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Addendum to the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11 to Include Benzophenones-2, -6, and -8

INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel initially reviewed the safety of Benzophenones-1, -2, -3, -4, -5, -6, -8, -9, and -11 and concluded that there were insufficient data to evaluate the mutagenic potential of Benzophenones-2, -6, and -8. All other test data needed for the safety evaluation of these three ingredients were considered adequate. The Expert Panel released the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11 (December 18, 1981) stating that these ingredients were safe as used in cosmetic products. As required by the CIR Procedures, a Notice of Insufficient Data Report on Benzophenones-2, -6, and -8 was issued (November 25, 1981), and it indicated that additional mutagenesis data would be required before a safety evaluation could be made on these three ingredients.

This Addendum contains the additional data supplied by industry in response to the Expert Panel's request. The mutagenesis data initially available to the Expert Panel and included in the Final Report have been extracted and reported here for convenience in reviewing this Addendum.

Chemical, biological, toxicological, and clinical data for Benzophenones-2, -6, and -8 can be found in the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11.

SHORT-TERM TESTS

Data from the Final Report

The mutagenicity of Benzophenones-2, -6, and -8 was investigated with the Ames *Salmonella*/mammalian-microsomal assay. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were used; all tests were performed

in the presence and absence of Aroclor-induced rat liver microsomal S-9 cell fraction to observe the mutagenic effect of each compound following metabolic activation. Preliminary cytotoxicity studies determined the dose range of each compound to be used. Benzophenones-2, -6, and -8 were nonmutagenic when assayed directly and were mutagenic with metabolic activation in *Salmonella* strain TA1537. Benzophenone-8 was weakly mutagenic in *Salmonella* strain TA1537, whereas Benzophenone-6 was determined to be mutagenic at three doses in the same strain. Benzophenone-2, in the presence of rat liver microsomes, induced a "small but fairly consistent increase in the number of mutants" in four *Salmonella* strains tested. At doses of 100–300 μg, Benzophenone-2 induced mutant increases of 50%–100% in TA100 and 200%–500% in TA1537. A mutant increase of 50% was observed in strains TA98 and TA1535, but these strains had not been tested enough times to provide conclusive results. The investigator suggested that "the small and somewhat erratic nature of the (mutagenic) response we have seen raises the possibility that the observed effect may be due to the presence of an impurity." The purity of the test sample was 99%, (lab-grade) and was assumed to be purer than that of the cosmetic-grade. Additional tests using lab-grade Benzophenone-2 found this ingredient to be mutagenic in TA1537 at doses of 200 and 750 μg when activated by Aroclor-induced hamster liver enzymes. Preliminary assays of cosmetic-grade Benzophenone-2 revealed mutagenic activity not differing significantly from that of the purer lab-grade.[1-5]

Benzophenone-2 was reported to be positive for strains TA98, TA100, TA1535, and TA1537 with S-9 metabolic activation in Table 9 of the Final Report based on textual comments on the unpublished data submitted by industry to CIR for that report. The interpretation of these data, which have since been published, was clarified by the investigators who reported that Benzophenone-2 is "clearly mutagenic" only in strain TA1537.[3] It was suggested that this may indicate that Benzophenone-2 causes frameshift mutations by intercalating between DNA bases without covalent bonding, and that caution should be taken in extrapolating from mutagenicity to carcinogenicity of such agents in the Ames test. An amended Table 9, Table 9A, is included in this Addendum.

An in vitro cytogenic assay was used to evaluate the ability of Benzophenone-2 to induce sister chromatid exchange (SCE) and chromosome aberrations (CA) in L5178Y mouse lymphoma cells. Assays were performed in the presence and absence of Aroclor-induced rat liver microsomal enzymes (S-9). The solubility of Benzophenone-2 in DMSO and its cytotoxicity were first determined. For the mutagenesis assays, doses of 6.250–200.00 μg Benzophenone-2 per plate were used. When assayed in the absence of S-9, Benzophenone-2 induced small, "biologically insignificant" increases in SCE frequency at 100 and 200 μg; CA frequencies were not elevated at any dose. With metabolic activation, however, Benzophenone-2 produced "statistically and biologically significant" increases in SCE frequency at the three highest dose levels indicating a dose-response relationship. The author noted that Benzophenone-2 was more toxic to cells with the activation system; only 17 scorable cells were found at the 100 μg dose. The investigator reported that ten CAs (including a quadriradial, a translocation, and two triradials) were observed among the 67 cells scored at the two highest doses with activation. He concluded that Benzophenone-2 does not directly induce

TABLE 9A. Ames *Salmonella* Mutagenesis Assay.

Benzophenone	Dose range (μg) (solvent)	Results[a] without S-9 metabolic activation					Results[a] with S-9 metabolic activation					Comment	Ref.
		TA98	TA100	TA1535	TA1537	TA1538	TA98	TA100	TA1535	TA1537	TA1538		
-1	0.1-500 Dimethyl sulfoxide (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	1
-2	0.1-10,000 (DMSO)	(-)	(-)	(-)	(-)	-[b]	(-)	(-)	(-)	(+)	-	Clearly mutagenic only in TA1537 with S-9 activation	2,3,6
-2	10-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	4
-3	1.0-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	1
-4	1.0-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	1
-6	1.0-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	Mutagenic only in TA1537 with S-9 activation at 10 and 100 μg. Toxic to TA1537 at 500 and 1000 μg with S-9	1
-8	7.0-700 (ETOH)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	Dose-dependent, weak but significant mutagenicity in TA1537 with S-9 activation only	1
-9	1.0-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	1
-11	10-1000 (DMSO)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	Nonmutagenic with and without S-9 activation	5

[a](-) = nonmutagenic; (+) = mutagenic.
[b]No data.

significant SCE or CA increases but does, under metabolic activation, induce these changes.[7]

A mouse lymphoma forward mutation assay was used to test Benzophenones-2 and -8 for mutagenesis. The L5178Y TK+/− cell line was used; assays were performed in the presence and absence of an Aroclor-induced rat liver microsomal preparation (S-9). Materials were dissolved in DMSO and tested for preliminary cytotoxicity to determine doses to be used in the assays. Without activation, Benzophenone-2 was mutagenic at "highly toxic" doses. In the presence of S-9, Benzophenone-2 became more toxic. An increase in the mutant frequency (3.0–6.8 times) was observed with the three most toxic doses. A dose-response relationship was not demonstrated. The investigator suggested that Benzophenone-2 "appears to react with microsomal system to yield a mutagenic product that induces mutants at lower applied concentrations and toxicities than under nonactivation conditions." It was concluded that Benzophenone-2 induced an increase in mutations at the TK locus in L5178Y mouse lymphoma cells only for highly toxic doses with or without metabolic activation and that this material is weakly mutagenic under the conditions of the test.[7] These findings need to be reconfirmed since there was no dose response pattern of toxicities over the preferred relative growth range in any of the trials; increases in mutant frequency in the assays occurred only at levels bordering total lethality; and the lethal dose was poorly reproduced from one trial to another with metabolic activation. When assayed directly, Benzophenone-8 did not induce mutant frequencies significantly greater than those of controls. With metabolic activation, however, Benzophenone-8 induced dose-dependent mutant frequency increases of 3.8 and 2.0 times for the two highest doses (24 and 32 μg, respectively). The investigator concluded that, under the test conditions, Benzophenone-8 is nonmutagenic when assayed directly, but under metabolic activation it induces a significant, dose-dependent increase in mutant frequency.[1]

New Data

The gradient plate test of McMahon et al.,[8] a modification of the Ames test, was used to investigate the effects of Benzophenone-2 and -6 on bacterial mutation.[9,10] The chemicals were dissolved in DMSO and tested at concentrations of 0.1–1000 μg/ml with and without rat liver S-9 metabolic activation. Benzophenones-2 and -6 were tested with *Salmonella typhimurium* strains G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98 (histidine auxotrophs) and *Escherichia coli* strains WP2 and WP2 uvrA-(tryptophan auxotrophs). Benzophenone-2 inhibited the growth of all the bacterial strains but did not induce mutations with or without metabolic activation. Benzophenone-6 did not inhibit bacterial growth and did not produce mutations in any of the bacterial strains with or without metabolic activation.

The induction of unscheduled DNA synthesis (repair synthesis) in primary cultures of adult rat hepatocytes was studied after exposure of the cultures to Benzophenones-2 and -6 dissolved in DMSO and at concentrations of 0.5–1000 nmoles/ml.[11,12] Cytotoxicity was observed at the 500 and 100 nmoles/ml con-

centrations of both chemicals. Benzophenones-2 and -6 did not induce DNA repair synthesis.

Female Chinese hamsters were orally administered 62.5–500 mg/kg Benzophenones-2 and -6 in a 10% aqueous acacia solution.[13,14] The animals were sacrificed, and the frequency of bone marrow SCE exchange was determined. Benzophenones-2 and -6 did not induce SCE in vivo in the bone marrow of Chinese hamsters. Cytotoxicity was not observed with either chemical.

The effect of Benzophenone-8 on SCE in Chinese hamster ovary cells was studied with and without rat liver S-9 metabolic activation.[15] The chemical was tested at concentrations of 333 ng/ml to 1 mg/ml in DMSO. Without metabolic activation, concentrations of 100–1000 μg/ml Benzophenone-8 were almost completely lethal to the cells, there was a reduction in monolayer confluency at 10 and 33 μg/ml, and concentrations of 1 μg/ml and greater caused cell cycle delays. There was no significant increase in SCE without metabolic activation at concentrations of 333 ng/ml to 10 μg/ml except for a slight increase at 10 μg/ml. With no evidence of a positive dose relationship, this small increase at a toxic dose was not thought to be meaningful by the investigators. With metabolic activation, Benzophenone-8 was extremely cytotoxic at concentrations of 100–1000 μg/ml, and there was some growth reduction at concentrations of 25–50 μg/ml. No cell cycle delay was noted. There was no increase in SCE at concentrations of 3.1–50 μg/ml Benzophenone-8 with metabolic activation.

Benzophenone-8 was tested in a mammalian cell forward gene mutation assay with and without an S-9 metabolic activation system.[16] The assay measured the ability of a chemical to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells. Benzophenone-8 was dissolved in DMSO and tested at concentrations of 33 ng/ml to 1 mg/ml. No cells survived concentrations of 100–1000 μg/ml Benzophenone-8 without metabolic activation or concentrations of 333.3–1000 μg/ml with metabolic activation. At a concentration of 100 μg/ml with metabolic activation, 9.3% of the cells survived. At all concentrations from 33 ng/ml to 33.3 μg/ml, there was greater than 58% survival with or without metabolic activation. Mutations were not observed at Benzophenone-8 concentrations of 2.2–66.6 μg/ml with or without metabolic activation.

Benzophenone-8 in corn oil was administered by gavage to mice, and its effect was investigated with a micronucleus test.[17] Micronuclei result from chromosome breakage. In a preliminary dose range study, groups of two male and two female mice received 50–5000 mg/kg Benzophenone-8 daily for two days, were observed for 48 further hours, and were sacrificed. No toxic signs or deaths were observed after the 50 mg/kg doses. Signs of toxicity including decreased activity, piloerection, and exophthalmus were observed at doses of 166–5000 mg/kg. At doses of 1666.6 and 5000 mg/kg, abnormal gait was also observed, and there was one death in each of these groups. A dose of 1500 mg/kg Benzophenone-8 was selected for the micronucleus assay. Two groups of eight mice received one dose of Benzophenone-8, and two groups of eight mice received two doses of Benzophenone-8 separated by 24 hours. Body drop, decreased activity, and abnormal gait were observed in all four groups. Benzophenone-8 did not

significantly increase the number of bone marrow micronuclei and was not cytotoxic.

CONCLUSION

The Panel concludes that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic and that the conclusion for the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11, which states "On the basis of the available animal and clinical human experience presented in this report, the Panel concludes that Benzophenones-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics" is also applicable to these three ingredients.

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