Comments on Draft Report 05 Dated March 21, 2021

NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Hydroxy-4-methoxybenzophenone (CASRN 131-57-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal and Reproductive Performance Assessments in F1 Offspring

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Issue: 2-Hydroxy-4-methoxybenzophenone (2H4MBP), also known as oxybenzone and benzophenone-3, is approved by the U.S. Food and Drug Administration for use in sunscreens and other personal care products in concentrations of <6%, either alone or in combination formulations, and as an indirect food additive in acrylic and modified acrylic plastics that come into contact with food.

Concern: To avoid confusion with the public "**approved by FDA**" should be removed. On February 26, 2019 FDA removed 2H4MBP and 11 other chemicals from Category I GRASE status to Category III "Insufficient data" for use in sunscreens based on the following concerns:

> "For example, the available literature includes studies indicating that oxybenzone is absorbed through the skin to a greater extent than previously understood and can lead to significant systemic exposure, as well as data showing the presence of oxybenzone in human breast milk, amniotic fluid, urine, and blood plasma. The significant systemic availability of oxybenzone, coupled with a lack of data evaluating the full extent of its absorption potential, is a concern, among other reasons, because of questions raised in the published literature regarding the potential for endocrine activity in connection with systemic oxybenzone exposure."

Also, FDA does not approve chemicals for use in **"other personal care products"** only Over-The-Counter drugs – please remove this statement. Also, 2H4MBP is commonly used at 6% (60,000 ppm) or less **(≤6%**), not less than 6% **"<6%"** as noted – please correct.

Issue: Under the conditions of this modified one-generation (MOG) study, there was equivocal evidence of reproductive toxicity of 2-hydroxy-4-methoxybenzophenone (2H4MBP) in Hsd:Sprague Dawley® SD® rats based on a decrease in F2 litter size in both the prenatal and reproductive performance cohorts.

Concern: There is no identification of the test levels used in the study in the conclusion. 2H4MBP use in sunscreens/antiaging products represents the highest use level and greatest risk to humans. Other than the recent absorption studies conducted by Matta et. al.[2,3], there has not been toxicity studies conducted on actual consumer products containing 2H4MBP. The maximum allowable level of 2H4MBP is 6% which is used in combination with other common sunscreen/antiaging actives, such as 3% Avobenzone + 15% Homosalate + 5% Octisalate + 10% Octocrylene + 7.5% Octinoxate (EHMC) - totaling a possible allowable use of actives in a product equal to 46.5% or 465,000 ppm. All of these actives have demonstrated, in the published literature, to possess some endocrine disrupting activity as well as may act synergistically with each another to cause toxicity[3-6]. Review of several SPF 50 or above products currently in the marketplace indicates a 35% - 45% active level of which 2H4MBP is commonly used at 6%. Therefore, greater emphasis needs to be placed on how this data is summarized in the conclusion, which can be taken out of context by the press and others. For example: in the Scientific Committee on Consumer Safety (SCCS) opinion on benzophenone-3[7] they state "it needs to be noted that the SCCS has regarded the currently available evidence for endocrine disrupting properties of BP-3 as inconclusive, and at best equivocal." Unfortunately, there were 42 DART publications

(Table 1 below), several in humans, that were ignored in the review allowing one to believe that there are no endocrine disrupting effects associated with benzophenone-3.

In summary, one should not simply state that "there was equivocal evidence of reproductive toxicity" and "some evidence of developmental toxicity". More emphasis needs to be placed on "when 2H4MBP was tested individually at one-half the concentration commonly used in sunscreen/antiaging products there was equivocal evidence of reproductive toxicity and some evidence of developmental toxicity. Additionally, it should be noted that 2H4MBP was not tested in conjunction with other OTC actives commonly used in sunscreen/antiaging products that may react synergistically with one another increasing the potential risk of reproductive and/or developmental toxicity. The shorter version of these statements would be that **this research does not represent the reproductive and/or developmental toxicity potential of 2H4MBP, which maybe greater than what is reported in this document, in humans at the levels used in sunscreen/antiaging products**.

Issue: "2-Hydroxy-4-methoxybenzophenone (2H4MBP) was obtained from Ivy Fine Chemicals (Cherry Hill, NJ) in a single lot (20100801), which was used for the dose range-finding and modified one-generation (MOG) studies … The HPLC/UV analysis showed a single impurity with a peak area <0.1%, indicating an 2H4MBP **purity of approximately 100.0%."**
Concern: Although most research utilizes the highest purity of a chemical available for testing, usually obtained from a specialty house, companies mostly purchase ingredients based on price not purity. A simple review of available grades of 2H4MBP in the marketplace demonstrates a purity range from 95% to 103%[8]. This should raise concern since impurities can have a profound impact on the toxicity produced by a chemical and, therefore, chemicals should be selected based on what companies purchase and not the highest purity levels available. Perhaps a footnote can be made in this section of the report stating that "**this ingredient may or may not represent the actually purity level of 2H4MBP that is used in consumer products**".

Additionally, based on the Maximum Tolerated Dose Studies conducted to determine the dose levels in feed it should be noted in the conclusion that levels of 30,000 ppm or 3% - which is half the human exposure - were found to produce **maternally toxicity** and that levels of 10,000 ppm or 1% - also a fraction of human exposure - were found to produce **fetal toxicity** based on the body weight data. Lastly, this data takes on a stronger significance when studies like Buckley et al.[9] are considered who observed in a nine year study following the children of mothers who tested positive for 2H4MBP in their third trimester - "After adjustment, phenol biomarkers were not associated with percent fat mass. However, the association between benzophenone-3 and percent fat mass was modified by child's sex: benzophenone-3 concentrations were inversely associated with percent fat mass in girls (beta = −1.51, 95% CI = −3.06, 0.01) but not boys (beta = −0.20, 95% CI = −1.69, 1.26). Although we did not observe strong evidence that prenatal environmental phenols exposures influence the development of childhood adiposity, the potential antiadipogenic effect of benzophenone-3 in girls may warrant further investigation."

References:
1) Matta MK et al. Effect of sunscreen application under maximal use conditions on plasma concentration of sunscreen active ingredients: a randomized clinical trial. JAMA 2019; 321:2082-2091.

2) Matta MK et al. Effect of sunscreen application on plasma concentration of sunscreen active ingredients. JAMA 2020; 323:256-267.
3) Rehfeld A et al. Chemical UV Filters Mimic the Effect of Progesterone on Ca 2+ Signaling in Human Sperm Cells. Endocrinology 2016; 157:4297-4308.
4) Christiansen S et al. Mixtures of endocrine disrupting contaminants modelled on human high end exposures: an exploratory study in rats. Int J Androl 2012;35:303-16.
5) Jang GH et al. Sequential assessment via daphnia and zebrafish for systematic toxicity screening of heterogeneous substances. Environ Pollut 2016; 216:292-303.
6) Kunz PY and Fent K. Estrogenic activity of UV filter mixtures. Toxicol Appl Pharmacol 2006; 217(1):86-99.
7) Scientific Committee on Consumer Safety SCCS OPINION on Benzophenone-3 - Opinion Adopted 30-31 March 2021. https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_247.pdf
8) Representative Technical Data Sheet for Benzophenone-3. https://www.ulprospector.com/documents/1555264.pdf?bs=34096&b=747069&st=20&r=na&ind=personalcare.
9) Buckley JP et al. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study. Environ Int 2016; 91:350-356.

Table 1 - DART Papers Omitted from 2021 Opinion - Scientific Committee on Consumer Safety on 2H4MBP:

No.	Omitted References
1	Aker AM, Watkins DJ, Johns LE, Ferguson KK, Soldin OP, Anzalota Del Toro LV, et al. 2016. Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environ Res 151:30-37.
2	Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.
3	Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Cordero JF, et al. 2019. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. Environ Res 169:41-51.
4	Arya S, et al 2020. Exposure of U.S. population to endocrine disruptive chemicals (Parabens, Benzophenone-3, Bisphenol-A and Triclosan) and their associations with female infertility*. Environ Pollut 265(Pt A):114763.
5	Balázs A, Krifaton C, Orosz I, Szoboszlay S, Kovács R, Csenki Z, et al. 2016. Hormonal activity, cytotoxicity and developmental toxicity of UV filters. Ecotoxicol Environ Saf 131:45-53.
6	Berger K, Gunier RB, Chevrier J, Calafat AM, Ye X, Eskenazi B, et al. 2018. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. Environ Res 165:379-386.
7	Binder AM, Corvalan C, Calafat AM, Ye X, Mericq V, Pereira A, et al. 2018. Childhood and adolescent phenol and phthalate exposure and the age of menarche in Latina girls. Environ Health 17:32.
8	Blüthgen N, Zucchi S, Fent K. 2012. Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*). Toxicol Appl Pharmacol 263:184-194.
9	Broniowska Ż, Pomierny B, Smaga I, Filip M, Budziszewska B. 2016. The effect of UV- filters on the viability of neuroblastoma (SH-SY5Y) cell line. Neurotoxicology 54:44-52.
10	Buckley JP, Herring AH, Wolff MS, Calafat AM, Engel SM. 2016. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study. Environ Int 91:350-356.
11	Buttke DE, Sircar K, Martin C. 2012. Exposures to endocrine-disrupting chemicals and age of menarche in adolescent girls in NHANES (2003-2008). Environ Health Perspect 120:1613-1618.
12	Campos D, Silva ARR, Loureiro S, Grabicová K, Staňová AV, Soares A, et al. 2019. Two-generational effects of benzophenone-3 on the aquatic midge *Chironomus riparius*. Sci Total Environ 669:983-990.
13	Coronado M, De Haro H, Deng X, Rempel MA, Lavado R, Schlenk D. 2008. Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4- methoxyphenyl-methanone) in fish. Aquat Toxicol 90:182-187.
14	DiNardo JC, Downs CA 2019. Can oxybenzone cause Hirschsprung's disease? Reprod Toxicol 86:98-100
15	Ferguson KK, 2018. Environmental phenol associations with ultrasound and delivery measures of fetal growth. Environ Int 112:243-250.
16	Guo J, Wu C, Zhang J, Li W, Lv S, Lu D, et al. 2020. Maternal and childhood urinary phenol concentrations, neonatal thyroid function, and behavioral problems at 10 years of age: The SMBCS study. Sci Total Environ 743:140678.
17	Harley KG, Berger KP, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2019. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod 34:109-117.
18	Hines EP, Mendola P, von Ehrenstein OS, Ye X, Calafat AM, Fenton SE. 2015. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reprod Toxicol 54:120-128.
19	Huo W, Cai P, Chen M, Li H, Tang J, Xu C, et al. 2016. The relationship between prenatal exposure to BP-3 and Hirschsprung's disease. Chemosphere 144:1091-1097.
20	Iribarne-Durán LM et al 2020. Menstrual blood concentrations of parabens and benzophenones and related factors in a sample of Spanish women: An exploratory study. Environ Res. 183:109228.
21	Joensen UN, Jørgensen N, Thyssen JP, Szecsi PB, Stender S, Petersen JH, et al. 2018. Urinary excretion of phenols, parabens and benzophenones in young men: Associations to reproductive hormones and semen quality are modified by mutations in the filaggrin gene. Environ Int 121:365-374.
22	Kim S, Jung D, Kho Y, Choi K. 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka (*Oryzias latipes*)--a two generation exposure study. Aquat Toxicol 155:244-252.
23	Kinnberg KL, Petersen GI, Albrektsen M, Minghlani M, Awad SM, Holbech BF, et al. 2015. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, *Danio rerio.* Environ Toxicol Chem 34:2833-2840.
24	Krause M, Frederiksen H, Sundberg K, Jørgensen FS, Jensen LN, Nørgaard P, et al. 2018. Maternal exposure to UV filters: Associations with maternal thyroid hormones, IGF-I/IGFBP3 and birth outcomes. Endocr Connect 7:334-346.
25	Krzyżanowska W, Pomierny B, Starek-Świechowicz B, Broniowska Ż, Strach B, Budziszewska B. 2018. The effects of benzophenone-3 on apoptosis and the expression of sex hormone receptors in the frontal cortex and hippocampus of rats. Toxicol Lett 296:63-72.
26	Lee J, Kim S, Park YJ, Moon HB, Choi K. 2018. Thyroid hormone-disrupting potentials of major benzophenones in two cell lines (GH3 and FRTL-5) and embryo-larval zebrafish. Environ Sci Technol 52:8858-8865.
27	Long J, et al. 2019. Maternal urinary benzophenones and infant birth size: Identifying critical windows of exposure. Chemosphere 219:655-661
28	Meng Q, Yeung K, Kwok ML, Chung CT, Hu XL, Chan KM. 2020. Toxic effects and transcriptome analyses of zebrafish (*Danio rerio*) larvae exposed to benzophenones. Environ Pollut 265:114857.
29	Messerlian C, Mustieles V, Minguez-Alarcon L, Ford JB, Calafat AM, Souter I, et al. 2018. Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment And Reproductive Health (EARTH) Study. Environ Int 114:60-68.
30	Nakamura N, et al. 2018 Transcript Profiling in the Testes and Prostates of Postnatal Day 30 Sprague-Dawley Rats Exposed Prenatally and Lactationally to 2-Hydroxy-4-methoxybenzophenone. Reprod Toxicol. 2018 December ; 82: 111–123
31	Philippat C, Wolff MS, Calafat AM, Ye X, Bausell R, Meadows M, et al. 2013. Prenatal exposure to environmental phenols: Concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. Environ Health Perspect 121:1225-1231.
32	Philippat C, Heude B, Botton J, Alfaidy N, Calafat AM, Slama R. 2019. Prenatal exposure to select phthalates and phenols and associations with fetal and placental weight among male births in the EDEN cohort (France). Environ Health Perspect 127:17002.
33	Polinski KJ, et al 2018. Distribution and predictors of urinary concentrations of phthalate metabolites and phenols among pregnant women in the Healthy Start Study. Environ Res. 162:308-317.
34	Santamaria CG, et al. 2020. Dermal exposure to the UV filter benzophenone-3 during early pregnancy affects fetal growth and sex ratio of the progeny in mice. Arch Toxicol 94(8):2847-2859
35	Scinicariello F, Buser MC. 2016. Serum testosterone concentrations and urinary bisphenol a, benzophenone-3, triclosan, and paraben levels in male and female children and adolescents: NHANES 2011-2012. Environ Health Perspect 124:1898-1904.
36	Seidel F, 2020. Reproductive toxicity of benzophenone-3. Arch Toxicol 94(10):3593-3594.
37	Tang R, Chen MJ, Ding GD, Chen XJ, Han XM, Zhou K, et al. 2013. Associations of prenatal exposure to phenols with birth outcomes. Environ Pollut 178:115-120.
38	Tao J, Bai C, Chen Y, Zhou H, Liu Y, Shi Q, et al. 2020. Environmental relevant concentrations of benzophenone-3 induced developmental neurotoxicity in zebrafish. Sci Total Environ 721:137686.
39	Wang Z, et al. 2020. Perinatal urinary benzophenone-3 concentrations and glucose levels among women from a fertility clinic. Environ Health 19(1):45
40	Wnuk A, Rzemieniec J, Lasoń W, Krzeptowski W, Kajta M. 2018a. Apoptosis induced by the UV filter benzophenone-3 in mouse neuronal cells is mediated via attenuation of Erα/Pparγ and stimulation of Erβ/Gpr30 signaling. Mol Neurobiol 55:2362-2383.
41	Wnuk A, Rzemieniec J, Litwa E, Lasoń W, Kajta M. 2018b. Prenatal exposure to benzophenone-3 (bp-3) induces apoptosis, disrupts estrogen receptor expression and alters the epigenetic status of mouse neurons. J Steroid Biochem Mol Biol 182:106-118.
42	Wnuk A, Rzemieniec J, Staroń J, Litwa E, Lasoń W, Bojarski A, et al. 2019. Prenatal exposure to benzophenone-3 impairs autophagy, disrupts RXRS/PPARγ signaling, and alters epigenetic and post-translational statuses in brain neurons. Mol Neurobiol 56:4820-4837.