I would like to thank the NTP for conducting a 2-year carcinogenicity assay, in addition to the various microarray and endocrine disrupting assays, on 2-Hydroxy-4-methoxybenzophenone (HMB) and allowing for a comment period and subsequent webinar on December 12, 2019. With that said, I have the following questions:

1) Dose levels used in NTP feeding studies:
NTP conducted a 2- and 13-week feeding study in rats and mice on HMB (NIH Publication No. 92-3344, October 1992), between June 1985 and July 1988. The dosages used were 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm. The more recent 2-year feeding study (Draft Technical Report 597, December 12, 2019) was conducted between July 2010 and 2012 and utilized doses of 0, 1,000, 3,000 and 10,000 ppm.

Review of the FDA NDC sunscreen database identifies that HMB is commonly used in ingested products (SPF lipsticks, lip balms and lip glosses) between 30,000 ppm and 60,000 ppm. Although the studies conducted in the 1980's are closer to the actual human exposure for OTC drugs, none of the NTP studies appear to represent the maximum expose and/or any exaggeration of the human experience. The dose levels used in the newer study are more in-line with levels commonly found in cosmetic products that use HMB as a photo-stabilizing agent and not a sunscreen active. Additionally, it should be noted that ~155 sunscreens containing lip balms/glosses were also found in the database that are directly (cartoon character based) or indirectly (various flavors) marketed to children. The HMB levels used in these drug products are the same as noted above, however the mg/Kg dose would be higher based on children's weight.

Why were these doses selected and how do you reconcile these differences with respect to human exposure?

2) Genetic toxicology:
There appears to be some discrepancies in the results observed between the first (1980's) NTP study and second (2000's). In the first study 1,000 ug/plate of HMB was considered to be "weakly mutagenic in Salmonella with metabolic activation, and induced sister-chromatid exchanges and chromosomal aberrations in CHO cells in the presence of a metabolic activation system." In the second study, up to 6,000 ug/plate of HMB was used and considered negative for genetic toxicity.

As noted in the 1980's report "HMB was tested at doses up to 1,000 µg/plate. Higher concentrations were toxic to the cells". How would NTP reconcile the differences in strain tolerance levels between the studies spanning ~25 years?

Also, the 1980's study found weak mutagenic activity only when 30% hamster S9 was used. Why was only 10% rat S9 used in the more recent experiments?

3) HMB supplier information:
HMB used for the 1980's testing was obtained from Aldrich Chemical Corporation (Milwaukee, WI) and the HMB used in the 2000's testing was obtained from Ivy Fine Chemicals Corporation (Cherry Hill, NJ). Both are excellent specialty chemical houses, however, they may or may not be reflective of the actually HMB used by many companies in consumer products, plastic components and/or foods/food wraps.

In past days, both pharmaceutical and cosmetic companies conducted in-house analyses of each lot received of a raw material before incorporating it into a batch. Unfortunately, today many cosmetic companies and 3rd party manufacturers merely review a COA and release the lot for production as long as it appears to be within the specifications set by the manufacturer. Additionally, many companies by materials like HMB based on price and not purity and, therefore, purchase materials mostly manufactured in Asia, followed by the EU and/or the US.

How does NTP reconcile the difference between possible impurity levels of a chemical that may exist between specialty chemical and mass chemical manufacturers?

4) There appears to be a number of linear and non-linear dose related effects associated with HMB exposure impacting brain/spinal cord, bone marrow, spleen, liver, kidney, adrenal cortex, testis and uterus. Likewise, multiple effects were observed in microarray analysis (with various levels of statistical significance) and mostly weak endocrine disruption effects.

How does this data correlate to human toxicity (systemic and reproductive) at the concentrations tested (cosmetic level 1,000 – 10,000 ppm) in addition to the actual levels of human exposure from OTC sunscreens (up to 60,000 ppm) plus additional levels from foods (direct and indirect), and other structurally related HMB drugs (ketoprofen)?

More specifically, how does the data reported relate to the potential impact of HMB on Hirschsprung's disease (Huo et al, Chemosphere 2016; 144:1091-7 and DiNardo/Downs, Reprod. Toxicol. 2019; 86:98-100) and the impact HMB demonstrated on the decrease of fat mass in female children exposed in utero and followed for up to 9 years (Buckley et al, Environ Int. 2016; 91:350-6)?

Were the microarray analysis and endocrine disrupting studies concurrently run with the carcinogenic testing or were they a later addition?

5) Lastly, sunscreen products have become significantly more complicated then they were 40+ years ago. High SPF topical products often contain 35% - 45% actives and ingestible products 10% - 20%. Therefore, it is uncertain if testing only one sunscreen active at a time is relevant to the human experience, especially since many have been known to react among themselves and with other chemicals.

Additionally, a few published studies demonstrate that HMB in conjunction with other potential endocrine disruptors can act in a synergistic manner causing toxic reactions in yeast cells, rats and in human sperm at levels below their know individual NOAEL (Kunz - Aquat Toxicol. 2006, 79:305-24; Christiansen - Int J Androl. 2012, 35:303-16; Rehfeld - Endocrinology 2016, 157:4297-4308). Several other scientists have demonstrated this phenomenon with various combinations of pharmaceuticals, pesticides, fungicides, plasticizers and/or preservatives (Brander - PLoS One 2013, 8:e74251; Christiansen - Environ Health Perspect. 2009, 117:1839-46; Hultman - J Toxicol Environ Health 2017, 80:987-1001; Jacobsen - Int J Androl. 2010, 33:434-42; Katchy - Toxicol Sci. 2014, 138:21-35; Kortenkamp - Int J Androl. 2010, 33:463-74; Rider - Int J Androl. 2008, 31:249-62; Rider - Int J Androl. 2010, 33:443-62; Silva - Environ Sci Technol. 2002, 36:1751-6; Zhou - Chemosphere 2017, 178:378-83).

What is NTP's position on testing mixtures of chemicals to determine carcinogenic and endocrine disrupting potential?

Respectfully,
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