Miller, Diane M. (CDC/NIOSH/EID)

From: Kendall B. Wallace <kbwallace@stratatox.com>
Sent: Friday, November 18, 2011 4:13 PM
To: NIOSH Docket Office (CDC)
Subject: 245 - Criteria for a Recommende
Attachments: NIOSH_StrataTox. diacetyl criteria doc.pdf

Please see attached pdf file. Thank you

Kendall B. Wallace, Ph.D.
Diplomate, American Board of Toxicology
Fellow, Academy of Toxicological Sciences

President, StrataTox, LLC

5775 South Pike Lake Road
Duluth, MN 55811

(218) 393.8899

STRATATOX, LLC
5775 S. Pike Lake Road Duluth, MN 55811
(218) 393-8899

Lauralynn Taylor McKernan, ScD, CIH
Commander, U.S. Public Health Service
NIOSH Mail Stop C-34
Robert A. Taft Laboratory
4676 Columbia Parkway, Room 111
Cincinnati, OH 45226

November 18, 2011

Re: NIOSH Docket Number 245
NIOSH Diacetyl and 2,3-Pentanedione - Draft Criteria Document

Rebuttal to the remarks by Dr. Egilman and correction of the errors in his interpretation of the calculated LUMO values and their application to setting safe levels for diacetyl and 2,3-pentandione.

Reference the proceedings record entitled:
PUBLIC MEETING REGARDING NIOSH DRAFT DOCUMENT: CRITERIA FOR A RECOMMENDED STANDARD OCCUPATIONAL EXPOSURE TO DIACETYL AND 2,3-PENTANEDIONE

FRIDAY, AUGUST 26, 2011
The Public Meeting was held in the Hampton Ballroom in the Omni Shoreham Hotel, 2500 Calvert Street N.W., Washington, D.C., at 8:00 a.m., T.J. Lentz, Moderator, presiding.

Dr. McKernan,

As authors of the evidence in question, we would like to correct a significant error in Dr. David Egilman's comments to NIOSH regarding the use of ELUMO calculations to support the proposed REL for diacetyl of 5ppb. These comments begin on page 215 of the document and our rebuttal addresses specifically the errors made on pages 218-219 where Dr. Egilman made the following statement:

> *"I am just going to talk about some other data that supports the TLV as it exists. ConAgra hired an ex-EPA person to do a structure activity analysis. By the way, all of the data that I am talking about is in a peer review paper by myself and Hank Schilling, which I will drop off here. It is titled A Proposal for Safe Exposure Levels*

of Diacetyl. It is peer reviewed, and it came out about four months ago, but it is not mentioned in the document.

And it is not a personal thing but I have data in there that is relevant to the discussion, the data that I have been referring to over and over again. This data, for example, only appears in that published paper. And what they have found was that structure activity relationship of this material was similar to TDI, which is not the most toxic of the isocyanates; HDI probably is. And that based on that analogy, the TLV would be about one part per billion because that is what the TLV is for isocyanates. So that is another piece of independent analysis performed at the funding of ConAgra that comes out with a 1.0 ppb number."

In his statement to NIOSH, Dr. Egilman is referring to sophisticated and theoretical quantum chemical calculations performed by ToxDx, LLC to first distinguish reactive from non-reactive chemicals identified in complex mixtures such as flavors and fragrances and then to rank-order chemicals within a group sharing a common reaction mechanism, such as Michael addition, nuclear substitution, SN2, etc. The ultimate objective was to identify those compounds in the mixture that might present the greatest concern for potential adverse human health effects. As the authors of this approach, it is our opinion that Dr. Egilman misinterpreted the scientific meaning and application of the results; he then published his misinterpretation in a journal devoted to occupational and environmental health. At the NIOSH public meeting, Dr. Egilman referred to his publication as being reviewed and authenticated by peers. Unfortunately, this erroneous interpretation of our results was not recognized during the review of his article prior to publication. In the following paragraphs we hope to shed light on the theory and proper interpretation of these calculations.

Chemicals that share similar structural and physical chemical properties tend to have similar behaviors when administered or exposed to humans. Application of these principles of quantitative structure-activity relationships (QSAR) has enormous potential for informing regulatory decisions, especially for the thousands of chemical identities for which little if any toxicity data is available; diacetyl and 2,3-pentadione are good examples. When untested chemicals are grouped and compared with well-studied chemicals that share similar structural and physical chemical properties, initial estimates of chemical hazard and safety can be formed. Although strictly theoretical and not sufficient for final rule-making, such projections, when properly applied, offer important insight into identifying those chemicals within a complex mixture that warrant the greatest concern for further toxicity testing.

One such measure of chemical similarity is the ability of chemicals to "react" with biological molecules to form a stable (covalent) bond. Such chemicals are referred to as "reactive" because they have the potential to form stable bonds with possible critical biologic target molecules (protein, DNA, etc.); this distinguishes them from "nonreactive" chemicals that cannot form stable bonds with molecular targets.

Grouping chemicals based on chemical reactivity can be accomplished by calculating parameters that describe what might happen when a chemical comes in contact with body tissues. For chemicals to be reactive, a stable bond is formed by an exchange of electrons between biological molecules and the chemical. Chemicals have greater tendencies to accept

those electrons (become reactive) when the energy of certain empty molecular orbitals of the chemical is lower than the energy of the electrons of the donor molecule (biological target), and a chemical bond may form spontaneously. A calculated parameter called LUMO (defined as the energy of the lowest unoccupied molecular orbital) is used to identify chemicals that could react with biological molecules and distinguish them from chemicals that are not reactive. There are many quantum chemical computer programs publically available that can be used to calculate these LUMO energies, as well as many other important theoretical parameters of chemicals.

However, while LUMO may be a useful parameter to identify reactive chemicals among complex mixtures of hundreds of chemicals, LUMO only describes the potential of a chemical to react; It is a measure of chemical reactivity. LUMO does not predict how rapidly the chemical will react nor do LUMO values predict which specific sites in membranes, proteins, or DNA will be the likely targets of the chemical. Since the toxicological effects of reactive chemicals depend on both the reaction rate and the reaction site (physical chemical properties of the molecular target) not to mention exposure and dosimetry, LUMO is a necessary first step in grouping chemicals based on potential to react but it does not indicate that two reactive chemicals that differ significantly in other important chemical structure or physical properties will have similar biological targets or potential adverse effects.

Based on his understanding, Dr. Egilman asserted in his comments to NIOSH and in his published manuscript, that chemicals with similar LUMO values should have the same toxicological effects, and that one can rank-order the degree of risk based only on LUMO values. Dr. Egilman advocates that since diacetyl and the isocynates have comparable LUMO values, they should have similar safety levels (exposure limits). Unfortunately, this fails to recognize the limitations of LUMO. 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-substituted diketones such as diacetyl and isocyanates.

Chemicals like diacetyl and 2,3-pentanedione can be detected by olfaction at low levels because they belong to a special class of reactive chemicals with a highly specialized reactivity mechanism. In this case, LUMO might suggest that diacetyl is reactive, but it cannot be used to predict how selective it is for certain binding sites in the airway. The acrylates used in the coatings industry are also reactive, but they bind with sulfur atoms in cysteine residues whereas diacetyl will not react with sulfur moieties. Consequently, diacetyl and acrolein may have similar LUMO values and share some short-term effects such as irritation of the nasal-pharyngeal surfaces, but the long-term effects are completely different due to the different binding capabilities.

In the case of the isocynates, the differences are even greater. Isocynates are highly reactive with a wide variety of biological tissues and react by a mechanism completely distinct from diacetyl. Diacetyl reacts with guanidine moieties whereas isocynates react with a wide variety of biological molecules and are classified as carcinogens (1). Thus, TDI (toluene diisocyanate)

poses a significant long-term risk of cancer, which is not the case for diacetyl or 2,3-pentanedione. To suggest that LUMO values can be applied in read across approaches to set safety limits of dramatically dissimilar chemicals like diacetyl and TDI is inappropriate on the basis of fundamental scientific principle.

In conclusion, based on a sound scientific understanding of LUMO, we assert that Dr. Egilman's testimony that the calculated LUMO for diacetyl and 2,3-pentanedione support the proposed REL for diacetyl of 5ppm is scientifically flawed.

Respectfully submitted,

StrataTox has my approval to sign the original on my behalf.
Gilman D. Veith

Kendal B. Wallace, Ph.D., DABT, FATS
President, StrataTox, LLC

Gilman D. Veith, Ph.D.

(1) Bolognesi C, Baur X, Marczynski B, Norppa H, Sepai O, Sabbioni G., Carcinogenic risk of toluene diisocyanate and 4,4'-methylenediphenyl diisocyanate: epidemiological and experimental evidence. Crit Rev Toxicol. 2001 Nov;31(6):737-72.