Comments submitted on behalf of:

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Introduction

We thank the State of California and the Office of Environmental Health Hazard Assessment for the opportunity to comment on the proposed No Significant Risk Level (NSRL) for diethanolamine (DEA).

However, we respectfully do not agree with the proposed NSRL value of 6.4 µg/day for exposure via the dermal route and we consider the derived value overly conservative. The approach used assumes a non-threshold mode of action for carcinogenicity for DEA which is considered not appropriate and relies on results obtained from cancer studies which have significant methodological flaws.

DEA is not a genotoxic carcinogen

Choline is an essential nutrient for humans and animals but the requirement varies by species. Choline plays important roles in cell membrane integrity, neurotransmission, regulation of gene expression, cell membrane signalling, lipid transport and metabolism, and early brain development. Whilst choline can be synthesized endogenously, most species require an exogenous source of choline to maintain adequate levels in particular during pregnancy, growth and development. The typical requirements for choline vary by species with mice and rats requiring significantly more choline than humans due to increased choline turnover as evidenced by circa 60-fold greater choline oxidase levels in these species versus humans.

In this regard it is also very important to note that choline restricted diets alone are able to induce liver tumours (Zeisel et al., 2000), in a non-genotoxic mode of action involving accumulation of triglycerides, oxidative stress, DNA damage and impaired apoptosis (Hensley et al., 2000) and mice have been shown to be distinctly susceptible to disturbances in choline homeostasis.

DEA is structurally analogous to choline and exerts its effects via:

Competitive inhibition of choline uptake in the diet leading to lower absorbed choline

Competitive inhibition of choline uptake in tissues via organic cationic transporters (OCT)

Competitive inhibition of the Kennedy pathway for synthesis of phosphotidylethanolamine and phosphotidylcholine

Disruption of choline oxidation to betaine, an important methyl donor in various biological processes and also important for maintaining cellular osmolarity

Numerous in vitro and in vivo studies have demonstrated the potential for DEA to perturb normal choline homeostasis. None of the available data indicate a genotoxic or non-threshold mode of action and as such use of multistage modelling and linear regression is considered not appropriate for effects which are threshold mediated.

In addition to the known species differences in choline requirements, the relevance of carcinogenic findings in rodent models is also considered questionable due to differences in methyl donor groups involved in DNA-methylation reactions and subsequent gene regulation. Methyl-donor metabolism in humans is not as susceptible to the effects of choline deficiency as compared to mice. The reason for this is that whereas betaine, a choline metabolite, is in rodents a major donor of methyl groups for DNA methylation reactions, this is not true for humans. Instead, humans rely predominantly on the methyl donor tetrahydrofolate (THF) for this purpose. As THF concentrations are independent of the level of choline in the body, the key initiating event to induce carcinogenicity in mice is not relevant in humans (Leung et al 2005).

NTP studies are methodologically unsound

The use of the rodent cancer bioassays conducted by NTP for classification of DEA as a carcinogen is considered problematic in that the studies have several significant flaws:

In these studies DEA was administered dermally in ethanol, a known carcinogen. The types of liver tumours observed are also known to occur following administration of ethanol alone. Furthermore, ethanol administration is known to increase liver requirements for choline and cause betaine depletion in the animals. The presence of ethanol therefore significantly exacerbates the effects of DEA on choline homeostasis.

There is a high incidence of tumours in the control animals most likely the result of ethanol exposure; indeed studies with ethanol alone in the same strain of mouse led to identical tumour types reported in the NTP study.

Although applied dermally, the dose sites were unoccluded allowing for oral exposure due to grooming. This would no doubt maximise systemic exposure to both ethanol and DEA. Derivation of dermal NSRL's using studies where exposure would have occurred via multiple parenteral routes concomitantly is considered not appropriate.

No dose response in tumour formation is observed for both sexes.

The 100%

occurrence of liver tumours at all dose levels tested indicate inadequate dose spacing which precludes use for establishing a point of departure.

In the mouse study, the maximum tolerated dose was exceeded at all dose levels in the females and in the top two dose levels in the males as evidenced by a >10% reduction in bodyweight gain at termination (males, 96%, 84% & 75% vs controls and females 80%, 70% & 64% vs controls low, mid & high dose respectively.) According to OECD guidance document 116, significant bodyweight reductions should be avoided in carcinogenicity studies and the top dose limited to causing a reduction in bodyweight

gain

of <10%. In the low dose group male mice, where reductions in bodyweight gain >10% did not occur, the incidence of tumours did not achieve statistical significance except for non-cancerous adenomas in the liver.

As a result of these flaws the evidence suggesting that DEA is potentially carcinogenic in humans and indeed whether DEA should be classified as a carcinogen is questionable.

In light of the methodological deficiencies in the NTP studies, Kirman et al. (2016) used the NTP studies to derive an appropriate NSRL (dermal) for DEA taking into account four cancer bioassays conducted for both DEA and DEA-containing condensates. Using the pooled data from the 4 cancer bioassays allowed better characterization of dose response and by taking into consideration species differences in dermal absorption a resulting NSRL value of 3400 ug/day was derived. This value is considered protective of human health.

References

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