Dragon, Karen E. (CDC/NIOSH/EID)

From: Dana Hollins <DHollins@chemrisk.com>
Sent: Thursday, November 17, 2011 8:13 PM
To: NIOSH Docket Office (CDC)
Subject: 245 - Criteria for a Recommended Standard: Occupational Exposure to Diacetyl and 2,3-pentanedione
Attachments: NIOSH COMMENTS - 11-17-2011.pdf

Greetings – Attached please find written comments regarding the "245 - Criteria for a Recommended Standard: Occupational Exposure to Diacetyl and 2,3-pentanedione".

Many Thanks,

Dana

Dana M. Hollins, MPH
Senior Health Scientist
Chem***Risk***, LLC
Pioneers in Science. Leaders in Solutions.
101 2nd Street, Suite 700
San Francisco, CA 94105
(415) 618-3213 Direct
(415) 312-2810 Mobile
(415) 896-2444 Fax
www.chemrisk.com

COMMENTS TO THE AUGUST 12, 2011 DRAFT NIOSH CRITERIA DOCUMENT REGARDING PROPOSED DIACETYL OCCUPATIONAL EXPOSURE LIMITS

I, Dana Hollins, am submitting comments regarding the August 12, 2011 DRAFT NIOSH criteria document regarding a proposed occupational exposure limit for diacetyl. I attended the public meeting on August 26, 2011 in Washington, DC and presented oral comments at that meeting. The enclosed written comments are a follow-up to these oral comments.

Our firm, who is engaged in consulting, believes we have a professional responsibility to share information with government bodies. We have in the past consulted and testified for flavorings manufacturers and as a result we have developed a body of knowledge about this issue. Scientists in our firm have studied this matter for the past 4 years and have published numerous papers or letters to the editor on the toxicological and medical aspects of this family of chemicals.

Thank you for your time and consideration of these comments.

1) **The animal toxicology data indicate that the effects of diacetyl are limited to the upper respiratory tract and there is no evidence to indicate that diacetyl is a cause of *bronchiolitis obliterans.***

Diacetyl vs. BO inducers: comparison of physico-chemical parameters and other metrics

There are several well-established chemical risk factors for BO in humans. These chemicals are highly reactive (or are converted to highly reactive compounds in the body) and exert clearly demonstrable destructive effects on the small, lower airways and alveoli of animals at relatively low concentrations. For example, nitrogen dioxide (NO_2), which is one of the most common chemical agents associated with BO in humans, is often present in silo gases and every year there are numerous case reports of farm workers developing BO as a result of silo gas exposures. Nitrogen dioxide is highly toxic because it is hydrolyzed to a reactive and biologically destructive acid (nitric acid) throughout the respiratory tract, including the alveoli. In studies involving rats and other animal species, 4-hours of exposure to NO_2 concentrations as low as 0.25-2.0 ppm will cause significant protein leakage into alveoli and injury to Type 1 alveolar lining cells (IPCS, 1977). Other inducers of BO include: mustard gas, sulfur dioxide, methyl isocayanate, ammonia, hydrogen fluoride, and hydrogen chloride. These compounds are all severe respiratory irritants and/or they are converted to biologically destructive compounds in the respiratory tract. In each case, the mechanism of toxic action is well understood.

Diacetyl clearly does not fit this profile. It is an organic compound, not an inorganic compound. It is not an acid. It is neither biologically reactive nor caustic at low concentrations nor is it metabolized to any compounds that are known to be reactive or caustic. In fact, diacetyl has been "generally recognized as safe" (GRAS) by the FDA since 1983. Furthermore, unlike the known BO inducers, diacetyl is present naturally in many foods, naturally occurs in the body (as a biochemical intermediate), and is a common food additive. If diacetyl exposure was analogous to exposure to mustard gas, it is reasonable to expect that adverse effects on workers handling even small volumes would have been easily recognizable.

Even if one were to believe that diacetyl is an "atypical" inducer of BO, any reasonable scientist would expect that a "mechanism of action" would be suggested or established to explain how diacetyl causes destruction of the deep lung in humans. As noted above, known inducers of BO have clearly understood mechanisms of toxic action. Yet no such theories have been put forth that satisfactorily explains how a relatively benign compound such as diacetyl should be considered an inducer of BO that ranks with the likes of chlorine and mustard gas.

Animal inhalation studies with diacetyl indicate that only the upper respiratory tract is affected

As noted above, the physico-chemical properties of diacetyl are highly inconsistent with those of known risk factors for BO and in fact they suggest diacetyl is not particularly reactive. Animal exposures involving diacetyl-containing artificial butter flavoring (ABF) and pure diacetyl show that inhalation of diacetyl vapor causes effect on the upper respiratory tract but does not cause alveolar or any other deep lung effects, even at concentrations that are:1) far beyond those measured in the workplace, and 2) high enough to cause severe necrosis of the upper respiratory tract and even death.

In 2002, the National Institute for Occupational Safety and Health (NIOSH) published a study in which male Sprague-Dawley rats were exposed to heated ABF vapors for six hours at diacetyl concentrations of 203, 285, and 352 ppm (Hubbs et al, 2002). The total VOC levels were: 298, 446, and 578 ppm, respectively. Other animal groups were exposed to intermittent "pulses" of ABF vapor with diacetyl concentrations ranging up to 940 ppm. The pulsed exposures were intended to represent intermittent workplace exposures to high ABF concentrations.

The investigators described the pathology of different segments of the nasal epithelium (the beginning of the respiratory tract), the large upper airways, and the alveoli. The most consistent morphologic change was necrosis of the nasal epithelium, which was observed in all animals in all exposed groups. Severe necrosis of the of the upper pulmonary airways was observed in all animals in the 285 ppm and 352 ppm-diacetyl groups, and in one rat in the 203 ppm-diacetyl group. Two rats died (post-exposure) in the 285 ppm and 352-ppm diacetyl groups. The authors indicated that the alveoli "were unaffected" in all groups.

The authors then repeated the above study, this time with pure diacetyl (Hubbs et al., 2004 and 2008). Consistent with the findings of the ABF study, they reported that continuous 6-hour exposure to heated vapors of pure diacetyl (at 99, 198, and 295 ppm) resulted in necrosis of the nasal epithelium in all animals at 198 and 295 ppm, but that such effects were not observed at 99 ppm. Effects on the upper respiratory epithelium were observed in 2/6 animals in the 295 ppm group; no such effects were noted in the 99 and 198 ppm group. Furthermore, as the authors note, the findings at 295 ppm "only bordered on statistical significance" using the exact p value of 0.054. In other words, even at the highest pure diacetyl concentration of 295 ppm the authors failed to note a clear biologically significant response in the upper airway epithelium. Also, *none of the diacetyl exposures were reported to cause any effects on the alveoli or deep lung tissue*. This is true even for rats that were exposed to numerous 15 minute "pulses" of *1,800 ppm diacetyl.*

In another inhalation study of diacetyl, Morgan et al. (2008) exposed mice to 200 and 400 ppm

diacetyl for 6 hours/day for 5 days. These exposures were longer than those used in the Hubbs studies (single 6 hour exposure), and many of the mice either died or were euthanized before the end of the exposure period. Not surprisingly, there was extensive necrosis of the upper airways; and yet, as indicated by the authors "No lesions of the bronchioles [lower airways] were noted". Similarly, no deep lung effects were observed in rats exposed to multiple 15 minute pulses of 1200 ppm diacetyl.

Comparison of animal diacetyl exposures to workplace conditions

It is critical to note that the exposure concentrations employed in the Hubbs and Morgan studies are far higher than those that could be considered representative of workplace diacetyl exposures. The diacetyl concentrations used by Hubbs et al and Morgan et al. (99-400 ppm constant exposures; multiple pulsed exposures of up to 1800 ppm) were in fact much higher than the levels measured in the ABF mixing rooms, where the highest airborne diacetyl concentrations have been found. Specifically, I have examined all of the mixing room data from the numerous microwave popcorn facilities examined by NIOSH (Kanwal et al., 2006; Kullman et al., 2005; NIOSH, 2003a, 2004), and I find that few time-weighted-average (TWA) samples of 60 min or longer exceed 3 ppm and in fact most TWA samples are less than 1 ppm.

Comparison of diacetyl no-effect levels to adverse effect levels for known BO inducers

As noted earlier, 4-hours of exposure to NO_2 concentrations as low as 2.0 ppm will cause significant protein leakage into alveoli and injury to Type 1 alveolar lining cells in rodents (IPCS, 1977). These findings indicate that NO_2 has an "effect level" on the alveoli that is at least as low as 8 ppm-hours, and may in fact be much lower. Similarly, 30 minutes of exposure to 65 ppm (32.5 ppm-hours) of hydrogen fluoride or 5 minutes of exposure to 75 ppm (about 4 ppm-hours) sulfur dioxide will cause alveolar necrosis in mice (IPCS, 2002 and ATSDR, 1998). In short, known BO inducers wdestroy the alveoli of rodents at doses less than 50 ppm-hours.

In contrast, Morgan et al. (2008) exposed mice to 400 ppm diacetyl for 6 hours/day for 5 days and did not observe even minimal alveolar effects. Hence, diacetyl has a "no effect level" at least as high as 10,200 ppm-hours, and in fact the no-effect level is likely to be much higher.

Summary

None of the inhalation studies conducted to date have reported any effects, even minimal effects, on the deep lung (terminal bronchioles or alveoli) in animals exposed to very high diacetyl concentrations, including concentrations that are both well beyond workplace levels and are high enough to kill the animals. Conversely, damage to the deep lung in animals has been observed with every single known inducer of BO, even at low exposures. While it is unclear whether direct affects on the alveoli or deep lung are a pre-requisite for induction of BO, certainly it cannot be concluded that a chemical (such as diacetyl) that only causes irritation of the upper respiratory tract, even at very high concentrations, "fits the profile" of a BO risk factor.
If one is to maintain that diacetyl is a risk factor for BO, then one must argue that diacetyl is a unique compound with toxic properties never before observed with any other chemical. There is no reason to believe diacetyl possesses such novel characteristics.

2) ***The epidemiology data do not support the conclusion that diacetyl is a cause of obstructive effects***

Diacetyl has been manufactured and used in bulk quantities in the United States since the early 1900s. According to a 2004 Flavor and Extract Manufacturer's Association (FEMA) report, approximately 211,000 pounds of diacetyl were used by American industries in 1995 (FEMA 2004). In the mid-1980s, microwave popcorn became a highly popular snack item and diacetyl-containing artificial butter flavoring (ABF) was a frequent additive for a substantial fraction of the microwave popcorn products. Diacetyl is also used in numerous other food-related industries, such as baked goods, candy, cake mixes, some syrups, certain cheeses and other dairy products.

As described below, workers in popcorn packing and other food industries have been studied to assess the possible role of diacetyl as a risk factor for obstruction and other respiratory disorders. To date, these studies, individually and in aggregate, have failed to demonstrate a causal relationship between diacetyl exposures and respiratory disorders, particularly obstruction. BO is an obstructive disease and therefore it stands to reason that, if diacetyl is truly causing BO in workers in popcorn plants, flavoring facilities, and diacetyl manufacturing facilities, there would be a measurable increase in obstructive effects in the workplace.

Thus far, the published studies on worker health in diacetyl and flavorings facilities have relied exclusively on the National Health and Nutritional Examination Survey (NHANES) III data as a source of "general population" information for comparison purposes. While NHANES is arguably the largest available source of "background" health status in the U.S., it may not adequately represent a relatively small, blue-collar population in a discrete geographical area, particularly one in a farming or agrarian setting (where many of the popcorn packing facilities are located). Agrarian populations are often exposed to numerous respiratory toxicants not related to their profession that affects pulmonary health, such as: organic dusts, endotoxins, fungal toxins, silo gases, microbial toxins, pesticides, fertilizers, disinfectants, and feed additives (ATS, 1998). Accordingly, and as described in detail in the American Thoracic Society's "Respiratory Health Hazards in Agriculture" (and many other publications), it is well understood that those who live and work in agrarian settings typically have a higher incidence of numerous respiratory disorders, including decreased FEV_1 and FVC:

> "Increased prevalence rates for chronic bronchitis have been reported in farmers and agricultural workers in many parts of the industry (page 29)."
>
> "Dosman and colleagues also reported significantly lower FVC, FEV_1, and FEV_1/FVC in farmers compared with control subjects (page 29)."

Thus, for diacetyl and flavorings workers who live in a farming community, a more apt comparison group would be an internal reference group (e.g. office workers at the facilities that are not exposed to diacetyl) or a comparison group of individuals from communities that represent the diacetyl workers.

Kreiss et al (2002)

Kreiss et al. (2002) was the first study to examine respiratory disorders in a group of popcorn workers (as opposed to case reports of single individuals). The authors [that are from the National Institute for Occupational Safety and Health (NIOSH)] evaluated 117 workers from the Gilster Mary-Lee (GML) facility in Jasper, Missouri via spirometric analyses and questionnaire responses. Over one hundred VOCs were measured in the mixing room; many of which are known respiratory irritants. The authors reported a statistically significant increase in the prevalence of airway obstruction (defined simply as a "low" FEV_1/FVC ratio) and self-reported symptoms (e.g. chronic cough, wheezing, shortness of breath, asthma, and chronic bronchitis) when the workforce was compared to expected rates from NHANES.

However, when the prevalence of these respiratory disorders was compared to those in an internal reference group of unexposed workers (office workers, etc.), many of the symptoms were no longer significantly increased in exposed workers (chronic cough, phlegm, wheezing, attacks of wheezing, chest tightness, fever, chills, night sweats, influenza-like achiness, and mucus membrane irritation). This change strongly suggests that the NHANES comparison was confounded by the aforementioned higher prevalence of respiratory disorders in those living in farming communities. Indeed, Kreiss et al (2002) noted that:

> "57% of the participants had exposures outside the popcorn plant to other possible causes of occupational lung disease; the leading sources of exposure were farming (40%), grain dust (32%), irritant gases (14%), and nitrogen oxides (8%)."

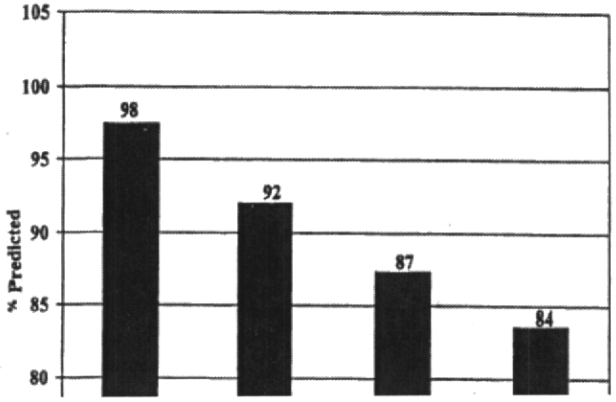
As noted earlier, nitrogen oxides are the primary cause of *bronchiolitis obliterans* in humans. Unfortunately, it does not appear that NIOSH seriously evaluated alternative risk factors in this or study any of the Human Health Evaluations (HHE) that they conducted soon after their investigation of the GML facility.

Also, it is clear that even the internal control group had a much higher than normal prevalence of respiratory disorders. For example, as can be seen in Table 4 of the paper, 25% of the control group reported wheezing, and 50% reported mucous membrane irritation. Given the fact that 1) the majority of the study participants reported that they had been exposed to known respiratory toxicants outside the popcorn plant, and 2) even the internal control group (unexposed to diacetyl) had a very high rate of self-reported symptoms, it is clear that NHANES was not an appropriate reference group for this cohort. Indeed, it could be argued that most or all of the respiratory disorders in these workers were entirely unrelated to the GML facility. In short, the results of Kreiss et al. (2002) likely reflect the predictable outcome of an investigation of respiratory effects in an agrarian, blue collar cohort that routinely worked with heated organic vapors under poorly ventilated conditions.

In short, most or all of the "increased prevalences" of self-reported respiratory symptoms are far more likely to have been the result of exposures to agents other than diacetyl. For reasons that are not explained, Kreiss et al. (2002) did not present a statistical comparison of the abnormal spirometry prevalence (airway obstruction) in workers versus the internal control group.

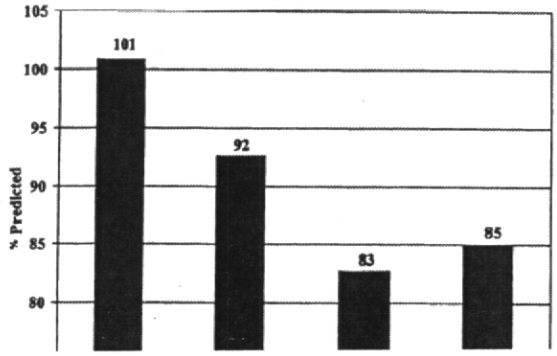
Kreiss et al. (2002) present a figure which they believe indicates a statistically significant relationship between cumulative diacetyl exposure and decreased FEV_1 in the GML workers

From Kreiss et al (2002). Average percent predicted FEV_1 by quartile of cumulative exposure to diacetyl [Figure 9 from GML HHE, Interim Report dated August 22, 2011 (NIOSH, 2006))]



Unfortunately, no information was provided as to how this figure or the underlying dose calculations were derived. More importantly, the figure itself is relatively meaningless because the same figure could be derived for most or all of the analytes at the GML plant. Specifically, airborne dust and chemical levels were all highest in the mixing room, lower in the quality control areas, and lower still in other areas of the facility. Indeed, as shown in the NIOSH HHE report for the GML facility (NIOSH, 2006), The figure below describes the relationship between cumulative dust exposure and decreased FEV1 in these same individuals:

Average percent predicted FEV_1 by quartile of cumulative exposure to respirable dust [Figure 9b from GML HHE, Interim Report dated August 22, 2011 (NIOSH, 2006)]



Also, the diacetyl levels measured in the GML facility (overall mean = 8.1 ppm; mean in the mixing room = 32.3 ppm) are well below those that *failed to cause alveolar effects in animals* (up to 1800 ppm). Finally, it is important to note (in Figure 3 above) that the FEV_1 decreases in most of the workers were low even though the diacetyl exposures were very high: the highest quartile of exposure (11-126 ppm-year) was associated with only a 12.5% decrease in FEV_1. As noted earlier, the ATS states that a change of less than 12% in FEV_1 or FVC is likely due to natural variability in the participant and instrumentation rather than due to a change in lung function (Pelligrino et al., 2005).

In summary, regarding Kreiss et al. (2002)

- use of the NHANES database as a "control group" was problematic due to: 1) the high percentage of GML workers with non-occupational exposures to known respiratory risk factor (including known inducers of BO), and 2) the high prevalence of respiratory symptoms in GML workers who were not exposed to diacetyl

- when the more appropriate internal control group was used as the basis of comparison, there were few significant differences between the exposed versus unexposed workers

- this study provided no evidence of a statistically significant increase of abnormal spirometry in the workers versus the internal control group

- the results of Kreiss et al. (2002) likely reflects the predictable outcome of an investigation of respiratory effects in an agrarian, blue collar cohort that routinely works with heated organic vapors under poorly ventilated conditions

- there was inadequate consideration of alternative causes; the prevalence of respiratory symptoms in these workers could also be explained by exposures to non-occupational toxicants or other compounds (e.g., VOCs, dusts, endotoxins) in the facility

- there is no evidence of a causal relationship between diacetyl exposure and prevalence of respiratory disorders in the GML workers

- the decreased FEV_1 values were not biologically significant even at very high exposures; this study therefore suggests that diacetyl did not pose a respiratory risk in these workers

Kanwal et al. (2006)

Kanwal et al. (2006) examined 708 workers across six microwave popcorn plants, including the workers from the Kreiss et al. (2002) study. Hence, this study is a far more robust version of the original Kreiss et al. (2002) analysis. Airborne diacetyl levels were much lower in the other five facilities (relative to the GML facility).

Unlike Kreiss et al. (2002), Kanwal et al. (2006) compared the spirometric outcomes in the exposed workers versus internal control groups. This is arguably the most valid approach for assessing whether diacetyl-related respiratory obstruction is occurring in the workforce. The

cohort was stratified in several ways, thereby permitting numerous evaluations of the potential contribution of diacetyl to obstructive disease: 1) "ever mixers" versus "never mixers," 2) smoking status, 3) mixers who worked > 12 months versus those who worked < 12 months, and 4) individuals who worked in the packaging area in plants with isolated tanks versus those who worked in the packaging area in plants with non-isolated tanks. *In every case, there was no difference between the exposed and control groups.* Specifically, there was no difference in % obstruction on spirometry in:

- smokers who had worked as mixers versus those who had never been mixers
- non-smokers who had worked as mixers versus those who had never been mixers
- smokers who had worked as mixers <12 months versus those who had worked as mixers > 12 months
- nonsmokers who had worked in the as mixers <12 months versus those who had worked as mixers > 12 months
- smokers who had worked in the packaging area in plants with isolated tanks versus those who had worked in plants with non-isolated tanks
- nonsmokers who had worked in the packaging area in plants with isolated tanks versus those who had worked in plants isolated tanks

In short, no matter how many different ways the authors "sliced the deck", there was no relationship between chemical exposure and obstruction in a pooled cohort of 708 workers from six different popcorn-packing facilities. Furthermore, a majority of the comparisons involving % predicted FEV_1 also showed no difference between control and exposed groups, i.e., there was no difference in % predicted FEV_1 in:

- smokers who had worked as mixers versus those who had never been mixers
- nonsmokers who had worked as mixers <12 months versus those who had worked as mixers > 12 months
- nonsmokers who had worked in plants with isolated tanks versus those who had worked in plants with non-isolated tanks
- smokers who had worked in plants with isolated tanks versus those who had worked in non-isolated tanks

Finally, there was no difference in a majority of the exposed versus control comparisons involving self-reported symptoms such as shortness of breath, chronic cough, and wheezing.

In summary, the study by Kanwal and colleagues demonstrates a clear lack of evidence that exposure to diacetyl is a risk factor for obstruction. Its results are arguably more relevant than

the initial study at the GML facility (Kreiss et al, 2002). As was the case in Kreiss et al. (2002), the reported % predicted FEV_1 values in Kanwal et al. (2006) represented relatively healthy individuals. Further, the small FEV_1 decrements that were observed could be due to oils, other components of ABF, dusts, endotoxins, and many other toxicants that would be elevated in the mixing room relative to other parts of the facility.

Lockey et al 2009

Similar to Kanwal et al. (2006), Lockey et al. (2009) examined a pooled cohort of over 700 individuals from multiple facilities that used flavoring chemicals. Also, like Kanwal et al. (2006), they evaluated the prevalence of obstruction in different job categories relative to an internal control group comprised of workers with little or no mixing experience (and therefore little to no occupational exposure to flavoring chemicals). Unlike Kanwal et al. (2006), however, Lockey et al. (2009) claim to have measured a high prevalence of obstruction in workers (mixers) exposed to diacetyl.

The reasons for this inconsistency can likely be explained by the very different methodologies used to characterize the findings. Kanwal et al. (2006) presented direct comparisons between % obstruction in those with high diacetyl exposures (ever mixers, mixers > 12 months, workers near nonisolated tanks) versus those with relatively less or no diacetyl exposures (never mixers, mixers < 12 months, and workers near isolated tanks, respectively). Lockey et al. (2009) does not present any such transparent comparisons. Instead, they present a "logistic regression model" that is claimed to "explain" how respiratory function of the different workers is influenced by various factors.

The problem is that the reported statistic for model fit, r^2, was poor across all the tested models. The largest reported r^2 values associated with the tested regression models (0.41 and 0.16, respectively) accounted for an insignificant amount of variability in the data. Therefore, variables that were *not* included in the regression models better predict pulmonary health decrements in the workers, so no conclusions can be drawn from the reported effect estimates. In other words, the values are so low they indicate that a vast majority of the variability in the respiratory function measurements *cannot* be explained by smoking status, diacetyl exposure, job title, or any other of the variables that are input to the model.

It is worth noting that in the one instance in which Lockey et al. (2009) do directly compare number of cases with obstructive respiratory function in the cohort versus the expected rate from NHANES (stratified by smoking status and age), *there is no difference.* Differences only arise when the Lockey et al. (2009) model is employed.

It is also critical to note that all five of the "obstructive cases" reported in the pre-PAPR mixing group (mixers that never used respirators) could have been due to pre-existing asthma, smoking, or some other condition. Specifically, as noted by the authors, one individual had pre-existing asthma and another had respiratory symptoms prior to work. There were current and former smokers in the group, and all of the individuals who were tested (three of the five) with a bronchodilator showed a positive response (individuals with BO do not respond to bronchodilators).

There are many other shortcomings present in the Lockey et al. (2009) analysis, and it is beyond the scope of this submittal to detail them all, but I believe that Lockey et al. (2009) fails to provide compelling evidence of either an increased risk of obstruction in mixers or a causative relationship between diacetyl exposure and obstruction for the following reasons:

- In Table 2 of the paper, it can be seen that Asian females, who have almost no mixing experience, have the worst FEV_1 % pred and FVC% pred values; this suggests a lack of association between diacetyl exposure and decreased respiratory function in this study.

- The results of Table 2 (in Lockey et al., 2009) indicate that cumulative diacetyl exposure (ppm-years) does not correlate at all with either FEV_1% pred or % obstructive PFT pattern, but smoking (pack-years) correlates very well with both of these respiratory endpoints.

- In Table 3 of the paper it can be seen that, in non-Asian males and females (the vast majority of the workforce), diacetyl exposure does not correlate at all with FEV_1% pred (e.g., in non-Asian males, intermittent pre-PAPR mixers actually have a significantly *increased* FEV_1% pred and FVC% pred)

- Lockey et al. (2009) reported a mean FEV_1 decrement of almost 13% in non-Asian females; this is a group that essentially had no diacetyl exposure. This decrement is larger than every other decrement that Lockey et al. (2009) attempt to ascribe to diacetyl.

- There was actually a greater % of obstruction PFT pattern (3/20=15%) in the PAPR group than in the pre-PAPR group (5/39=13%); this suggests that diacetyl exposure is not associated with obstruction.

- As shown in Table 6 from the paper, diacetyl exposure does not correlate with obstructive PFTs in non-Asian males.

van Rooy et al. (2009)

The van Rooy et al. (2009) study is unique in that it is the only study thus far to examine diacetyl *manufacturers*. Also, the study took place in the Netherlands. Hence, this study avoided the problems of confounding exposures to numerous other workplace respiratory irritants (dusts, endotoxins, hundreds of other VOCs, etc.) and also the NHANES-related shortcomings (described earlier) inherent in the NIOSH studies. The findings can be summarized as follows:

- when the worker population was compared to national averages or an internal reference population, there was no difference in the % with self-reported symptoms for a vast majority of the symptoms

- contrary to the claims of Kreiss et al. (2002) and Lockey et al. (2009), there was no clear association between FEV_1 (% predicted) and exposure to diacetyl

- contrary to the claims of Kreiss et al. (2002) and Lockey et al. (2009), lung function was actually *better than predicted* with diacetyl exposure

Like the Kanwal et al (2006) study, van Rooy et al (2009) examined PFT results as a function of diacetyl exposure using many different comparisons: smokers vs nonsmokers, years worked at the plant post-1995, number of years worked at the plant, and cumulative weighted number of years. Like the Kanwal et al (2006) study, in every case the authors failed to find a causative relationship:

In short, the van Rooy et al. (2009) study certainly does not indicate that cumulative diacetyl exposures are associated with an increased risk of serious respiratory disorders.

Summary

There are three relatively robust studies that have evaluated respiratory disorders (including obstruction) in workers potentially exposed to diacetyl: Kanwal et al. (2006), van Rooy et al. (2009), and Lockey et al. (2009). Kanwal et al. (2006) reported no increase in % obstructive patterns in workers. van Rooy et al. (2009) actually reported that lung function improves as a function of diacetyl exposure. Lockey et al. (2009) claims to have observed an increased risk of obstruction in mixers who had no respiratory protection. An unbiased weight of evidence evaluation of these studies would reach a conclusion that the preponderance of data do *not* show a relationship between diacetyl exposure and serious respiratory disorders. When one then considers the animal data (discussed previously), wherein numerous studies have failed to show evidence of any deep lung effects even following very high diacetyl exposures, it appears that diacetyl is unlikely to be a cause of BO or any other serious respiratory disease in humans.

3) The animal studies of Morgan et al are an appropriate basis for establishing an OEL.

Maier et al. (2010) evaluated the available health effects data on microwave popcorn and food flavoring manufacturing workers and concluded that the results would not support the development of a valid threshold. Specifically, of the worker studies, they concluded that Lockey et al. (2009) was the most robust data set and noted that the Lockey et al. (2009) findings appeared to suggest a threshold for airway obstruction of ≥ 0.8 ppm-years. However, Maier et al. (2010) concluded that the Lockey et al. (2009) investigation suffered from too many uncertainties, including: 1) the diacetyl measurements underestimated the actual airborne concentrations due to humidity complications, 2) the airborne measurements likely underestimated historical exposures, 3) the lack of controlling for the numerous other compounds to which the workers were exposed, 4) difficulty in controlling for exposures that occurred in previous employments, 5) difficulty in controlling for non-occupational risk factors, and, 6) as I mentioned earlier, the magnitude of the changes in the PFT values were small according to the definitions used by ATS and NIOSH (and likely of no biological significance). Hence, a threshold of ≥ 0.8 ppm-years would likely be an overly conservative estimate of a threshold for diacetyl-related obstructive effects. I have noted several other issues associated with the Lockey et al. (2009) study above, most of which concern the lack of an observed diacetyl exposure-response relationship. For those reasons alone I do not believe that the results

from the Lockey et al. (2009) study serve as a valid basis for which a cumulative-exposure threshold for diacetyl could be established.

Maier et al. (2010) also evaluated the results from the available animal studies and concluded that the subchronic mouse study conducted by Morgan et al. (2008) provided sufficient data for the derivation of a diacetyl exposure threshold. As detailed in their analysis, they conducted what is commonly referred to as a "benchmark concentration" analysis using "minimal to mild" peribronchial lymphocytic inflammation (PLI) as the critical adverse effect endpoint. This yielded a human equivalent concentration for occupational exposure of 2 ppm; the 2 ppm value was then adjusted by an aggregate uncertainty factor of 100 to arrive at the proposed occupational exposure limit of 0.2 ppm.

It is important to note that this threshold is based on minimal effects in the URT; any threshold based on obstruction or actual *bronchiolitis obliterans* would arguable be higher. Further, it is worth noting that some of the "minimal to mild" PLI effects considered to be "significant" by Maier et al. (2010) may have in fact not been significant at all. Specifically, Morgan et al. (2008) did not provide any measures of statistical significance in their effects table (Table 6), and it is unclear whether any effects observed in the 25 ppm exposure group, and possibly even the 50 ppm exposure group, were greater than in the controls. Further, it could be argued that 25 ppm is a NOAEL for PLI, rather than a LOAEL, given the fact that 3/5 mice *in the control group* experienced PLI effects 6 weeks post-exposure, with a frequency and severity *greater than* those in the 25 ppm group at both 6 weeks and 12 weeks. Clearly, the PLI effects at 25 ppm are probably not treatment-related. Finally, if one assumes that a severity score of 2.0 ("mild") or greater is required to qualify as a true adverse effect (as opposed to the "minimal" scores of 1.0 included by Maier et al. (2010), which appear to be indistinguishable from background in the Morgan study), the LOAEL is actually 100 ppm. Along these lines, I am not aware of any occupational standard, set by any regulatory agency in the world, that is based on only "minimal" URT effects that actually occur with *greater* severity and frequency in the unexposed population. In short, the modeling exercise employed by Maier et al. (2010) is a valid but very conservative exercise, and other just as reasonable and valid approaches would yield much higher thresholds.

Egilman et al. (2011) proposed a "safe" occupational limit of "around or below 1 ppb" (equivalent to a 45-year occupational exposure of 0.045 ppm-years or less), based on "evidence from multiple epidemiology studies." The authors also suggest that 1 ppb, or even levels below 1 ppb, may still pose a risk of BO to the consumer. The concept of a 1 ppb (or less) threshold for BO is clearly not valid. For example, according to Egilman et al (2011), the odor threshold for diacetyl is 1.5 ppb. By extension, a $\leq$1 ppb threshold would infer that an individual who sniffed a glass of California chardonnay might end up needing a lung transplant. Similarly, Rothweiler et al. (1992) characterized the "long term" emissions from carpets and water-based adhesive in a residential setting, and reported indoor diacetyl concentrations of 9 ppb and 15 ppb for carpet with polyurethane and latex backings, respectively, four days following the carpet installation. According to the threshold proposed by Egilman et al. (2011), individuals living in newly carpeted settings may be at risk of developing BO. Further, airborne diacetyl levels in the offices and warehouses of the popcorn and flavorings facilities routinely exceeded 1 ppb by several orders of magnitude yet clearly these workers are not at risk of developing BO.

In summary, based on the weight of the scientific evidence, the 0.2 ppm threshold is a reasonable, and likely conservative, cumulative exposure threshold to use in the characterization of health risks to workers exposed to diacetyl.