Safe exposure level for diacetyl

In 2011, we published a proposal for a safe exposure level for diacetyl (2,3-butanedione) and submitted similar material to NIOSH for their consideration in setting legal limits.^{1,2} Included in those proposals was an interpretation of a confidential structure–activity analysis of diacetyl conducted for ConAgra by Kendall B. Wallace and produced in legal discovery.³ Recently, Wallace and Gilman Veith submitted to the NIOSH diacetyl docket a critique of our interpretation of Wallace’s analysis.⁴ Wallace and Veith did not accept our invitation to submit their critique to IJOEH as a letter to the editor in order to advance the scientific exchange. However it may be found, as of the 10th of June 2014, at <http://www.cdc.gov/niosh/docket/archive/pdfs/NIOSH-245/0245-111811-wallace.pdf>

Egilman and Schilling Respond

In his initial analysis for ConAgra, Wallace used a lowest unoccupied molecular orbital (LUMO) analysis, a mathematical model that compares the structure–activity relationships between different chemicals, and found that the LUMO energies of diacetyl and toluene diisocyanate (TDI) were comparable.³ In both our paper and NIOSH presentation, we framed the LUMO analysis as convergent, supplemental data supporting the occupational exposure limit derived from our analysis of animal and human data.^{1,2} Since the threshold limit value (TLV) for TDI is 1–3 parts per billion (ppb), we found that the TLV for diacetyl should be at the low end of this range, at 1 ppb. Others who have analyzed this question have come to similar estimates for the diacetyl TLV; ACGIH recommended 10 ppb and NIOSH recommended 1–5 ppb.^{2,5,6} ConAgra sponsored a company, Toxicology Excellence for Risk Assessment, (TERA) to develop a TLV for diacetyl.⁷ They recommended a level of 200 ppb, orders of magnitude higher than the ACGIH and NIOSH recommendations. TERA did not consider Wallace's analysis.⁷ We have previously critiqued the TERA study.²

We found nothing amiss with Wallace and Veith's original LUMO analysis, finding their methodology and results credible and consistent with other methods of evaluating diacetyl toxicity. However, in their recent submission to NIOSH, they claim that LUMO levels cannot be used to compare diacetyl to isocyanates because they have different mechanisms of action. Wallace and Veith, in their critique state that 'although LUMO can be used to distinguish between reactive and nonreactive chemicals across broad chemical groups, its use in rank ordering of individual chemicals is valid only when applied within a group of chemicals that share the same chemical reaction mechanism, which is not the case for α,β diketones such as diacetyl and isocyanates' [emphasis added].⁴ Wallace and Veith failed to provide any citation for this assertion. They fail to provide any evidence that LUMO and HOMO (highest occupied molecular orbital) numbers are not independent predictors of toxicity, irrespective of the biological/chemical mechanism involved. Current scientific analysis never provides a complete understanding of the mechanism through which toxins cause effects.

They reported their understanding of the differences between the mechanism of action of diacetyl and isocyanates to ConAgra:

*This [mechanism of action] is of particular concern when interpreting the data for the isocyanates, which possess a distinct and characteristic asthmatic response, believed to involve formation of a chemical 'hapten' while diacetyl evokes 'a chemical irritant pulmonary response.'*³ [emphasis added]

In vitro, animal and human studies have offered a great deal of evidence that diacetyl toxicity is related to hapten formation which is the same mechanism which Wallace and Veith posit for isocyanate toxicity. For example, Morgan *et al.* found that diacetyl reacts with *N*-alpha-acetylgarginine to form haptens.⁸

Morgan *et al.* explain that their results 'suggest that injury to the airway epithelium may involve alteration of cellular proteins containing arginine, including those on cell membranes. An immunological response to these modified cell membrane proteins may contribute to the development of obliterative bronchiolitis.'⁸ Isocyanates have been found to generate a similar immune response.⁹ Thus, whatever the 'chemical reaction,' diacetyl and isocyanates appear to act through at a similar immune mechanism.

Diacetyl and Formation of Citrulline Antigens: A Hapten Model for Diacetyl Toxicity

There is evidence that diacetyl may contribute to respiratory bronchiolitis in smokers. Diacetyl is added to tobacco and is present in cigarette smoke.¹⁰ Fourteen commercial cigarette brands and one reference cigarette released 301–433 μg of diacetyl per cigarette.¹⁰ Peak exposures in smokers were not measured. Almost all smokers have been found to have respiratory bronchiolitis interstitial lung disease (RBILD), and some develop other interstitial lung disease (ILD) as well.¹¹ Smoking enhances peptidyl arginine deaminase-2 expression. Peptidyl arginine deaminase-2 is an enzyme that catalyzes the conversion of arginine to citrulline in the bronchial mucosal and alveolar compartment, which in turn generates citrullinated proteins.¹² Diacetyl is used to react with protein-bound arginine to produce protein-bound citrulline.^{13,14} Researchers have treated citrullinated residues with diacetyl and antipyrine to create citrullinated proteins which react with anti-cyclic citrulline protein antibodies (ACPAs).^{13,15}

Recently several authors have suggested that ACPA production accompanies or contributes to

the development of ILD and bronchiolitis in rheumatoid arthritis (RA) or in patients who develop ILD or bronchiolitis obliterans in the absence of RA.^{16–18} Giles *et al.* suggested that ACPA play a ‘possible role’ in the pathogenesis of RA-associated ILD.¹⁷ Fischer *et al.* suggested that a subset of patients with ILD and bronchiolitis produce ACPA.¹⁶ Most of these patients (58%) smoked; however, the authors did not report other potential exposures to diacetyl or 2,3-pentanedione that have been and are present in microwave butter flavoring fumes.^{19,20}

NIOSH researchers found peak exposures of 4 and 13 million particles of diacetyl per cubic foot in the breathing zone of quality control workers while opening freshly popped microwave popcorn.² Consumer peak exposures to diacetyl from microwave popcorn bag opening should be comparable to those experienced by quality control workers.²¹ Elci *et al.* reported that repeated short-term, high exposures to diacetyl increased the risk of obstructive lung disease in these workers.²² Diacetyl which is inhaled as a result of exposure to microwave popcorn flavoring and tobacco smoke and e-cigarettes may enhance formation of citrulline antigens and thus play a role in the pathogenesis of RBILD and bronchiolitis.²³ This may only occur in patients who have a particular genetic pattern(s).¹⁸

For comparative toxicity purposes, LUMO values can be used to compare the relative reactivity and toxicity of chemicals that produce the same disease in humans. Isocyanates have been shown to induce bronchiolitis and bronchiolitis obliterans in animals and humans.^{24–26} We think that comparisons of toxicity based on LUMO and other chemical modeling methods are strengthened when the chemicals induce similar health effects in human and animal models, and as in this case, appear to trigger their toxicity through similar ‘mechanism(s) of chemical reactions.’

Wallace and Veith also predict that based on its ‘chemical reaction mechanism’, TDI poses a significant long term risk of cancer in contrast to diacetyl and 2,3-pentanedione.⁴ One of us (DE) helped design a study to address this concern.²⁷ Fortunately, isocyanate-exposed workers did not have an increase in cancer rates. Two other studies have also failed to find an increase in cancer rates in isocyanate-exposed workers, while one other has found an increase in lung cancer limited to women.^{28–30} The lack of clear evidence of carcinogenicity of isocyanates in exposed workers is further evidence that Wallace and Veith’s ‘mechanism of action’ theory is incorrect.

We think that it was a shame that Dr. Wallace and ConAgra failed to publish their analysis, which would never have seen the light of day had ConAgra not been forced to produce it in litigation. LUMO analysis

appears to be an important tool that can be used to predict chemical toxicity and prevent unnecessary deaths and injuries.

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