Summary of Toxicology Data

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The Chemical Structure of Diacetyl

- Reactive
- Can cause protein cross-links
- Can inactivate proteins
The Chemical Structure of 2,3-Pentanedione

- Reactive
- Can cause protein crosslinks
- Can inactivate proteins
- Reported to be somewhat more reactive with arginine groups than diacetyl
Metabolism

\[ \text{O} \quad \text{C} \quad \text{C} \quad \text{O} \quad \text{R} \quad + \quad \text{NADPH} \quad \xrightarrow{\text{DCXR}} \quad \text{O} \quad \text{C} \quad \text{C} \quad \text{R} \quad \text{OH} \quad + \quad \text{NADP} \]
Experimental Inhalation Toxicology
Normal Airway Epithelium

10 µm
Acute Diacetyl (2,3-butanedione) Effect

10 µm
Comparable 2,3-Pentanedione Effect

![Graph showing pathology score vs concentration for different conditions.](image)
Experimental Inhalation Toxicology: Summary of Morphology Data

- Butter flavoring vapors that contain diacetyl cause airway epithelial damage (Hubbs et al, 2002)
- Diacetyl causes airway epithelial damage
  - Rats (Hubbs et al, 2008)
  - Mice (Morgan et al, 2008)
- In rats and mice the nose is the most-affected site (Hubbs et al, 2002, 2008; Morgan et al, 2008)
- Bronchi and bronchioles are affected at higher exposures or exposures of longer duration (Hubbs et al, 2002, 2008; Morgan et al, 2008)
- Bronchiolitis obliterans is produced by experimental aspiration of diacetyl (Palmer et al, 2011)
- Acute exposures to 2,3-pentanedione are comparable to diacetyl in causing airway epithelial damage (Hubbs et al, 2010; Morgan et al, 2010)
The Pharmacokinetic Model Predicts More Diacetyl is Removed by the Nose of Rats

<table>
<thead>
<tr>
<th></th>
<th>Anterior Trachea</th>
<th>Posterior Trachea</th>
<th>Air Exiting Trachea</th>
<th>% Absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1.2 mM</td>
<td>1.1 mM</td>
<td>61 PPM</td>
<td>39%</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose-Breathing</td>
<td>1.2 mM</td>
<td>1.2 mM</td>
<td>79 PPM</td>
<td>21%</td>
</tr>
<tr>
<td>Mouth-Breathing</td>
<td>1.5 mM</td>
<td>1.5 mM</td>
<td>96 PPM</td>
<td>4%</td>
</tr>
</tbody>
</table>

Morris and Hubbs, 2009
A New Pharmacokinetic Model which Includes Airways of the Deep Lung

- Dose to the bronchiolar epithelium of humans under light exercise conditions is predicted to be more than 40-fold greater than the dose to the bronchiolar epithelium of experimental exposed rats

Gloede et al, 2011
Diacetyl Instillation Causes Bronchiolitis Obliterans in Rats

- Large single dose of diacetyl by intratracheal instillation bypassed the rodent nose
- Abnormal repair of the injured bronchiolar epithelium
- Produced bronchiolitis obliterans

Palmer et al, 2011
Human Relevance

• Damage to the respiratory epithelium of the small bronchioles is believed to cause bronchiolitis obliterans (King, 1989)
• The respiratory epithelium is damaged by butter flavoring vapors, diacetyl and 2,3-pentanedione (Hubbs et al, 2002, 2008; Morgan et al, 2008)
• Inhalation of diacetyl produces a higher dose to the bronchioles of humans than rodents (Gloede et al, 2011)
• Diacetyl instillation causes bronchiolitis obliterans in rats (Palmer et al, 2011)
• Clinical bronchiolitis obliterans is seen in workers inhaling diacetyl (Kreiss et al, 2002; Akpinar-Elci et al, 2004; Van Rooy et al, 2007).
Human Relevance

Akpinar-Elci et al, 2004
In vivo Pulmonary Function Changes After Diacetyl Experimental Exposures

• Acute diacetyl inhalation decreased tidal volume and mid-expiratory flow rate (Larsen et al, 2009)
• Acute high dose exposures decreased the sensory irritation effect of a subsequent exposure (Larsen et al, 2009)
• Acute high dose exposure increases the number of substance P positive neurons in the jugular ganglia (Goravanahally et al, 2010)
• Mice exposed to 50 or 100 ppm diacetyl have decreased respiratory rate after a 6 week exposure (Morgan et al, 2008)
• Mice exposed to 100 ppm diacetyl have decreased minute volume after a 6 week exposure (Morgan et al, 2008)
Diacetyl and 2,3-Pentanediione Effects on Tracheas *in vitro*

- Mild airway contractions at diacetyl concentrations from $10^{-7}$ to 1 mM in guinea pig trachea (Fedan et al., 2006)
- *In vitro* methacholine response was mildly increased in rat tracheas after inhaling 360 ppm diacetyl (Fedan et al., 2010)
- *In vitro* methacholine response was mildly increased in rat tracheas after inhaling 240, 320 or 360 ppm 2,3-pentanediione (Fedan et al., 2010)
- The effect of diacetyl and 2,3 pentanediione on *in vitro* tracheal reactivity does not involve the epithelium (Fedan et al., 2011)
- *In vivo* methacholine challenge of 2,3-pentanediione exposed rats results in a decreased response to the methacholine (Fedan et al., 2010)
- High diacetyl concentrations (3mM) may affect tracheal epithelial ion transport (Fedan et al., 2006)
Additional Toxicologic Considerations

• Diacetyl is mutagenic *in vitro* (Kim et al, 1987; Marnett et al, 1985; National Toxicology Program, 2007; Whittaker et al, 2008)

• Prior skin exposure to diacetyl can sensitize to subsequent exposure (Anderson et al, 2007)
Toxicology Conclusions

• Diacetyl is a reactive alpha-diketone
• Diacetyl and mixtures of butter flavoring vapors damage airway epithelium
• Airway epithelial damage is believed to be the underlying lesion for bronchiolitis obliterans in humans
• Pharmacokinetic modeling studies indicate that at a given concentration in air, more diacetyl reaches the deep lung of humans than reaches the deep lung of rats
• The structurally related alpha-diketone flavoring, 2,3-pentanedione also damages airway epithelium