority of consumers, estimate “realistic worst case” exposure use using 95\textsuperscript{th} percentile data

- Topography data also informs testing regime to be used for chemical and biological testing, e.g.
  - When determining amount of ingredient per puff
  - Puffing regime used in aerosol in vitro testing model (80/3/30)
Modified SA7 Topography Holder (e-cigarette holder)

Spigot adapter to attach e-cigarettes to modified SA7 topography holder. Spigot *removes* jetting through orifice, which results in inaccurate puffing topography measurements.

- **Pressure ports on top to reduce excipients from blocking tubes**
- **Bracket to support larger e-cigarette modular devices**
- **Removable cap allows access to orifice plate for cleaning**
1. Analytical aspects
2. TTC
Analytical aspects

Generate aerosol
- For e-liquids sold separately, choose compatible device. Include information on test conditions in IFU

Targeted scan for carbonyls and metals

Developed untargeted scan: TD-GC/MS-TOF
- Specificity - Breadth of flavours, generally volatile
- Accuracy - Detection limit based on TTC for unknown contaminants: 1.5 µg/day
  - Eg. If consumer data suggest 95th %-ile for a specific product type is 300 puffs/day → LOD 5ng/puff

GC/MS of aerosol

Semi-quantify. Identify peaks resulting in estimated exposure above 1.5 µg/day.

Identify peaks not due to ingoing ingredients or nicotine-related.

Risk assess compounds identified based on semi-quantified levels
TTCs for contaminants, thermal breakdown and reaction products

- In contrast to ingredients, no pre-selection has taken place, i.e. CMR properties cannot be excluded.
- Oral TTC’s well established and based on large databases of carcinogenic compounds
- Joint EU scientific committees, international workshop on TTCs and Eurotox 2013 discussions: currently route to route extrapolation from oral values preferred
TTCs for contaminants, thermal breakdown and reaction products

Combined oral to vaping inhalation TTC (route and duration) safety factor: $0.33 \times 2 = 0.67$

- Round up to 1 given uncertainties in route extrapolation

Overall oral TTC to vaping inhalation TTC (route and vaping duration) safety factor: 1

<table>
<thead>
<tr>
<th>Non-ingredient vaping inhalation TTC [ug/day]</th>
<th>Compounds</th>
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<td>Cramer Class 2 &amp; 3</td>
</tr>
<tr>
<td>1800</td>
<td>Cramer Class 1</td>
</tr>
</tbody>
</table>
Robust yet practical approach to safety evaluations:

- Ingredient purity & hazard exclusion screening requirements
  - Pharma and food grade
  - Do not use CMRs and respiratory sensitizers

- Inhalation data gap –
  - In vitro cytotoxicity model
  - Ingredient inhalation TTC: 170 µg/day for Cramer class 2 & 3, 980 µg/day for Cramer class 1

- Exposure assessment data
  - Consumption and topography on relevant vaping product

- Potential reaction and thermal breakdown products
  - Targeted and untargeted scans
  - Contaminant vaping inhalation TTCs: 0.15, 1.5, 90, 1800
ACKNOWLEDGEMENTS

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