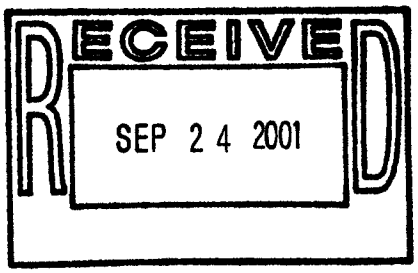
C T F A

THE COSMETIC, TOILETRY, AND FRAGRANCE ASSOCIATION

September 17, 2001



E. EDWARD KAVANAUGH
PRESIDENT

Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens
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P.O. Box 12233
Research Triangle Park, NC 27709

RE: Substances Under Review for Possible Listing in the Report on Carcinogens, Eleventh Edition (66 Federal Register 38430): Diethanolamine

Dear Dr. Jameson,

The Cosmetic, Toiletry, and Fragrance Association[1] (CTFA) appreciates the opportunity to provide comments on the above referenced topic. Diethanolamine-based ingredients are used within the personal care products industry, and thus, the review for possible listing in the Report on Carcinogens is of significant interest to CTFA members. This document addresses the basis of the nomination, and provides information that shows that the listing of diethanolamine (DEA) in the 11th Report on Carcinogens is not scientifically justified.

- Diethanolamine does not meet the NTP standard for listing as "reasonably anticipated to be a human carcinogen."
  The NTP standard to be listed as "reasonably anticipated to be a human carcinogen" based on studies in experimental animals is:
  "....there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset...."

[1]CTFA is the U.S. national trade association representing the personal care products industry. CTFA is comprised of over 300 active members that produce the vast majority of the cosmetics distributed in the U.S. and that also produce many over-the-counter drugs designed for dermal application. The association also has over 300 associate members that provide raw ingredients and supplies and services to the industry. Many of CTFA's members are international companies that do business in many foreign countries as well.

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The nomination of DEA is based on positive findings in one study carried out in $B6C3F_1$ mice. DEA tested negative ("no evidence of carcinogenic activity") in a chronic bioassay in F344/N rats conducted by NTP[2] and was negative in a transgenic mouse model (TgAC),[3] as will be discussed further below. Thus, the evidence for DEA carcinogenicity is from one study in one species, with DEA exposure occurring via one route of exposure.

In the mouse study, an increased incidence of tumors was seen in the livers of male and female mice. There was a marginal increase in renal tubule adenomas in males (only), which did not rise to the level of statistical significance until an extended analysis (step sectioning) was performed. No treatment-related increase was seen in kidney carcinomas, with or without step sectioning.

Mouse liver tumors are common spontaneous tumors, and $B6C3F_1$ mice are particularly susceptible. The historical background liver tumor rate in B6C3F1 mice in 72 studies conducted for NTP during the years 1981-1986 ranged from 2-70% in females, and 10% to 81% in males,[4] and in dermal studies conducted by NTP, the background rate for liver adenomas ranged from 56%-78%.[5] The incidence of adenomas in control animals in the DEA bioassay was 64% and 62% in females and males, respectively; and the incidence of adenomas and carcinomas combined was 66% and 78% in females and males, respectively. The typical high background liver tumor rate in $B6C3F_1$ mice, and the high control incidence in this study, preclude the conclusion that the increased incidence was to "an unusual degree." Furthermore, the mouse liver is the most common target site in rodent bioassays run by the NTP.[6] Thus, the incidence of liver tumors is not unusual with regard to either tumor type or incidence. Kidney adenomas are also known to occur spontaneously in $B6C3F_1$ mice, and were seen in the concurrent controls. Thus, the effects seen in the mice were increases in tumors that occur spontaneously as a consequence of the $B6C3F_1$ mouse genotype.

- <u>DEA was negative in chronic bioassays in F344/N rats, and was negative in transgenic mouse studies. DEA is non-genotoxic</u>.

As noted above, a chronic bioassay was carried out with DEA in F344/N rats. The results

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[2]NTP (1999) Toxicology and Carcinogenesis Studies of Diethanolamine (CAS No. 111-42-2) in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). NTP Technical Report Series No. 478.

[3]Spalding, J.W., French, J.E., Stasiewicx, S. Furedi-Machacek, M. Conner, F., Tice, R.R., and Tennant, R.W. (2000) <u>Toxicol. Sci.</u> Vol. 53(2), pp. 213-223.

[4]Haseman, J.K., Bourbina, J., and Eustis, S.L. <u>Fundam. Appl. Toxicol</u>. (1994) Vol. 23(1), pp. 44-52.

[5]See reference 2, p. 147.

[6]Huff, J., Cirvello, J., Haseman, J., and Bucher, J. (1991) <u>Environ. Health Perspect</u>. Vol. 93, pp. 247-270.

of this study showed "no evidence"of carcinogenic activity in either males or females. DEA was also negative in the Tg.AC transgenic mouse model. It has been suggested that the negative transgenic result, in contrast to the positive outcome of the chronic bioassay, "is not necessarily representative of a 'false-negative' result but rather, an indication that the conventional bioassay has given a false positive result. Thus, the response in transgenic animals may provide a more accurate assessment of potential human risk."[3] This statement was based on evidence of a species-specific response to DEA, which will be discussed further below.

DEA is non-genotoxic in a battery of assays, which included *Salmonella typhimurium* and mouse lymphoma gene mutation assays, tests for sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells in vitro, and a mouse micronucleus assay in vivo.[7]

- Mechanistic work has identified choline deficiency as the mechanism of action of tumorigenesis for DEA in the B6C3F$_1$ mouse, a highly susceptible species and strain. In contrast, humans are much less sensitive, and thus the outcome of the NTP study is not relevant to carcinogenicity in humans.

Research to elucidate the mechanism of action of liver tumor formation in B6C3F$_1$ mice exposed to DEA has been undertaken. Choline deficiency, a recognized cause of liver tumor formation in rodents,[8] has been investigated, based on DEA's ability to disrupt phospholipid metabolism by inhibiting the incorporation of ethanolamine and choline into phospholipids.[9] It has been shown that mice treated with DEA demonstrate changes in choline metabolites that are consistent with choline deficiency.[10,11] These effects were seen at all dose levels used in the mouse bioassay with severity that increased in relation to dose.[11] The changes were not seen in rats which did not develop tumors in the chronic bioassay.[11] In vitro studies have shown that DEA can block choline uptake into cells, alter the utilization of choline into phospholipid biosynthesis, and become incorporated in phospholipids directly.[12] These effects are competitive and reversible, and thus a critical dose must be exceeded in order to

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[7]See reference 2, p. 6 and Appendix E.

[8]Newberne, P.M., DeCarmargo, J.L.V., and Clark, A.M. (1992) Toxicol. Path., Vol. 10, pp. 95-109.

[9]Barbee, S.J. and Hartung, R. (1979) Toxicol.Appl. Pharm. Vol. 47, pp. 431-440.

[10]Stott, W.T., Bartels, M.J., Brzak, K.A., Mar, M-H., Markham, D.A., Thornton, C.M., and Zeisel, S.L. (2000) Toxico. Lett., Vol. 14(1-3), pp. 67-75.

[11]Lehman-McKeeman, L.D. (2001) Toxicology, in press.

[12]Lehman-McKeeman, L.D. and Gamsky, E.A. Biochem Biophys. Res. Commun. (1999) Vol. 262, pp. 600-604.

elicit the adverse effects. Choline supplementation inhibited morphological transformation in Syrian Hamster Embryo cells treated with DEA.[13] The mechanistic work has also demonstrated the lack of formation of nitrosamines from DEA under conditions designed to favor their formation.[10]

The B6C3F$_1$ mouse is relatively lacking in capacity to maintain methylation status, which is thought to contribute to its susceptibility to hepatocarcinogenesis.[14] Altered DNA methylation can result from changes in S-adenosylmethionone (SAM) levels. Studies looking at alteration of SAM levels by DEA showed greater sensitivity in the B6C3F$_1$ mouse strain compared to C57Bl/6 mice, a strain which is relatively resistant to liver tumor formation.[11] Data have also been developed showing that DEA does not effect SAM levels in rat liver, again demonstrating species differences.[11]

Humans differ markedly from rats and mice with respect to choline metabolism and with choline requirements. Rodents oxidize choline more rapidly than humans, a fact that is thought to contribute to species differences in susceptibility to choline deficiency.[15] Rodents require more methionine (part of the choline biosynthetic pathway) than humans do because of a greater demand for cysteine needed for hair growth.[16] In rodents, de novo synthesis of choline cannot keep up with the body demand for choline, and as such choline is a required dietary nutrient. In contrast, choline deficiency can be induced in humans only under extraordinary circumstances[17] which is reflected in the fact that a recommended daily allowance (RDA) for choline has not been established.

Rats are also known to be sensitive to choline deficiency; however, the F344 rats exposed to DEA did not develop tumors. The dose levels used in the rat study were less than those used in the mouse study, and it is known that DEA penetrates mouse skin more readily than rat skin.[18] Furthermore, rats do not exhibit the extensive grooming behavior seen in mice, which would have effectively increased the dose received by the mice in this dermal study. The use of higher DEA doses in the chronic rat study was precluded by effects seen in skin in a subchronic rat study conducted by NTP; a higher dose would have exceeded the MTD.

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[13]Lehman-McKeeman, L.D. and Gamsky, E.A. (2000) Toxicol. Sci., Vol. 55(2), pp. 303-310.

[14]Counts, J.L., Sarmiento, J.I., Harbison, M.L., Downing, J.C., McClain, R.M. and Goodman, J. (1996) Carcinogenesis, Vol. 17(6), pp. 1251-1257.

[15]Sidransky, H. and Farber, E. (1960) Arch. Biochem. Biophys. Vol. 87, pp. 129-133.

[16] Zeisel, S.H. and Blusztajn, J.K. (1994) Ann. Rev. Nutr. Vol. 14, pp. 269-296.

[17]Savendahl, L., Mar, M.-H., Underwood, L.E., and Zeisel, S.H. (1997) Am. J. Clin. Nutr., Vol. 66, pp. 622-625.

[18]Sun, J.D., Beskitt, J.L., Tallant, M.J., and Frantz, S.W. (1996) J. Toxicol.-Cut. & Ocular Toxicol., Vol. 15(2), pp. 131-146.

However, the dose of DEA that the rats received is still far in excess of a dose that humans would encounter in any realistic exposure scenario, and yet this sensitive species did not develop tumors. It is reasonable to conclude that the carcinogenic risk from DEA to humans, with a much lower sensitivity to choline deficiency, is theoretically nonexistent.

- The use of ethanol as a vehicle is a confounding factor for the bioassay.

The vehicle used in the mouse chronic bioassay was ethanol, a chemical which can disrupt phospholipid synthesis. Ethanol increases choline oxidation, limiting its availability to cells, and increases the breakdown of phosphatidylcholine. Further, ethanol stimulates the methylation of phosphatidylethanolamine which stresses the SAM pathway.[19] Therefore, ethanol, itself, represents a significant confounding factor for this study. Furthermore, the International Agency for Research on Cancer (IARC) lists alcoholic beverages as a known human carcinogen, and liver is the primary target organ for ethanol toxicity.[20]

- The International Agency for Research on Cancer (IARC) conducted a weight of the evidence review of DEA carcinogenicity and concluded that DEA was "not classifiable as to human carcinogenicity."

DEA underwent evaluation by IARC in 2000. The IARC review considered all of the available evidence on DEA in reaching a conclusion on its carcinogenicity. The outcome of that review was that there was "inadequate evidence in humans for the carcinogenicity of diethanolamine", and "limited evidence in experimental animals for the carcinogenicity of diethanolamine." DEA was classified as Group 3, "not classifiable as to its carcinogenicity to humans."[21]

- Bioassays of DEA/fatty acid conjugates do **not** provide support for the listing of DEA.

The results of the chronic bioassays carried out with DEA/fatty acid conjugates - coconut oil acid diethanolamine condensate, lauric acid diethanolamine condensate, and oleic acid diethanolamine condensate - are cited in the DEA NTP technical report as being consistent with the results of the DEA study.[2] The increase in liver tumors seen in mice in the coconut oil acid diethanolamine condensate and the lauric acid diethanolamine condensate (females only) is attributed to free DEA contained in the test material. However, all three condensate test materials are complex mixtures with many distinct and different components.

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[19]Barak, A.J.,Tuma, D.J., and Sorrell, M.F. (1973) Am. J. Clin. Nutr., Vol. 26, pp. 1234-1241.

[20]IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. (1998) Ethanol, Vol. 44, p. 35.

[21]IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. (2000) Diethanolamine, Vol. 77, p. 349.

Additionally, there is uncertainty around the level of free DEA in the test material. Unreacted "amine" levels were apparently provided by the manufacturer; however, it is unclear whether the identity of the amines was confirmed.[22] [23] DEA levels were not monitored during the study. It is not scientifically justified to attribute adverse effects seen in the condensate studies to free DEA, and thus these results do not provide additional evidence of the carcinogenicity of DEA in mice.

In summary, for the reasons stated above, CTFA strongly believes that DEA does not meet the NTP criteria to be listed as "reasonably anticipated to be a human carcinogen." An increased incidence of a common tumor type was seen in one species in a chronic bioassay. A second study in rats gave negative results, as did a study in transgenic mice. Mechanistic work has identified choline deficiency as the mechanism of action in $B6C3F_1$ mice, a uniquely susceptible species and strain. This mechanism would not be relevant to humans under realistic exposure conditions. Furthermore, the bioassay was confounded by the use of ethanol as a vehicle, which can itself disrupt choline homeostasis. Lastly, IARC reviewed all of the available information and concluded that DEA was "not classifiable as to its carcinogenicity to humans."

Thank you for your attention to these issues.

Sincerely,

[redacted]

Gerald N. McEwen, Jr., Ph.D., J.D.
Vice President - Science

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[22]NTP (2001) Toxicology and Carcinogenesis Studies of Coconut Oil Acid Diethanolamine Condensate (CAS No. 68603-42-9) in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). NTP Technical Report Series No. 479, Appendix I.

[23]NTP (1999) Toxicology and Carcinogenesis Studies of Lauric Acid Diethanolamine Condensate (CAS No. 120-40-1) in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). NTP Technical Report Series No. 480, Appendix I.