LETTER TO THE EDITOR


Brent L. Finley,* David A. Galbraith,* † and David Weill‡

*ChemRisk, Inc., San Francisco, California 94105; †Palo Alto Medical Foundation, Palo Alto, California 94301; and ‡Stanford University Medical Center, Division of Pulmonary and Critical Care Medicine, Stanford, California 94305-5236

Received May 12, 2008; accepted June 2, 2008

Dear Sirs

Morgan et al. (2008) evaluated respiratory responses in mice exposed to heated diacetyl vapors. This research is potentially relevant to the ongoing investigations into food-related industries where it has been suggested that artificial butter flavoring (ABF) and, specifically, diacetyl exposures are associated with serious respiratory effects in workers, including constrictive bronchiolitis, a form of obliterative bronchiolitis (OB).

For the sake of background, constrictive bronchiolitis is a peribronchiolar process of fibrosis, that “surrounds rather than fills the lumen,” resulting in “extrinsic compression and obliteration of the airway” (Ryu et al., 2003). The deposition of collagen and progressive fibrosis produces an “obliterated” appearance to biopsy specimens, reflecting irreversible distal airway damage with permanent loss of pulmonary function. One must be careful, however, in interpreting the results of tissue analysis. Although histologic signs of inflammation can be suggestive that constrictive bronchiolitis could eventually occur in an individual case, its presence should not be assumed to represent an “early form” of chronic severe lung disease (Chan and Allen, 2004; Colby, 1998; Laohaburanakit et al., 2003).

Because the existing diacetyl toxicology database is comparatively sparse, the Morgan 2008 study may be referred to in the process of setting occupational standards or for identifying and controlling inhalational risk factors in the flavorings and processing industries. Accordingly, we have several questions and comments.

1. Morgan et al. (2008) indicated that the animals in their study were exposed to diacetyl concentrations similar to those experienced by workers in popcorn manufacturing facilities. “Mixers were exposed to time-weighted average diacetyl concentrations of about 100 ppm, with potential exposure to peak concentrations greater than 1200 ppm when manually adding flavoring to heated oil” (Morgan et al., 2008). The National Institute for Occupational Safety and Health (NIOSH) investigation of the Gilster-Mary Lee popcorn facility described by Kreiss et al. (2002) was cited to support the authors’ exposure estimates.

Unfortunately, much of the NIOSH microwave popcorn facility data has not been accompanied by sample duration information, making it difficult to distinguish “peak” from Time Weighted Average (TWA) measurements. However, our review indicates that the TWA diacetyl concentrations used by Morgan et al. (25–400 ppm) were in fact much higher than the TWA levels measured in the ABF mixing rooms, where the highest airborne concentrations have been found. The 100 ppm concentration cited actually represents the rounded-up maximum of a range of values (2.26–97.9 ppm) measured in the mixing room at the Gilster-Mary Lee facility (NIOSH, 2006). NIOSH did not provide sampling duration information, making the 100 ppm estimate neither representative (an abstracted maximum value from a single facility), nor certain that it even reflects a TWA value. If one examines 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), one finds that no TWA sample of 60 min or longer exceeds 3 ppm and in fact most TWA samples are less than 1 ppm. Although animal studies using exposures that exceed standard human conditions are commonplace in toxicology, such studies should not be represented as reflecting “typical” worker exposures. Hence, we believe it would be more accurate to state that the respiratory effects observed in Morgan et al. (2008) occurred at diacetyl concentrations orders of magnitude higher than typical TWA values measured in popcorn plant mixing rooms.

1 To whom correspondence should be addressed at ChemRisk, Inc., 25 Jessie Street at Ecker Square, Suite 1800, San Francisco, CA 94105. Fax: (415) 896-2471. E-mail: dgalbraith@chemrisk.com.

© The Author 2008. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved.
For permissions, please email: journals.permissions@oxfordjournals.org
Morgan et al. (2008) correctly note that Hubbs et al. (2002) exposed rats to ABF vapors containing 203, 285, or 352 ppm diacetyl and observed necrosis of the epithelium in the mainstem bronchi above 285 ppm. However, a more thorough discussion of the animal toxicology literature would have also discussed the implications of later studies by Hubbs et al. (2004, 2008), where rats were exposed to pure diacetyl vapor. Briefly, the respiratory epithelial damage seen with pure diacetyl was limited to the nasal and tracheal mucosa and was of lesser magnitude. No deep lung effects were noted. Hubbs et al. (2008) noted that the ABF vapors caused more damage than pure diacetyl and stated: “In view of the somewhat greater damage to intrapulmonary airways noted in rats inhaling butter flavoring vapor mixtures (Hubbs, 2002), we cannot exclude the possibility that other vapors in butter flavoring contribute to the lung disease seen in workers exposed to butter flavoring” (emphasis added).

We also note that neither Morgan nor Hubbs employed a control group in which animals were exposed to other heated organic (nondiacetyl) vapors, and wonder if some of the observed effects could simply be due to inhalation of any heated organic material in sufficient concentrations.

Morgan et al. (2008) discuss diacetyl in the context of phosgene (a WW1 chemical warfare agent), oxides of nitrogen, and other known inducers of OB (Borak and Diller, 2001; Coalson and Collins, 1985; Parimoni et al., 2004; Persinger et al., 2002; Peters and Hyatt, 1986), but observed that, unlike these acutely toxic respiratory agents, “...a distinct episode of diacetyl overexposure preceding the symptoms was not documented by workers, indicating that OB was more likely caused by repeated and/or intermittent exposures to toxic concentrations of diacetyl.” First, we would like to point out that phosgene and nitrogen oxides have well-documented mechanisms of action that involve the formation of corrosive acids in the deep lung. We are unaware of any such proposed mechanisms for diacetyl but perhaps the authors could clarify.

Second, by asserting that diacetyl must be able to cause obstructive airway lesions in humans and that respiratory effects reported in popcorn workers must have somehow been caused by diacetyl, we feel the authors have employed circular logic, leading to conclusions that contradict recent statements by NIOSH scientists.

For example, in addition to the aforementioned observation by NIOSH scientist Hubbs, in a recent NIOSH evaluation of a microwave popcorn facility in Montana it was found that all of the workers had respiratory problems, some suggestive of OB (NIOSH, 2003b). However, diacetyl was found to be nearly absent in the environmental monitoring of the facility and company officials did not believe that any diacetyl-containing materials were ever used in making their products (NIOSH, 2003b). In their concluding comments NIOSH noted: “This investigation emphasizes that many flavoring chemicals, not solely diacetyl, may be responsible for respiratory disease among workers exposed to flavoring chemicals. ... The increasing body of evidence implicating flavoring chemicals as causes of occupational respiratory disease should motivate a systematic approach to identify the occupational respiratory disease among workers in industries where flavorings are used or manufactured, to measure flavoring chemical exposures other than diacetyl in these industries...” (NIOSH, 2003b) (emphasis added).

This is consistent with findings in other NIOSH investigations, including the initial investigation of the Gilster facility, where NIOSH discovered that half of the reported cases of severe respiratory disorders occurred in packagers and quality control workers, where measured airborne diacetyl levels were far too low to be plausibly associated with disease (NIOSH, 2006).

At a recent meeting, NIOSH proposed a survey of flavorings manufacturing and food processing facilities, where NIOSH staff would collect personal and area samples of numerous agents that could be causing respiratory problems, including 2-furaldehyde, acetalddehyde, diacetyl, benzaldehyde, isovaleraldehyde, acetoin, propionaldehyde and phosphoric, butyric, acetic, and propionic acid (NIOSH, 2008).

Federal agency scientists charged with identifying and controlling the cause of serious occupational respiratory disorders have thus opted to keep an open mind regarding possible causative factors. We would encourage other researchers generating potentially useful and relevant information to do the same.

2. Similar to findings reported by Hubbs et al. (2002, 2004, 2008), Morgan et al. (2008) found no signs of distal airway damage as a result of diacetyl inhalation, even at levels that caused death in some animals. “Oropharyngeal aspiration,” where concentrated diacetyl in solution was injected into anesthetized animals, was the only protocol where the authors observed any suggestion of deep lung effects (Morgan et al., 2008). As they noted, this method is nonphysiologic, bypassing the normal protective mechanisms that minimize vapor intrusion into the lower lung. The authors reasoned that the more convoluted nasal passages in mice and the high water solubility of diacetyl could result in a higher degree of upper airway diacetyl effects in mice relative to humans, and that instillation of diacetyl liquid directly into the mouse lung might have a better chance of demonstrating damage.

First, we are curious as to whether the authors have evaluated direct lung aspiration of any other compounds, as it is unclear whether the reported responses were specific to diacetyl or could be observed for other organic liquids. We note that Dr Morgan has conducted inhalation studies with other volatile ketones (Morgan et al., 2000) and wonder if he has conducted similar aspiration experiments with these or other compounds. Future investigations should consider the study of other common organic compounds to characterize the degree of “uniqueness” of the observed diacetyl responses.
Second, even in the event that liquid diacetyl could somehow reach the deep lungs and persist under the conditions simulated by the authors, we note they still did not observe any significant degree of inflammatory response in their aspiration studies; a condition that would be expected in lesions felt to be at risk for eventually leading to OB.

Lastly, macrophage-predominant airway infiltration, found in the aspiration studies, is a picture more associated with respiratory bronchiolitis (Colby, 1998) (commonly associated in the aspiration studies, is a picture more associated with cigarette smoking) than with constrictive bronchiolitis, where lymphocytic infiltration of the airway submucosa is generally seen in the early stages of disease (Chan and Allen, 2004).

3. Constrictive bronchiolitis in humans exhibits a relative sparing of the conducting airways and lung parenchyma (Laohaburakanit et al., 2003) but striking effects on terminal bronchioles and alveoli. Conversely, rodents exposed to diacetyl have a pattern of proximal respiratory tract damage with distal sparing. If, as is suggested, the explanation is a simple matter of species-related differences in respiratory physiology, one would expect that many chemicals could fit this profile of effects. We therefore ask if the authors are aware of any such agents.

4. The Morgan et al. (2008) findings raise several questions and possibly suggest an alternative strategy for future research. Morgan et al. (2008), like Hubbs et al. (2008), found that rodents exposed to heated diacetyl vapors, at levels suggested to be representative of the workplace, experience significant necrosis of the upper airways but no lesions or fibrosis in the small airways of the deep lung (terminal bronchioles and alveoli), even at concentrations that may result in death following short-term exposure. This finding appears to be inconsistent with the terminal bronchiolar/alveolar obliterative pathology allegedly observed in workers reported to have OB. This suggests to us that one or more of the following assertions must be true:

a. The animal exposure studies do not accurately simulate real workplace conditions;

b. Rodents are not the appropriate animal model to represent humans, possibly due to the differences in airway physiology discussed by the authors, or;

c. Diacetyl simply does not cause OB in animals or humans.

We believe a different animal model is ripe for exploration. We suggest that an inhalation study employing animals whose nasal passages are known to be less convoluted and more analogous to humans (e.g., guinea pigs, dogs or rabbits) would be of interest. Such studies should continue to evaluate continuous as well as “pulsed” exposures, heated “negative control” organic vapor exposures should be studied, and the design should employ airborne diacetyl concentrations that more accurately represent levels measured in the workplace.

5. Morgan et al. mention several other diacetyl toxicological studies in various stages of preparation. We are interested in these efforts, and urge extra precautions to prevent unintentional bias from creeping into conclusions. For example, the authors concluded that: “Collectively, the findings of severe epithelial injury, lymphocytic bronchiolitis, and fibrohistiocytic lesions in mice exposed to various concentrations of diacetyl support the hypothesis that workplace exposures to diacetyl contribute to the development of OB in humans” (Morgan et al., 2008). In our opinion, both the Morgan and Hubbs studies have actually provided strong support for the contrapositives of their original hypotheses. Specifically, rodent studies involving diacetyl have not provided persuasive histologic evidence of OB in its customary anatomic distribution, using experimental exposures far greater than those experienced by humans, thus supporting the notion that diacetyl is not a likely cause or significant contributor to OB at exposure levels measured in the occupational setting.

We agree that further research would be helpful to better characterize the possible human health hazards posed by diacetyl and other chemicals in the food and flavorings industry. We also believe that careful attention to appropriate study methods, selection of a proper animal model and meticulous data interpretation are essential elements of this research.

REFERENCES


