ACETALDEHYDE WORKING GROUP

Response to the Targeted Consultation on Acetaldehyde Dehydrogenase (ALDH2) Genetic Polymorphism and its Relevance to the Harmonised Classification and Labelling of Acetaldehyde (Submitted July 25, 2016)

Overview

The following comments are submitted in Response to the Risk Assessment Committee's (RAC) targeted consultation on acetaldehyde dehydrogenase (ALDH2) genetic polymorphism and its relevance to the harmonised classification and labelling (CLH) of acetaldehyde (EC 200-836-8; CAS 75-07-0) [1]. The comments were developed on behalf of the Acetaldehyde Working Group (AWG), which operates as a subgroup of the Vinyl Acetate Council (VAC). AWG/VAC is a not-for-profit association, located in Washington, DC (USA), whose mission is to address human health and relevant risk assessment/regulatory issues of interest to the membership[2].

This targeted consultation was initiated as an outgrowth of the June 2016 meeting of the RAC at which time the Committee "asked for further information to clarify the mode of action (MoA) of acetaldehyde - in particular, experimental and human studies that could clarify the influence of acetaldehyde dehydrogenase (ALDH2) genetic polymorphism on the physiological levels of acetaldehyde and possible health effects." AWG appreciates the RAC's desire to understand whether exposure to acetaldehyde could pose a systemic risk, including whether acetaldehyde effects are local and, in the case of inhalation exposure, confined to the upper respiratory tract. Since ALDH2 polymorphisms are known to occur among Asian populations showing an approximate 50% expression of the mitochondrial ALDH2*1/2 (the ALDH2+/-) heterozygous genotype, questions have been raised about systemic risks from acetaldehyde inhalation exposure.

AWG acknowledges the risk that exists from ethanol ingestion, with ethanol's systemic metabolism to acetaldehyde. In the case of ethanol ingestion, mitochondrial ALDH2 polymorphisms take on an important role in ethanol's hazard profile. At the same time, the systemic hazard posed by ingestion of ethanol in individuals with reduced capacity to metabolize acetaldehyde (e.g., ALDH2*1/2 genotypes) should not be assumed to be relevant to

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[1] http://echa.europa.eu/view-article/-/journal_content/title/echa-launches-targeted-consultation-on-the-harmonised-classification-and-labelling-of-acetaldehyde

[2] Members of AWG/VAC include: American Forest and Paper Association, Celanese Corporation, Dow Chemical Company, Eastman Chemical Company Kuraray America, LyondellBasell Industries, Wacker Chemie AG. These companies have global interest in these compounds, including EU initiatives pursuant to REACH. (Note that Celanese is the Lead Registrant for acetaldehyde.)

systemic risks posed by the inhalation of acetaldehyde. AWG is not aware of studies evaluating ALDH polymorphisms from the ingestion of the naturally occurring acetaldehyde in foods, flavorings or alcoholic beverages. These comments are focused on the inhalation route, given acetaldehyde's high vapor pressure (98 kPa at 20°C) and since inhalation is the most relevant route of exposure associated with industrial operations.

Presented in the attached are perspectives relevant to systemic toxicity concerns related to ALDH2 polymorphisms and the likely impact on upper respiratory tract injury. The comments give particular attention to addressing how genetic polymorphism impact toxicokinetics, in particular the distribution of acetaldehyde *in vivo*. There is a body of information indicating that ALDH activity is expressed in various tissues including those responsible for metabolism at sites of first contact (e.g., nose and lung) for inhalation exposure to acetaldehyde. While ALDH has been detected in various tissues of both humans and rodents, comparative mapping of ALDH in the respiratory tract are lacking for humans, making comparison of respiratory tract risk to acetaldehyde difficult. However, a physiologically-based pharmacokinetic (PBPK) model was developed in collaboration with the US EPA (Teeguarden et al, 2008, attached) that allows comparison of absorbed acetaldehyde dosimetry in the nose of rats and humans. According to the PBPK model, a very limited role was found for the high affinity, mitochondrial ALDH (ALDH2) in the metabolism of acetaldehyde. The modeling suggested that other ALDH isoforms, such as ALDH1, which is a low affinity but higher capacity enzyme, effectively manage the acetaldehyde load to the nasal mucosa.

Teeguarden et al. (2008) also reported that reduced acetaldehyde clearance in heterozygote intermediate activity individuals had a minimal effect on acetaldehyde concentrations and acetaldehyde flux in respiratory and olfactory epithelium and on pH or H+ concentration in the respiratory epithelium and olfactory submucosa. They reported that the effect was greatest at low exposures, where tissue acetaldehyde concentrations were near the Km of 30 µM. The acetaldehyde concentrations in dorsal olfactory epithelial tissue were only 6% higher for the zero activity genotype than for the full activity genotype at exposures of 10 ppm acetaldehyde, and 0.6% higher at exposures of 150 ppm acetaldehyde. Again, this is evidence for other ALDH isoforms with lower affinity but higher capacity relative to ALDH2, in metabolizing acetaldehyde and essentially contributing to a first-pass-like barrier to systemic entry of acetaldehyde into systemic circulation.

Based on these findings, acetaldehyde tissue and systemic concentrations are not expected to be markedly increased at inhaled exposure concentrations of 50 ppm acetaldehyde, which corresponds to the histopathological NOAEL in a 90-day inhalation study with acetaldehyde (Dorman et al., 2008) and a level well below the saturation of ALDH detoxification in rodent nasal mucosa, estimated to be at acetaldehyde concentrations of 300 ppm (Stanek & Morris, 1999).

Collectively, these data show a minimal role for ALDH2 in the metabolic clearance of acetaldehyde and metabolic acidification of nasal tissues following exposure to acetaldehyde, even at low exposures. The data suggest that the ALDH1 pathway is responsible for a

preponderance of the total acetaldehyde metabolism. While ALDH1 polymorphisms are known to exist in humans, we are not aware of studies showing any impact of ALDH1 polymorphisms on tissue dosimetry or on upper respiratory tract injury.

Based on the available information, AWG concludes that ALDH2 polymorphisms are not expected to significantly change nasal tissue dosimetry of inhaled acetaldehyde and that the contribution of heterozygous or homozygous deficient ALDH2 phenotypes should have minimal, if any, effect on the inhalation or systemic toxicity of acetaldehyde.

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Issues Relevant to Potential Impacts of ALDH2 Polymorphisms on the Upper Respiratory Tract Following Inhalation Exposure

Acetaldehyde is metabolized to acetate by aldehyde dehydrogenase (ALDH) (Jakoby et al., 1982), a detoxifying pathway in most tissues, including the nasal tissues of rodents and humans. Although ALDH metabolism is relevant for acetaldehyde exposure by both inhalation and oral exposures, the information presented is focused on inhalation exposure, including occupational and general populations.

Is ALDH expressed in different forms?

Two key variants of the wild-type ALDH2 allele have been identified: ALDH2*1, an active form of the enzyme that is dominant in most populations studied, and ALDH2*2, an inactive form of the enzyme that is relatively common only in Asians. Three genotypes and their corresponding phenotypes are expected: a fully active homozygote (ALDH2*1/1, full activity), an intermediate activity heterozygote (ALDH2*1/2, intermediate activity), and a zero activity homozygote (ALDH2*2/2, zero activity). Differences in the distribution of ALDH genotypes along the respiratory tract may be relevant for human risk assessment.

In rodent nasal olfactory and respiratory tissues, low-affinity (cytosolic, ALDH1) and high-affinity (mitochondrial, ALDH2) pathways for acetaldehyde metabolism have been described (Bogdanffy et al., 1998; Casanova-Schmitz et al., 1984; Morris, 1997). These same low- and high-affinity pathways (Yoshida, 1992) are present in human tissues (Franklin & Johnson, 1994; Ginsberg et al., 2002; Oyama et al., 2005; Stewart et al., 1996). ALDH2 protein or mRNA has been measured in human liver, kidney, muscle, heart, esophagus, spleen, thymus, prostate, testis, ovary, peripheral blood lymphocytes, and intestinal mucosa (Franklin & Johnson, 1994; Oyama et al., 2005; Stewart et al., 1996).

Is ALDH expressed differently between human and rodent tissues?

The distribution of ALDH2 expression in humans is similar to that in rodents for tissues that have been studied. ALDH2 is expressed in multiple mouse tissues including nasal tissues, liver, lung, testis, esophagus, stomach, colon, and pancreas (Morris, 1997; Oyama et al., 2005). In the rat, ALDH2 expression is similarly distributed— kidney, liver, lung, heart, spleen, and nasal tissues (Morris, 1997; Yoon et al., 2006). Hamster and guinea pig nasal tissues also show evidence of an ALDH2 pathway (Morris, 1997). Comparative data on human nasal tissue ALDH2 is unavailable since ALDH2 distribution has not yet been assayed, but given the wide tissue distribution of ALDH2 in humans and in mice and rats, and the evidence of a high-affinity pathway in the nasal tissue of multiple rodent species, it is plausible that ALDH2 is expressed in human nasal tissues.

What are the projected consequences of ALDH polymorphisms on acetaldehyde toxicokinetics?

PBPK model simulations (Teeguarden et al., 2008) suggest a more limited role for the high affinity ALDH in the metabolism of acetaldehyde, and as such limited contribution to the risks associated with exposure to acetaldehyde. This should not be surprising because the polymorphic form of ALDH has a low capacity for acetaldehyde metabolism and a low Km (30 µM). At inhaled acetaldehyde concentrations above 50 ppm, estimated tissue acetaldehyde concentrations are more than 10 times the Km for the polymorphic form of ALDH, and metabolism by this enzyme is fully saturated. Further, because of its low capacity, at these exposure levels the ALDH2 form of the enzyme contributes trivially to acetaldehyde metabolism. Therefore, its presence (e.g. the fully functional homozygote) or absence (e.g. the zero activity homozygote) is not important to estimated tissue concentrations of acetaldehyde or protons (i.e., increased local cytotoxicity from increased intracellular pH).

Do data exist comparing projected rodent and human toxicokinetics of acetaldehyde with ALDH polymorphisms?

Teeguarden et al. (2008) developed an acetaldehyde upper respiratory tract PBPK model to evaluate interspecies differences in tissue acetaldehyde and H+ dosimetry and explore the potential impact of ALDH2 polymorphisms on human nasal tissue dosimetry. These authors reported that the impact of presumed ALDH2 polymorphisms on human olfactory tissue concentrations was negligible; the high-affinity, low-capacity ALDH2 does not contribute significantly to acetaldehyde metabolism in the nasal tissues. The human equivalent acetaldehyde concentration for homozygous low activity was 66 ppm, 1.5% lower than for the homozygous full activity phenotype.

This model assumed metabolism of acetaldehyde by ALDH in the nasal cavity is modeled as two saturable processes comprising one high-capacity, low-affinity (ALDH1) and one low-capacity, high-affinity pathway (ALDH2), both following Michaelis–Menten kinetics. Acetaldehyde metabolism is assumed to be distributed in the nasal compartments according to previously reported histochemical data (Bogdanffy et al., 1986). Unidirectional airflow is assumed in the nasal cavity. The analytical expressions that describe the transport of an inhaled chemical across the lumen and tissue subcompartments in the nasal cavity have been described elsewhere in detail (Andersen et al., 1999; Bogdanffy et al., 1999b).

The presence of this polymorphism is well studied and has been characterized in hepatic but not nasal tissue. It was assumed that polymorphisms observed in human hepatic ALDH2, as described by Ginsberg (Ginsberg et al., 2002), are qualitatively and quantitatively predictive of those in the nasal tissues. Ginsberg reports the hepatic activity associated with each of the genotypes (Ginsberg et al., 2002), which are 0.54, 0.07, and 0.0 µmol/min/g liver for the fully active, intermediate activity, and zero activity genotypes, respectively. Assigning a relative activity of 100% to the fully active homozygote, the activity of the intermediate activity heterozygote was 13% of the fully active homozygote. The zero-activity homozygote was assigned a relative activity of zero for the ALDH2 Vmax.

Reduced acetaldehyde clearance in heterozygote intermediate activity individuals had a minimal effect on acetaldehyde concentrations and acetaldehyde flux in respiratory and olfactory epithelium and on pH or H+ concentration in the respiratory epithelium and olfactory submucosa. The effect was greatest at low exposures, where tissue acetaldehyde concentrations were near the Km of 30 µM. For example, the acetaldehyde concentrations in dorsal olfactory epithelial tissue were only 6% higher for the zero activity genotype than for the full activity genotype at exposures of 10 ppm acetaldehyde, and 0.6% higher at exposures of 150 ppm acetaldehyde. The olfactory submucosa tissue pH was only 1% lower (more acidic) in the full activity compared with the zero activity group for exposures of 10 ppm acetaldehyde and 1.5% lower for exposures of 150 ppm acetaldehyde. This suggests a minimal role for ALDH2 in the metabolic clearance of acetaldehyde and metabolic acidification of nasal tissues following exposure to acetaldehyde and other compounds producing acetaldehyde in the course of normal metabolism, even at low exposures. The ALDH1 pathway is responsible for a preponderance of the total acetaldehyde metabolism, at least at these experimental doses and flow rates.

The human equivalent concentration (HEC) to the rat NOAEL of 50 ppm based on steady-state acetaldehyde concentrations from continual exposures was 67 ppm. <u>The impact of presumed ALDH2 polymorphisms on the HEC calculation was negligible; the human equivalent acetaldehyde concentration for homozygous low activity was 66 ppm, 1.5% lower than for the homozygous full activity phenotype.</u>

Are systemic acetaldehyde concentrations likely to be affected for inhaled acetaldehyde by nasal tissue ALDH polymorphisms?

<u>PBPK modeling</u>

Teeguarden et al. (2008) utilized the hepatic Vmax for three variants of the human the high-affinity, low-capacity pathway for acetaldehyde metabolism (ALDH2) to explore the impact of corresponding, but unmeasured, reductions in nasal tissue ALDH2 activity would have on nasal tissue metabolism and tissue dosimetry. Measured ALDH2 activities show a limited capacity for acetaldehyde metabolism by ALDH2 in rat nasal tissues, which suggests a more limited role for ALDH2 and polymorphisms in ALDH2 to impact tissue acetaldehyde levels and risks from exposure to acetaldehyde. Human simulations, as expected, support this finding, demonstrating that polymorphisms in genes coding ALDH2 have very little impact on tissue aldehyde or H+ concentrations. This should not be surprising; data in multiple rodent species (Morris, 1997) indicate that in nasal tissues, ALDH2 does not seem to be the major contributor to acetaldehyde metabolism as it may be in the liver. Teeguarden et al. (2008) concludes that in the nose, ALDH1 activity is dominant compared to ALDH2 for the majority of the acetaldehyde metabolized. While ALDH1 polymorphisms are known to exist in humans, we are not aware of studies showing impact of ALDH1 polymorphisms on tissue dosimetry or on upper respiratory tract injury.

In vivo studies

Oyama et al (2007) evaluated the *in vivo* histopathology and blood levels of acetaldehyde in normal (Aldh2+/+) and acetaldehyde dehydrogenase-2 knockout (Aldh2-/-) mice. The sites harmed by acetaldehyde inhalation centered in epithelial tissues from the nose to trachea but not in the lung. This suggests that acetaldehyde is mostly absorbed upstream of the lung under the experimental conditions. The incidence of both epithelial erosion and subepethelial hemorrhage appeared higher in ALDH2-/- mice compared to Aldh2 +/+ mice. The authors concluded that the respiratory epithelium and subepithelium in the nose and squamous epithelium in the dorsal skin showed clear differences in damages between wild type and knockout mice, suggesting that in these tissues a certain level of ALDH2 that is enough for detoxifying acetaldehyde is expressed or induced. However, in the liver, inflammatory cell nests were similarly observed near interlobular vessels in both wild type and knockout mice, suggesting that a low level of ALDH2 is expressed or that other enzymes may metabolize acetaldehyde in these tissues. Since there are no PBPK modeling predictions available for mice, no conclusions can be drawn for whether these *in vivo* data are more relevant for human risk assessment.

Oyama et al. (2007) also showed acetaldehyde bioavailability depends upon functional ALDH2 activity. They noted that mean blood acetaldehyde concentrations of ALDH2−/− mice and ALDH2+/+ mice were 2.39 vs. 1.65 uM, respectively, in mice exposed to 125 ppm acetaldehyde, and 8.90 vs. 1.72 µM, respectively, in the 500 ppm acetaldehyde group. These data indicate that acetaldehyde in ALDH2 deficient mice, can be found at low levels in circulating blood, and at least theoretically, reach organs distal to the nose. In contrast, no change in circulating acetaldehyde was noted in ALDH2 competent mice indicating lack of bioavailability of acetaldehyde following inhalation exposure. These data are difficult to interpret since no blood acetaldehyde concentrations were reported for unexposed control mice; thus, it is unknown whether there were statistically significant differences observed, particularly in the low exposure group. Interestingly, ALDH metabolism is thought to be saturated at concentrations exceeding 300 ppm in the rodent nose (Stanek and Morris, 1999) and a concentration well below the histopathological NOAEL in a 90-day inhalation study with acetaldehyde (Dorman et al., 2008).

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