Toxicology Report: 2,3-Butanedione “Diacetyl”

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Executive Summary

In May 2000, an unusual fixed airways obstruction in eight workers of a microwave popcorn plant in Missouri brought up national attention. However, the cause of this lung disease, resembling bronchiolitis obliterans, was due to exposure to the predominant vapor of diacetyl that gives the smell and flavor of butter to popcorn. Following that, many more cases of lung disease were reported in 2002 -2006 which resulted in passing the Popcorn Workers Lung Disease Prevention Act in 2007.

Diacetyl can be made through fermentation, and a copper catalyst. It gives the butter flavor and taste to some foods such as popcorn, dairy products, coffee, and alcoholic beverages. Diacetyl can be a product from decomposition of methyl ethyl ketone.

The companies primarily synthesize diacetyl to use it as a food additive. A large number of workers are exposed to diacetyl, as a result of its production and usage in the food industries. NIOSH performed an experiment in the 1980’s and found that thousands of workers were potentially exposed to diacetyl in the United States. Exposure to diacetyl may occur through inhalation of butter flavoring vapors, ingestion of foods and drinking water containing diacetyl, and dermal contact with consumer products containing this compound. These exposures may pose some significant health risks to the related workers particularly for those working directly with the substance.

Due to its relatively low vapor pressure, diacetyl is an airborne chemical in butter-flavoring for popcorn. Studies showed that diacetyl inhalation causes some lung problems in the workers who mix oil and butter flavoring at the plant depending on length of exposure. These issues can be mentioned as severe injury of the epithelium airway, shortness of breath, and cough as a result of manufacturing buttered popcorn. The prominent upper respiratory tract issues including sensory and nasal tissue irritation and inflammation were considered. Testing of rats for acute and subchronic studies indicate the upper respiratory tract to be a significant target for symptoms due to cytotoxic effects of diacetyl vapor. Workers chronic exposure to diacetyl may result to a non-reversible lung disease called bronchiolitis obliterations. Those who work in facilities with open containers of artificial butter flavoring are more susceptible to diacetyl exposure. Smokers and children if exposed to the vapor would be affected more when considering their forced expiratory volume.
Introduction

Chemical and Physical Properties of diacetyl

Diacetyl or butane- 2, 3- butanedione is an α-diketone compound with four carbons, six hydrogens and two adjacent carbonyl groups. Diacetyl is soluble in all common organic solvents while it is soluble in four parts of water.

Diacetyl is responsible for the buttery flavor of popcorn. It is a greenish-yellow liquid with a strong rancid butter odor and taste. Diacetyl is a low molecular weight, organic compound with four carbon atoms and two adjacent carbonyl groups. It is easily vaporized at temperatures historically used in microwave popcorn production, resulting in potentially high gas-phase concentrations in the workplace (Harber et al. 2006).

Chemical Structure of Diacetyl

![Diacetyl Chemical Structure](http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html)

How is it made?

Diacetyl is made by oxidation of 2-butane over a copper catalyst at 300 degree C in a yield of about 60% (Ramos 2011). It can also be obtained from methyl ethyl ketone by converting to the isonitroso compound and then decomposing to diacetyl by hydrolysis with HCl (Ramos 2011). It is produced during the yeast fermentation process of glucose as well as the alcoholic fermentation process that L-malic acid is converted to L-lactic acid. Diacetyl reduces the acidity of the wine and changes its scent by imparting a buttery flavor to wine. Low levels of diacetyl give a slick or slippery texture to beer and wine.
Uses of diacetyl

Diacetyl is naturally present in a variety of fermented foods and beverages including all types of wine, alcoholic beverages, dairy products, coffee, and vinegar. It is primarily used as an artificial flavoring agent. It is added to some foods including popcorn, flours, chocolate, cooking oils, candy, chips, frosting, and more.

Exposure Pathway

Exposure to diacetyl may occur through inhalation of butter flavoring vapors such as microwave popcorn, ingestion of food and drinking water containing diacetyl flavor, and dermal contact with consumer products containing diacetyl. The occupational exposure to diacetyl vapors in popcorn plants has produced some respiratory problems in workers.

Background History

Diacetyl became prevalent in 2000 when bronchiolitis obliterans, a rare and usually non-reversible form of lung disease, was observed in eight workers of a microwave popcorn plant in Missouri. In 2002, the National Institute for Occupational Safety and Health (NIOSH) and the Missouri health department determined the rate of lung disease in a popular popcorn-making factory as 31 percent for mixers, 1 percent for packers, and zero elsewhere in the factory (NIOSH 2011). Many more cases and lawsuits were reported by industries and NIOSH regarding this issue from 2002-2006 led to passing the Popcorn Workers Lung Disease Prevention Act in 2007.

Health Effects

Monitoring results from the respiratory systems of popcorn workers illustrated that their lung function and structure had been altered. The alterations were including the air trapping, thickening of the bronchial walls, and volume loss in the upper lobe of their lungs. Furthermore, the results from animal studies confirmed that inhalation of diacetyl by rats can cause epithelial necrosis and inflammation in the nose, larynx, trachea, and bronchi while nose rated for having the greatest sensitivity to diacetyl. Based on these studies, the primary target site of inhaled diacetyl is the upper respiratory tract in rats. However, the human nasal cavity would be less sensitive to this exposure while the primary site of injury was reported as distal airways. Relative to the rats nasal cavity, the human nasal cavity is shorter, has fewer turbinates, and a smaller surface area for removal of reactive chemicals (Phalen, 1984). Moreover, rats and rodents are nose-breathers, while humans are nasal and oral breathers (Phalen 1984). Therefore, oral breathing avoids the scrubbing action of the nasal passages, which protects the bronchioles. As a
result, higher concentrations of diacetyl or other reactive chemicals reach the bronchioles and cause more severe damages. The chemical reactivity and exposure measurement data represented that diacetyl is a respiratory hazard.

Exposure Standards and Regulations

The U.S Food and Drug Administration (FDA) recognized diacetyl to be safe as a food additive. However, in order to minimize the adverse health effects on workers, NIOSH recommended the effective engineering control techniques for workers in microwave popcorn packaging plants include isolation of mixing processes (i.e. enclosures) and use of local and general exhaust ventilation to reduce inhalation exposure to diacetyl (NIOSH, 2003). Although there is no OSHA permissible exposure limit, NIOSH recommended an exposure rate on a national level but is not enforced. Wearing appropriate personal protective equipment such as respirators, safety goggles, and gloves are strongly recommended to protect workers from the hazard.

Overview

This paper outlines the chemical and physical properties of diacetyl as well as environmental sources of diacetyl and its application in the food industries while highlighting the NIOSH recommended exposure level for diacetyl in order to reduce health risks associated with diacetyl exposure. Also being discussed are the exposure pathway, occupational exposures, and adverse health effects of diacetyl.

ENVIRONMENTAL SOURCES

How Diacetyl comes into contact with the public as well as its source will be mentioned in this section. It is appropriate to first mention the compound itself and its background. Diacetyl, also known as butanedione, is the end product of fermentation. Chemically structured, it is a diketone; a molecule that has two adjacent ketones attached to one another. At room temperature, it is normally recognized by its greenish-yellow color. The liquid is not odorless, it is known to have a distinct odor ranging from a butter-like to chlorine-like smell. Diacetyl can be both naturally found and manufactured. Those handling the compound and working close with it, come the most into contact with the diacetyl. This means the workers at the factor level that produce diacetyl.

Industrial Sources of Diacetyl

Companies chemically synthesize diacetyl mainly for commercial use. Diacetyl is used in many foods; it is used to give butter and certain food flavorings a distinctive buttery flavor and smell.
A prime example of food that has diacetyl being used as an additive is popcorn. Not only are the workers making the diacetyl exposed to the compound, they are at a potential risk of being harmed by exposure.

Table below shows how much diacetyl is produced yearly in the United States From 1986- 2002. As the years progress, there is no indication of an increase in production of diacetyl.

<table>
<thead>
<tr>
<th>Year</th>
<th>Production Range (pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>10 thousand - 500 thousand</td>
</tr>
<tr>
<td>1990</td>
<td>10 thousand - 500 thousand</td>
</tr>
<tr>
<td>1994</td>
<td>10 thousand - 500 thousand</td>
</tr>
<tr>
<td>1998</td>
<td>10 thousand - 500 thousand</td>
</tr>
<tr>
<td>2002</td>
<td>10 thousand - 500 thousand</td>
</tr>
</tbody>
</table>

(Retrieved from National Library of Medicine)

Exposure for Workers

The National Institute for Occupational Safety and Health is a federal organization in the United States aimed at preventing injuries and ailment related to work. NIOSH is under the umbrella of the CDC or the Centers for Disease Control and Prevention. According to a survey done by NIOSH back in 1981-1983 approximately 3,437 workers were potentially exposed to diacetyl in the US. Exposure to diacetyl may come dermally through skin contact or through inhalation in the factories.

The NIOSH recommends an exposure limit of 5 ppb (parts per billion) per 40-hour workweek. NIOSH also suggests 15 minutes maximum for a short-term exposure of 25 ppb. In a certain microwave popcorn factory, workers that were affected, were exposed to an average of 20 parts per billion of diacetyl (Day et. Al 2011).

Representatives from NIOSH conducted a study in a commercial bakery mix factory. The company until 2008, had been using a type of butter-milk flavoring that contained 15-20% diacetyl (Day et. Al 2011). Since then they have been using a substitute in place of the flavoring that had contained diacetyl. Specifically NIOSH was evaluating the substitute and its usefulness as an alternative. Results from the study showed that the substitute flavoring contained ketones that share a similar structure with diacetyl. They concluded that the substitute flavoring and its toxicity have not been properly tested. It might not be safe and could pose a health risk to the workers just as much as the flavoring that had contained 15-20% diacetyl (Day et. Al 2011).
The table below is taken from the NIOSH study. It compares the structure and physical properties of diacetyl and the ketones present in the substituted flavoring. Majority of the ketones shown below share the same alpha-diketone group as diacetyl.

**TABLE III. Structures and Physical Properties of Ketone Compounds in Bulk Flavorings and Workplace Air Samples**

<table>
<thead>
<tr>
<th>Ketone Compound</th>
<th>Structure</th>
<th>Physical Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-Butanedione (Diacetyl)</td>
<td><img src="image1" alt="Structure" /></td>
<td>MW (g/mol)</td>
<td>86.09</td>
</tr>
<tr>
<td>3-Hydroxy-2-butanone (Acetoin)</td>
<td><img src="image2" alt="Structure" /></td>
<td>88.11</td>
<td>1.004</td>
</tr>
<tr>
<td>2,3-Pentanedione (Acetyl propionyl)</td>
<td><img src="image3" alt="Structure" /></td>
<td>100.12</td>
<td>0.957</td>
</tr>
<tr>
<td>2,3-Hexanedione (Acetyl butyryl)</td>
<td><img src="image4" alt="Structure" /></td>
<td>114.14</td>
<td>0.934</td>
</tr>
<tr>
<td>2,3-Heptanedione (Acetyl valeryl)</td>
<td><img src="image5" alt="Structure" /></td>
<td>128.17</td>
<td>0.919</td>
</tr>
</tbody>
</table>

(Table retrieved from Day et. Al 2011)

**Consumer Exposure**

Diacetyl earlier was mentioned as a very popular food additive and is an active ingredient in many foods. The table below shows the type of food categories diacetyl is in as well as the amount of diacetyl present. Units are in ppm or parts per million. In multiple cases, such as alcoholic beverages, hard candies, fats and oils, and non-alcoholic beverages; the maximum amount is more than twice the amount of usual content. The food categories with the highest usual content of diacetyl are baked goods, meat products and soft candy.
Results in the table shows diacetyl is used to add flavor to many foods ranging from alcoholic drinks to soft candy

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Usual (ppm)</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic beverages</td>
<td>6.33</td>
<td>15.28</td>
</tr>
<tr>
<td>Baked goods</td>
<td>28.17</td>
<td>49.94</td>
</tr>
<tr>
<td>Cheese</td>
<td>3.68</td>
<td>7.95</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>0.69</td>
<td>9.20</td>
</tr>
<tr>
<td>Fats, oil</td>
<td>6.01</td>
<td>62.18</td>
</tr>
<tr>
<td>Frozen dairy</td>
<td>11.17</td>
<td>35.08</td>
</tr>
<tr>
<td>Gelatin, pudding</td>
<td>13.45</td>
<td>26.58</td>
</tr>
<tr>
<td>Gravies</td>
<td>7.19</td>
<td>25.04</td>
</tr>
<tr>
<td>Hard candy</td>
<td>10.65</td>
<td>30.08</td>
</tr>
<tr>
<td>Imitation dairy</td>
<td>10.50</td>
<td>25.50</td>
</tr>
<tr>
<td>Meat products</td>
<td>27.81</td>
<td>27.81</td>
</tr>
<tr>
<td>Milk products</td>
<td>4.70</td>
<td>7.00</td>
</tr>
<tr>
<td>Nonalcoholic beverages</td>
<td>10.35</td>
<td>21.43</td>
</tr>
<tr>
<td>Snack foods</td>
<td>0.38</td>
<td>0.76</td>
</tr>
<tr>
<td>Soft candy</td>
<td>17.13</td>
<td>43.32</td>
</tr>
</tbody>
</table>

(Retrieved from National Library of Medicine)

An emission of diacetyl was closely researched in one study to figure out the level of exposure to diacetyl. The studied showed that diacetyl in powdered form gave lower emissions of diacetyl (Boylstein et. al 2006). But the study also claimed that the liquid emissions of diacetyl were 65 times larger than the powdered form of diacetyl (Boylstein et. al 2006).

### Health Effects of Diacetyl

The concentration of diacetyl produces adverse effects on human health primarily for those working directly with the substance. Diacetyl is most prominent as an airborne chemical in butter flavoring for popcorn providing negative health symptoms in the lungs directly correlating to workers who mix oil and butter flavoring at the plant. Further investigations display severe injury of the epithelium airway (Kanwal et. Al 2006). These injuries include respiratory tract symptoms such as shortness of breath and cough in workers exposed while manufacturing.
buttered popcorn (Boylstein 2012). If workers are subject to chronic exposure, symptoms may result to a disabling lung disease called bronchiolitis obliterations (Boylstein 2012).

Adverse health effects were prominent in the upper respiratory tract. Sensory and nasal tissue irritation and inflammation were considered. The sensory irritation resulted in a singing or burning sensation but may not be a clear critical effect in humans for butter flavoring (Maier et. al 2010). Inflammation of the upper nasal tract was a result of cell damage (Maier et. al 2010).

Lab rat testing for acute and subchronic studies indicate the upper respiratory tract to be a significant target for symptoms due to cytotoxic effects of diacetyl vapor (Maier et. al 2010). Histopathology and nasal inflammation findings occurred at shorter exposures and lower concentrations rather than the effects further in the respiratory tract.

A lab rat test conducted in 2008 exemplified a fibrotic response in the lungs of mice from an aspiration of diacetyl aerosols (Morgan et. al 2008). Diacetyl was incorporated to the lungs of the mice by an oropharyngeal aspiration giving higher doses to the airways and bypassing the nasal cavity. The mice grew fibrohistiocytic lesions in the bronchioles, alveolar ducts and bronchiole-alveolar junction due to the exposure of diacetyl (Morgan et. al 2008). Mice cannot be directly compared to humans when considering respiratory function because mice are obligate nasal breathers. Yet, this finding supports the idea that diacetyl may contribute to the fibrosis response of bronchiolitis obliterations to workers whiles manufacturing artificial flavored popcorn.

The National Institute for Occupational Safety and Health (NIOSH) performed eight medical and industrial hygiene surveys in one plant from 2000-2003. They performed spirometry testing and standardized questionnaires to employees at the plant. After surveying the amount of diacetyl exposure, NIOSH used ventilation and isolation to reduce concentration. As a result, the concentration reduced in one to three magnitudes (Kanwal et. al 2011). Longtime workers who initially had high exposure resulting in stable chest symptoms, nasal, eye, and skin; symptoms declined (Kanwal et. al 2011). Also, new workers who faced lower symptom prevalence did not worsen over time (Kanwal et. al 2011). Overall, the ventilation and isolation of diacetyl reduced the risk of lung disease and respiratory symptoms for the workers.

Respiratory Symptoms Correlated to Diacetyl

A research group directed by Dr. Richard Kanwal conducted a study on six microwave-popcorn plants and performed a health hazard evaluation under federal regulations. Three of the plants contained more than 100 workers and were owned by three of the top five producers of microwave popcorn in the United States (Kanwal et. al 2006). The investigation collected information through questionnaires and performing spirometry tests. Data collected considered symptoms, smoking history, work history, medical diagnoses and
work-related exposures. Symptoms that were evaluated were chronic cough, wheezing, and breath after exertion. The criteria for acknowledging a worker as a smoker was smoking more than 20 packs in a lifetime or smoking at least one cigarette a day for one year. The comparison groups were those workers that mix the oil and flavorings and those workers who perform other tasks away from the diacetyl chemical.

The investigation surveyed 537 of the 708 current employees working at popcorn manufacturing facilities (Kanwal et. al 2006). Of these workers, 86 of them reported to worker as an oil and butter flavorings mixer (Kanwal et. al 2006). The workers were tested and placed in four different categories for analysis. The four categories for comparison included: workers that mix the oil and butter flavor and do not smoke and those who mix and smoke; workers that do not mix oil and butter flavor who do not smoke with those who do not mix but do smoke.

![Figure 1 (Kanwal et. al 2006).](image)

The mixers had a higher prevalence for all respiratory symptoms containing a significant difference with shortness of breath, wheezing and chronic cough (Kanwal et. al 2006). The mixers who do not smoke had a larger forced expiratory volume (Kanwal et. al 2006). The data set that best exemplified the correlation of mixing and respiratory symptoms is wheezing proven to be statistically significant because the p-value=0.001 (Kanwal et. al 2006). Therefore, without the role of chance, there is a correlation between diacetyl vapor and wheezing in workers who mix the butter flavoring: those working directly with diacetyl may acquire adverse respiratory symptoms.
Vulnerable Population

Populations most vulnerable to diacetyl exposure are those who work in facilities with open containers of artificial butter flavoring. Smokers show a more exaggerated adverse respiratory affect (Figures 1 and 2) when considering their forced expiratory volume. This displays a further decrease in lung capacity that has potential due to the combination of both cigarette smoke and diacetyl. The graph comparing length of time employed by Dr. Kanwal demonstrates a large difference in wheezing symptoms from those who do or do not smoke. In theory, children would be vulnerable to diacetyl exposure because they take more inhalations per minute in comparison to adults, as well as a smaller body weight on average. Inhalation and body weight are two aspects used when considering the hazard, thus children increase their exposure to volatile chemicals in comparison to adults. Yet, this would only be a concern if they were frequently exposed to diacetyl, which is not the case.

Likelihood of Various Effects

The likelihood of an average citizen acquiring respiratory symptoms directly from diacetyl exposure is insignificant. Yet, the chance of employees obtaining respiratory illnesses while working with diacetyl vapors is increased. The California Department of Public Health (CDPH) collected serial spirometry data at 20 companies for flavoring manufacturing workers who were at risk of bronchiolitis obliterations (Kreiss et. al 2011). They analyzed the forced expiratory volume for one second.

CDPH looked at 416 workers who had two spirometry tests taken from commercial providers at different times. 40 of these 416 workers (9.6%) had an abnormal forced expiratory volume decline (Kreiss et. al 2011). The California Department of Public Health also looked at high quality spirometry tests of 289 workers (Kreiss et. al 2011). Of these workers, 21 (7.3%) had an abnormal decline in volume (Kreiss et. al 2011). One of these workers that were tested for this group was diagnosed with airway obstructions. On average, approximately 8.65% of workers faced a decline of forced expiratory volume when working directly with the diacetyl chemical.

Level and Length of Exposure

A correlation has been a result between the duration of time an employee is working for a popcorn company and their decline in respiratory health. An investigation directed by the National Institute of Occupation Safety and Health studied the respiratory symptoms comparing employees working for more than one year and those working for less than one year. They looked at 26 workers that had worked for more than a year compared to 45 that worked for less. The employees mixing the flavoring for more than one year had higher prevalence of all respiratory and airways obstructions (Kanwal et. al 2006).
The graph from the study displays a quite dramatic influence that diacetyl has on employees for a long duration of time. The shortness of breath demonstrated that those working for more than one year had prevalence later than three times the amount of those working less than one year. The data taken for shortness of breath was statistically significant with a probability value of .005 (Kanwal et. al 2006). The forced expiratory volume comparison had a 5% difference in volume with those working longer having a smaller lung capacity (Kanwal et. al 2006).

The data taken from the California Department of Public Health at the 20 companies also compared workers who worked with over 800lbs/year of diacetyl and those who worked with less that 800lbs/year. Their results showed a direct correlation between increases of diacetyl exposure to an increase in respiratory symptoms. The companies with workers using more than 800 lb/year of diacetyl showed a significant decline of forced expiratory volume, -113.6ml/year. Those workers using less than 800lbs/year of diacetyl, displayed a less significant decline of forced expiratory volume, -51.6ml/year. Both types of exposure result in a decline of forced expiratory volume, yet an increase in amount of diacetyl shows a more exaggerated decline.

Through these studies, the most notable respiratory symptoms include nasal tract cell damage, irritation and inflammation of the epithelial airway, coughing, wheezing and decreased lung capacity. In the most extreme cases, bronchiolitis obliterans are a result, which is defined as a fibrotic non-reversible lung disease that can be fatal.


Several of these studies are products from a handful of notable scientists. This group of scientist worked together, as well as with other researchers to perform multiple experiments that were peer-reviewed for academics. The reliability of the data are a result from the research of these scientists, which were consistent and sound. From each resource, there were no contradictions in the case studies about exposures and health effects. Data from the graphs from the articles did not contradict tables, nor were they exaggerated. Complete calculations were given for the numerical information. These articles also cited several other peer-reviewed journals. Conclusions from multiple articles were consistent. Some research, by Boylstein, Kanwal, Kreiss and Pendergrass are dated from years back, but these researchers continued investigations and published more recent articles. Further investigations are still underway.