Summary of Toxicology Data

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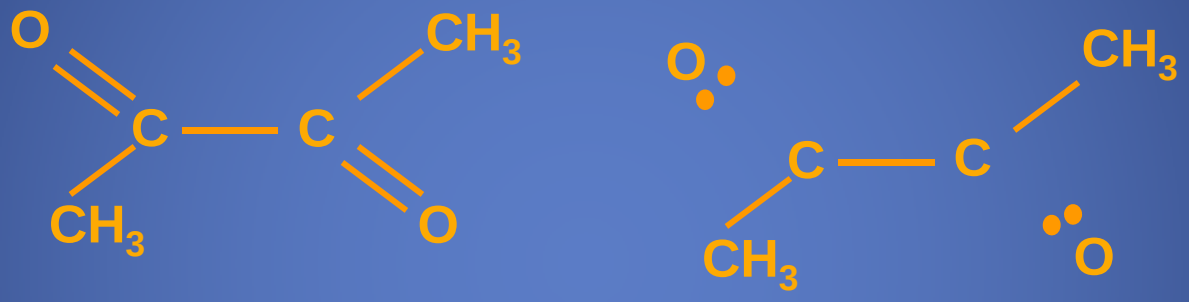
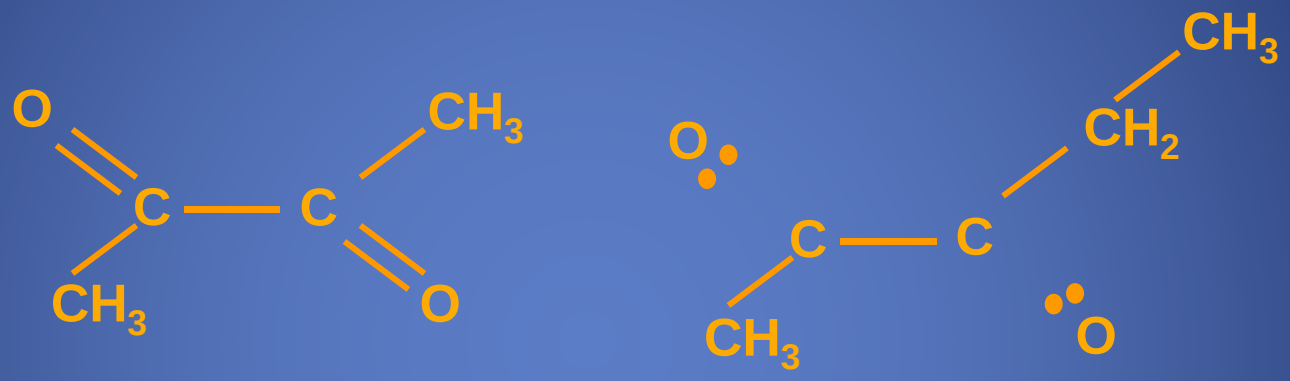
The Chemical Structure of Diacetyl



- Reactive
- Can cause protein cross-links
- Can inactivate proteins

The Chemical Structure of 2,3-Pentanedione



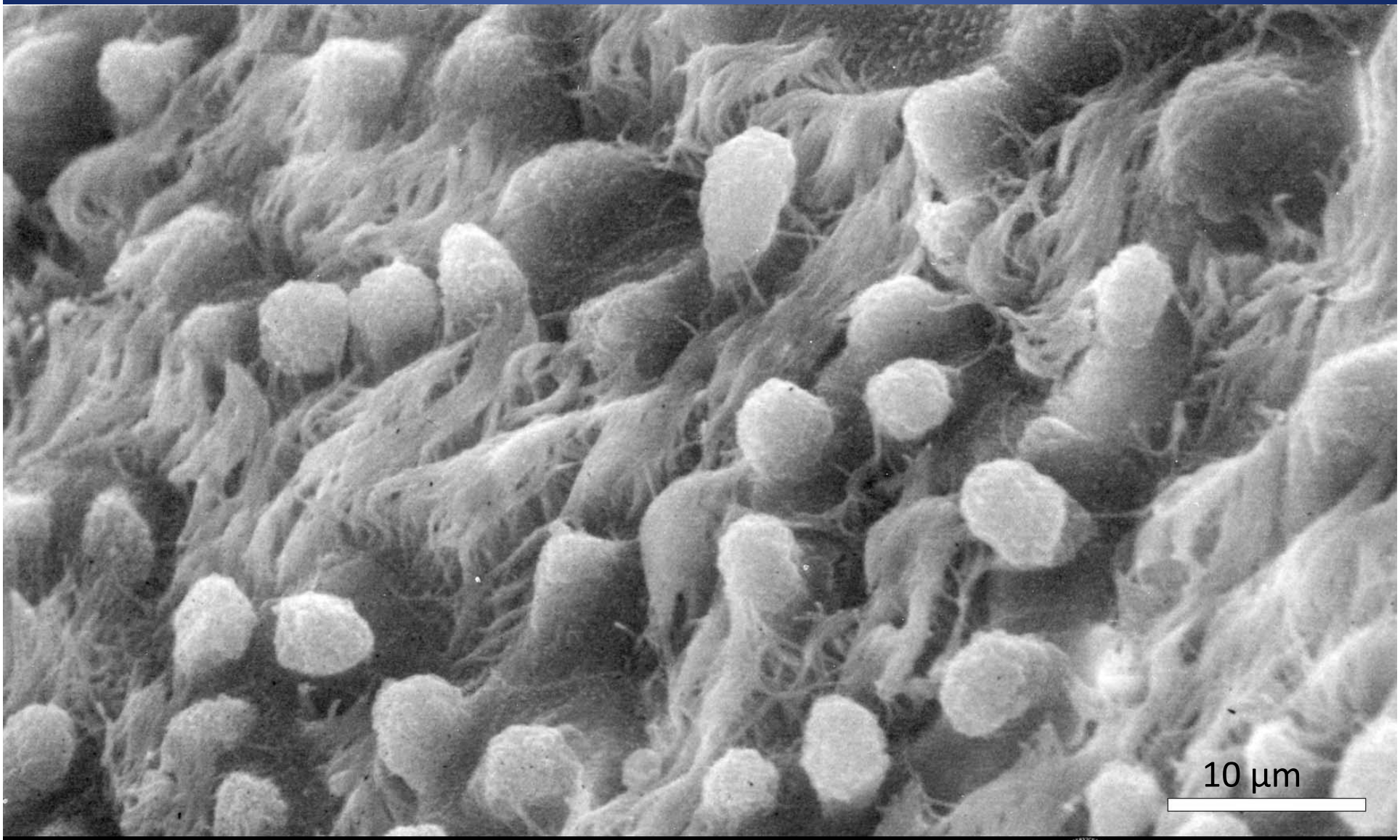
- Reactive
- Can cause protein crosslinks
- Can inactivate proteins
- Reported to be somewhat more reactive with arginine groups than diacetyl

Metabolism

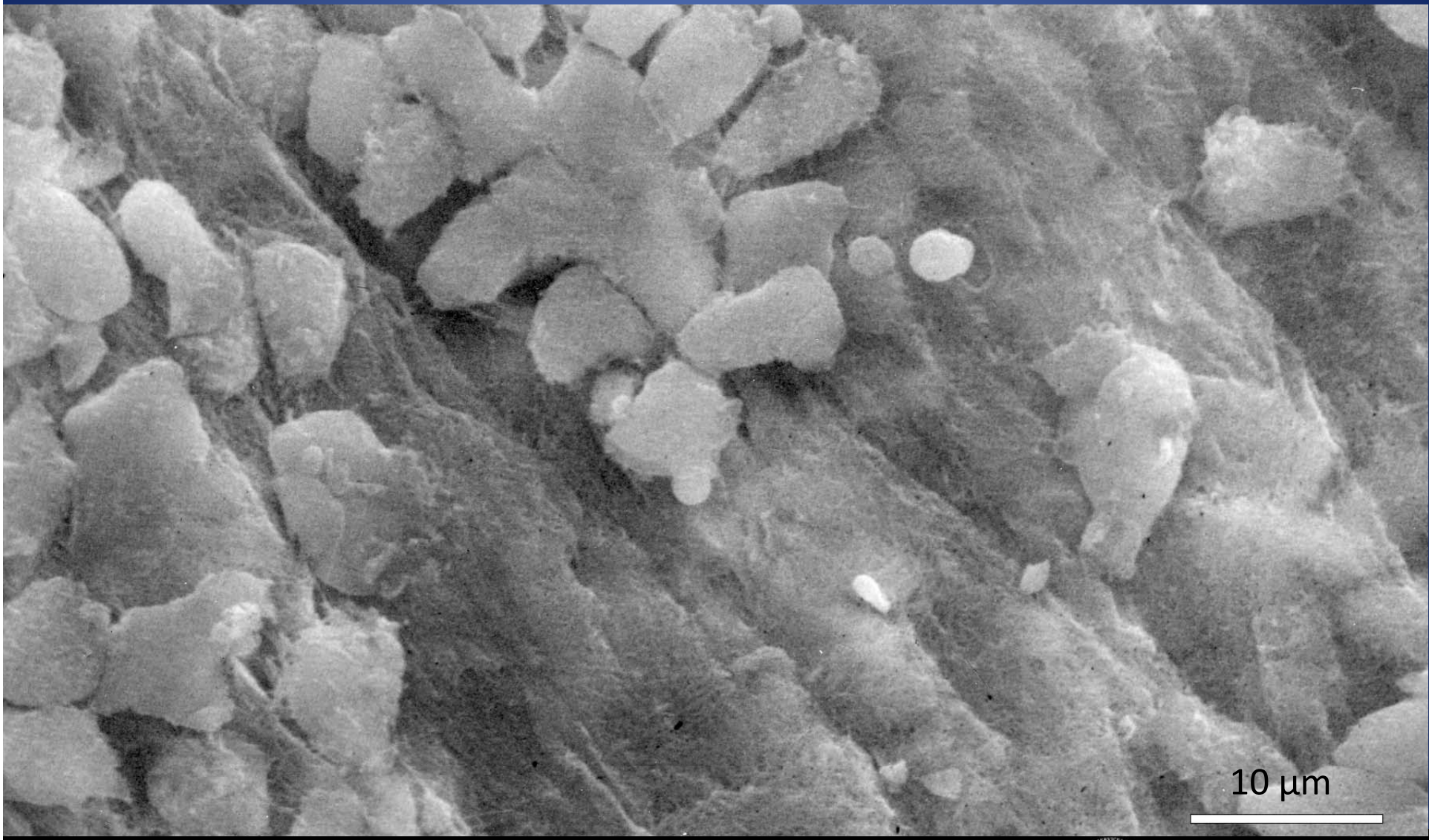
+ NADP

Experimental Inhalation Toxicology

Normal Airway Epithelium

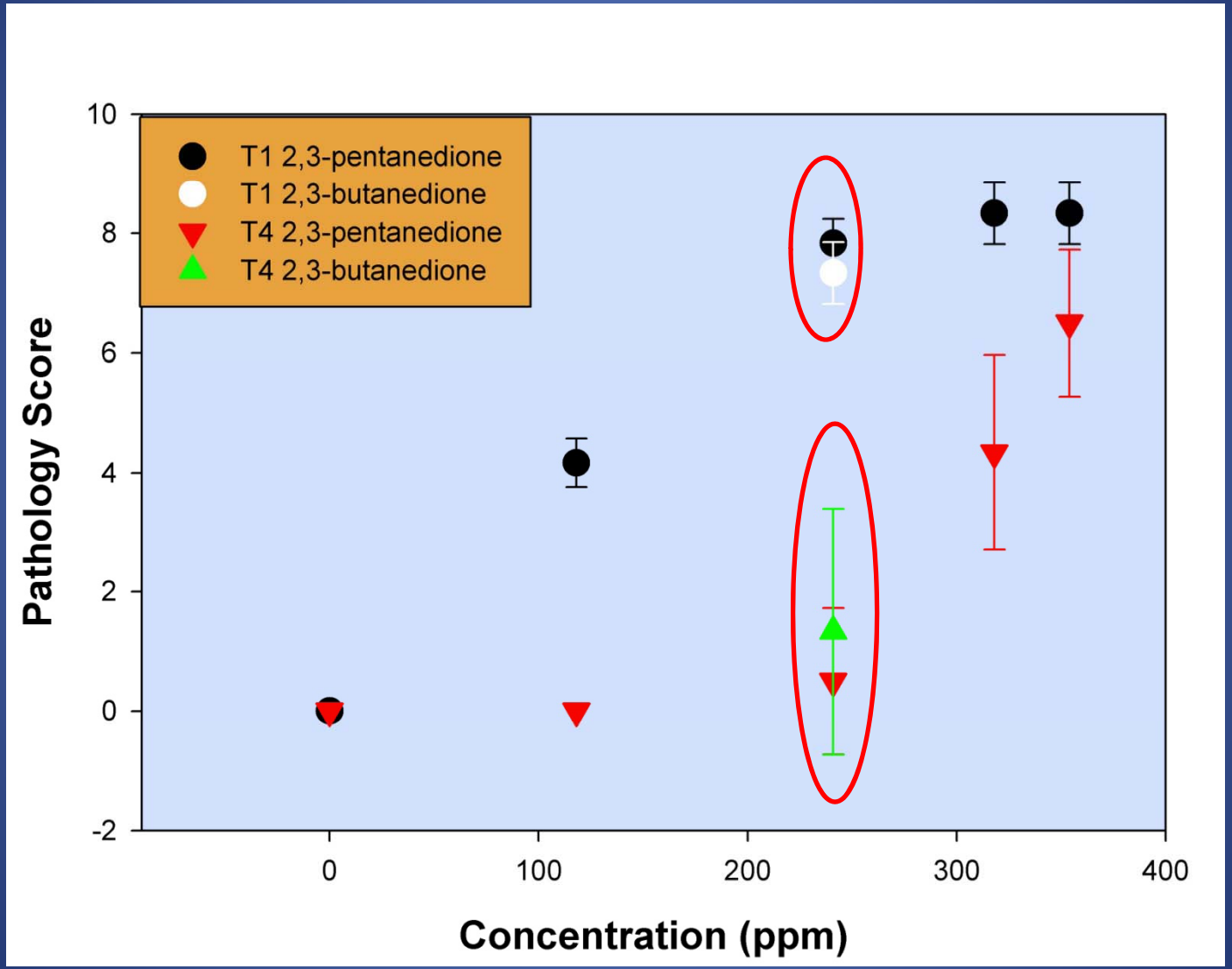


Acute Diacetyl (2,3-butanedione) Effect



Department of Health and Human Services
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

Comparable 2,3-Pentanedione Effect



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

100 ppm Exposure

	Anterior Trachea	Posterior Trachea	Air Exiting Trachea	% Absorbed
Rat	**1.2 mM**	**1.1 mM**	**61 PPM**	**39%**
Human				
Nose-Breathing	**1.2 mM**	**1.2 mM**	**79 PPM**	**21%**
Mouth-Breathing	**1.5 mM**	**1.5 mM**	**96 PPM**	**4%**

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

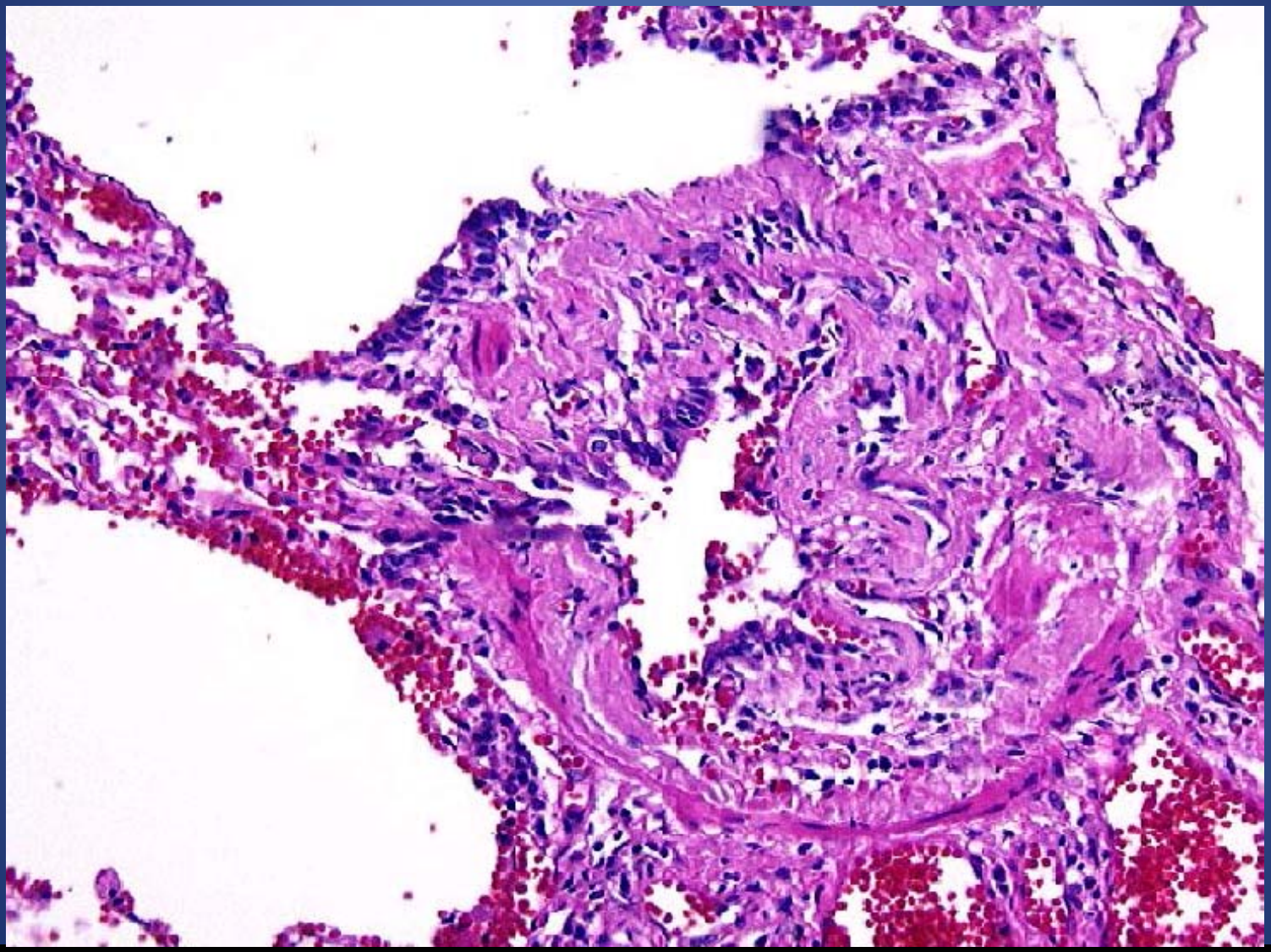
- Large single dose of diacetyl by intratracheal instillation bypassed the rodent nose
- Abnormal repair of the injured bronchiolar epithelium
- Produced bronchiolitis obliterans

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-Pentanedione 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-pentanedione (Fedan et al, 2010)
- The effect of diacetyl and 2,3 pentanedione on *in vitro* tracheal reactivity does not involve the epithelium (Fedan et al, 2011)
- *In vivo* methacholine challenge of 2,3-pentanedione 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