Amended Safety Assessment of Benzophenones as Used in Cosmetics

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ABSTRACT: The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of benzophenones in cosmetic products; these ingredients are reported to function mainly as light stabilizers in cosmetics. The Panel reviewed the relevant data relating to the safety of these ingredients in cosmetic formulations provided in this safety assessment, and data from the previously published safety assessments, and concluded that Benzophenone-1, -2, -3, -4, -5, -6, -8, -9, -10, -11, and -12 are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

The safety of Benzophenone-1, -2, -3, -4, -5, -6, -8, -9, -10, -11, and -12, as used in cosmetics, is being evaluated in this safety assessment. The Expert Panel for Cosmetic Ingredient Safety (Panel) originally published a safety assessment of 6 of these ingredients with the following conclusion in 1983: "On the basis of the available animal data 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."[1] During the same year, the Panel also published an addendum to this existing safety assessment, having concluded that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic ,and that the published conclusion on Benzophenones-1, -3, -4, -5, -9, and -11 is applicable to these 3 ingredients.[2] In accordance with the Cosmetic Ingredient Review (CIR) Procedures & Support to the Expert Panel for Cosmetic Ingredient Safety document, the Panel evaluates the conclusions of previously-issued reports every 15 years. Thus, the Panel re-evaluated the conclusion, and in 2005, published re-review summary that stated the Panel determined to not reopen the 1983 published safety assessment until results from National Toxicology Program (NTP) carcinogenicity studies on benzophenones are available.[3]

The NTP carcinogenicity study on Benzophenone-3 was published in May 2020, and accordingly, the Panel re-opened the review of the benzophenones listed above. Additionally, the Panel determined that it was appropriate to include Benzophenone-10 and -12 in this report. Results from the NTP study are included in the current safety assessment, as are other safety test data on benzophenones that have been identified in the published literature since the original safety assessment was published in 1983. Data from the original CIR safety assessments on benzophenones appear in *italics*, when available, at the beginning of each section in the report text. (This information is not included in the Summary section.) For complete and detailed information, please refer to the original documents, which are available on the CIR website (https://www.cir-safety.org/ingredients).

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the benzophenones reviewed in this safety assessment are reported to function mainly as light stabilizers in cosmetic products, but some are also reported to function as sunscreens (see Table 1).[4] In the United States (US), sunscreens are active ingredients in over-the-counter (OTC) drug products, and are not cosmetic ingredients (21 CFR 352.10); however, in Europe, sunscreens are classified as cosmetics.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. The published data in this document were identified by conducting an exhaustive search of the world's literature from year 1983 forward. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/ preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties. Dossiers for Benzophenones-1, -3, -4, -8, and -12 were found on the European Chemicals Agency (ECHA) website.[5-9] The ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

CHEMISTRY

Definition and Structure

Benzophenones-1 to -12 are substituted derivatives of 2-hydroxybenzophenone.[1] Substituents include hydroxy, methoxy, octyloxy, sulfonyl, and methyl groups. Benzophenones may be mono-, di-, tri-, or tetra-substituted.

Definitions, CAS numbers, and individual structures of the benzophenones included in this report are presented in Table 1.

Chemical Properties

An important property of benzophenones is their ability to absorb and dissipate ultraviolet (UV) radiation.[1] Most benzophenones are solid at room temperature, soluble in organic solvents, and insoluble in water.

The benzophenones reviewed in this safety assessment are solid compounds with molecular weights ranging from 214.21 Da (Benzophenone-1) to 366.44 Da (Benzophenone-12). Properties of benzophenones are presented in Table 2.[1,10]

Method of Manufacture

The most common method of production of benzophenones is the Friedel-Crafts reaction.[1] No further manufacturing information, specific to the cosmetic ingredients, has been found in the published literature or submitted as unpublished data.

Composition/Impurities

Values for the maximum moisture content of benzophenones have been reported as follows: Benzophenone-1 (2%), Benzophenone-2 (5%), Benzophenone-3 (13%), Benzophenone-4 (10% to 16%, trihydrate form), Benzophenone-6 (0.5%), Benzophenone-8 (2%), Benzophenone-9 (5%), and Benzophenone-11 (5%).[1]

A maximum concentration of 1 ppm arsenic as an impurity has been recommended for Benzophenones-1, -2, -3, -4, -6, -9, and -11.[1] *The following maximum concentrations for lead as an impurity in benzophenones have been recommended: Benzophenone-1 (18 ppm), Benzophenone-2 (8 ppm), Benzophenone-3 (13 ppm), Benzophenone-4 (18 ppm), Benzophenone-6 (13 ppm), Benzophenone-9 (8 ppm), and Benzophenone-11 (13 ppm).*

USE

Cosmetic

The safety of the cosmetic ingredients included in this report is evaluated based, in part, on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category. The concentration of use survey on benzophenones was conducted for ingredient use as a light stabilizer, but not as a sunscreen. It is important to note that sunscreens are classified as cosmetics in Europe, but not in the United States. It is within the Panel's purview to review cosmetic ingredients in relation to human health and safety, but not for environmental safety.

In the original (1983) report, Benzophenone-2 was the benzophenone with the highest reported use frequency (229 uses total);[1] in 2021, use frequency of Benzophenone-2 decreased to 55 uses.[11] In 2021, Benzophenone-4 was the benzophenone with the highest reported use frequency (1226 uses total); the use frequency of Benzophenone-4 in the original (1983) report was 240 uses. Benzophenone-4 had the highest concentration of use reported in both the original and current reports. In the original (1983) report, the highest use concentration reported was ≤ 10% in suntan gels, creams and liquids;[1] in 2020, the highest reported use concentration was substantially lower, at concentrations of up to 1.6% in other non-coloring hair preparations (leave-on products).[12] Complete frequency and concentration of use data are presented in Table 3.

According to VCRP and Council survey data, 5 of the benzophenones reviewed in this safety assessment are not currently in use in cosmetic products. These ingredients are presented in Table 4.

Cosmetic products containing benzophenones may be applied to the skin or, incidentally, may come in contact with the eyes (e.g., Benzophenone-4 in eye makeup preparations at concentrations up to 0.2%). Benzophenone-3 is used in products that come in contact with mucous membranes during product use (maximum use concentrations up to 0.5% in bath soaps and detergents). Additionally, Benzophenone-3 could be incidentally ingested (maximum use concentrations up to 0.5% in lipstick). In baby products, Benzophenone-3 is being used at maximum concentrations up to 0.25% (in baby lotions, oils, and creams (not powder)). Products containing benzophenones may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Benzophenone-3 is being used in aerosol hair spray (maximum concentration of 0.014%), hair spray (maximum concentration of 0.05%), and in pump deodorant spray (at maximum concentration of 0.08%). Higher concentrations (up to 0.5%) are used in fragrance formulations. Benzophenone-4 is also being used in aerosol hair spray (maximum concentration of 0.015%) and pump hair spray (maximum concentrations of 0.1%). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm, compared with pump sprays.[13-16] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[13,14] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[14] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Benzophenone-3 is also being used in face powders (use concentrations unknown). Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[17-19]

Benzophenone-3, Benzophenone-4, and Benzophenone-5, but not the other benzophenones in this safety assessment, are included on the European Union's list of ultraviolet light (UV) filters allowed in cosmetic products.[20] A maximum concentration of 6 % Benzophenone-3 (as a UV filter) is allowed in ready for use preparations. Not more than 0.5% Benzophenone-3 is allowed to protect the product formulation. Benzophenone-4 and Benzophenone-5 are allowed in ready for use cosmetic preparations at concentrations up to 5% (as acid).

Non-Cosmetic

In the US, sunscreens are active ingredients in over-the-counter (OTC) drug products, and are not cosmetic ingredients (21 CFR 352.10); however, in Europe, sunscreens are classified as cosmetics. According to the US FDA proposed rule issued in 2007, the following benzophenones were allowed in sunscreens as active ingredients within the concentration specified for each ingredient: Benzophenone-3 (a.k.a. oxybenzone, up to 6%), Benzophenone-4 (a.k.a. sulisobenzone, up to 10%), and Benzophenone-8 (a.k.a. dioxybenzone, up to 3%) (21 CFR 352.10). On February 26, 2019, the FDA published another proposed rule to establish final monograph regulations for OTC sunscreen drug products (84 FR (38) 6204).[21] The rule now proposes that the following 3 benzophenones would be excluded from the final monograph because there are insufficient data to determine whether they are generally recognized as safe and effective (GRASE): Benzophenone-3, Benzophenone-4, and Benzophenone-8. Particularly, given the available data showing significant transdermal absorption and systemic availability of Benzophenone-3, as well as the potential for endocrine activity, FDA proposes that Benzophenone-3 is not GRASE for use in sunscreens without further data. FDA has determined that the following data on Benzophenone-3 are needed: human absorption data (including metabolite study in humans); non-clinical safety studies (toxicokinetics, dermal carcinogenicity, and systemic carcinogenicity); developmental and reproductive toxicity (if developmental and reproductive toxicity (DART) studies do not resolve the concerns raised in the literature relating to potential endocrine disruption, it may be possible to resolve these concerns through additional testing); and FDA is seeking input on whether additional studies or contraindication are necessary to support the safety of sunscreens containing Benzophenone-3 for children under 2 years of age. FDA has determined that the following data on Benzophenone-4 and Benzophenone-8 are needed: dermal irritation and sensitization testing; phototoxicity and photoallergenicity testing; human maximal use bioavailability studies; post-marketing adverse event reports; dermal carcinogenicity; systemic carcinogenicity; DART; toxicokinetics; and additional testing when data suggest a concern about other long-term effects, such as endocrine effects.

According to the proposed rule, FDA expects that a systemic carcinogenicity study would not be needed to support a GRASE determination for a sunscreen active ingredient if an adequately conducted human pharmacokinetic maximal use trial (MUsT) resulted in a steady state blood level less than 0.5 ng/ml, and an adequately conducted toxicology program did not reveal any other safety signals for the ingredient or any known structurally similar compound indicating the potential for adverse effects at lower levels. The threshold value of 0.5 ng/ml is based on the assessment that the level would approximate the highest plasma level below which the carcinogenic risk of any unknown compound would be less than 1 in 100,000 after a single dose.

Benzophenone-3 is among the substances listed by FDA as indirect food additives (substances for use as basic components of single and repeated use food contact surfaces) (21CFR177.1010). Furthermore, Benzophenone-12 may be safely used as an antioxidant and/or stabilizer in polymers used in the manufacture of articles or components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, subject to the following limitations (21CFR178.2010): For use only at levels not to exceed 0.5% by weight of olefin copolymers complying with section 177.1520 I.

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Benzophenone-3

Sunscreen products were applied to excised human epidermis in Franz diffusion cells, with the amounts of sunscreen ingredients penetrating into and across the human epidermis assessed by high performance liquid chromatography (HPLC) for 8 h following application. The receptor fluid consisted of bovine serum albumin in phosphate-buffered saline.[22] Benzophenone-3 penetrated human skin to the receptor phase (0.08 g/m^2 or 10% of applied dose) after the 8-h study period.

The penetration of Benzophenone-3 across excised human epidermis and high-density polyethylene (HDPE) membrane was measured using in vitro Franz-type diffusion cells.[23] Human epidermal tissue (abdominal region of 1 female) was obtained by blunt dissection of full-thickness skin and heat separation. The tissue was mounted between the donor and receptor chambers of the diffusion cell, and the surface area available for diffusion was 1.18 cm^2. The receptor chamber volume was 3.4 ml, and the receptor fluid was bovine serum albumin (4%) in phosphate-buffered saline. Both penetration and epidermal retention were measured following application of infinite and finite (epidermis only) doses of Benzophenone-3 (2%) in the following 5 vehicles: liquid paraffin, coconut oil, 50:50 ethanol:coconut oil, aqueous cream, and oily cream. For the infinite dose studies, an aliquot (200 mg/cm^2) of each formulation was applied to the epidermal surface under occlusion. For the finite dose studies, an aliquot of each formulation (20 mg/cm^2) was applied without occlusion. Benzophenone-3 remaining in the epidermis (R_s, µg) was extracted twice with methanol and quantified using HPLC. The highest Benzophenone-3 skin retention was observed for the 50:50 ethanol:coconut oil combination. Maximal and minimal Benzophenone-3 fluxes were observed from liquid paraffin and coconut oil, respectively.

In the infinite dose study, statistically significant differences existed between all 5 formulations with respect to penetration of Benzophenone-3 across HDPE membrane, after application of an infinite dose in a range of formulation

vehicles. The order of flux (highest to lowest) was: liquid paraffin > oily cream > 50:50 ethanol:coconut oil > coconut oil. For Benzophenone-3 penetration across epidermal membrane, liquid paraffin was greater than 50:50 ethanol:coconut oil; however, the difference between the 2 vehicles was not statistically significant. For the remaining vehicles, the order of flux (high to low) was oily cream > aqueous cream > coconut oil. Statistically significant differences ($p < 0.05$) existed between the Benzophenone-3 fluxes across the epidermis for these formulation vehicles.

In the finite dose study (mimicking the real-life situation), the percentage of applied Benzophenone-3 dose absorbed ranged between 1.97% from coconut oil and 9.97% from liquid paraffin. A comparison of the maximum amount of Benzophenone-3 that penetrated indicated that liquid paraffin and coconut oil statistically significantly differed from each other and the remaining formulations. The highest Benzophenone-3 skin retention was observed for 50:50 ethanol:coconut oil . The alcohol-based vehicle showed low Benzophenone-3 release from the vehicle, but high skin penetration and retention. The authors concluded that sunscreen chemicals applied to the skin are substantially retained in the superficial layers of the stratum corneum. They also noted that the results of this study also indicated that the release and skin penetration of Benzophenone-3 was influenced by the formulation vehicle in which it was applied to the membrane.

Benzophenone-3 (10% in water-in -oil or oil-in-water emulsion) was evaluated in a skin penetration study involving full-thickness pig ear skin in vitro.[24] Both fresh and previously frozen pig ear skin were used. The skin permeation of Benzophenone-3 (in water-in-oil emulsion) was described as rapid, i.e., after 1 h of skin exposure to 2 mg/cm^2. After 1 h, skin permeation was ≥ the limit of quantification (0.615µg/cm^2). Approximately 0.5% of the applied dose passed into the receptor fluid (phosphate-buffered saline). The absorption rate was higher from the water-in-oil emulsion than from the oil-in-water emulsion. After 24 h of skin exposure, the amount of Benzophenone-3 that passed through the frozen-stored skin was 27.2 ± 1.3 µg/cm^2 (from water-in-oil emulsion) and 22.1 ± 1.1 µg/cm^2 (oil-in-water emulsion). Additionally, after 24 h of exposure, the amount of Benzophenone-3 that passed through fresh skin was 22.4 ± 0.9 µg/cm^2 (from water-in-oil emulsion) and 17.6 ± 0.8 µg/cm^2 (from oil-in-water emulsion).

Benzophenone-3 and Benzophenone-4

Static diffusion cells were used to evaluate the skin penetration of Benzophenone-3 and Benzophenone-4 in vitro.[25] The limits of detection were 20 µg/l (for Benzophenone-3) and 90 µg/L (for Benzophenone-4). Human skin from abdominal or breast surgery was used. The mean amount found in the receptor fluid was 1.0 ± 0.4 µg/cm^2 for Benzophenone-3, compared to 1.1 ± 0.8 µg/cm^2 for caffeine (known as a good penetrating compound). The amount of Benzophenone-4 in the receptor fluid was below the limit of detection.

The percutaneous absorption of Benzophenone-3 and Benzophenone-4 (each in an oil-in-water emulsion) was evaluated in vitro using fresh human skin of women who had undergone breast or abdominal surgery.[26] The skin (epidermal side up) was positioned on the lower part of the diffusion cell, and 3 mg/cm^2 of test formulation applied. Exposure times of 30 min and 16 h were observed. For Benzophenone-3, there was no difference between the mean quantity found in the stratum corneum after 30 min or 16 h. Benzophenone-3 penetrated very quickly and saturated the stratum corneum in less than 30 min, and was found in the receptor fluid. For Benzophenone-4, the quantity found after 30 min (2.1 ± 1.3 µg/cm^2) was statistically significantly less than that found after 16 hours (4.0 ± 1.8 µg/cm^2). Benzophenone-4 was found in the stratum corneum, epidermis, dermis, and receptor fluid.

Animal

Benzophenone-3

A study was performed to investigate whether long-wavelength UV (UVA; maximum wavelength from lamp = 365 nm) and mid-wavelength UV (UVB; maximum wavelength from lamp = 312 nm) affect the absorption of Benzophenone-3 through the skin.[27] The dorsal skin of female nude mice (ICR-Foxn^{nu} strain) was subjected to UVA (24 and 39 J/cm^2) or UVB (150, 200, and 250 mJ/cm^2) irradiation. UVA irradiation was performed every other day, and each mouse was exposed 3 times over a 5-d period. UVB irradiation was performed once a day for 5 d. The interval between each UVB irradiation was 24 h. Irradiated skin was excised from the mouse (back) immediately after the last UV exposure. Senescent skin (24 wk old) was used for comparative purposes. In vitro skin absorption was evaluated using a Franz cell. The donor compartment was filled with Benzophenone-3 (3.5 mg/ml in 30% ethanol/double distilled water). The receptor was loaded with 30% ethanol in pH 7.4 buffer. The duration of the experiment was 48 h. When compared to intact skin, a negligible change in skin absorption after UVA exposure was found, though there was a slight increase in flux at a dose of 24 J/cm^2. UVB exposure resulted in a decrease in skin deposition of Benzophenone-3 (statistically significant ($p < 0.05$) at dose of 250 mJ/cm^2); no statistically significant decrease was detected at doses of 150 and 200 mJ/cm^2. UVB exposure at doses of 200 and 250 mJ/cm^2 caused a slight, but statistically significant ($p < 0.05$) enhancement of Benzophenone-3 flux. The values for Benzophenone-3 flux were: 11.92 ± 0.74 µg/cm^2/h (normal), 14.05 ± 0.17 µg/cm^2/h (UVA at 24 J/cm^2), 12.02 ± 0.11 µg/cm^2/h (UVA at 34 J/cm^2), 8.04 ± 1.40 µg/cm^2/h (UVB at 150 mJ/cm^2), 13.98 ± 0.06 µg/cm^2/h (UVB at 200 mJ/cm^2), and 20.73 ± 0.03 µg/cm^2/h (UVB at 250 mJ/cm^2). The skin absorption parameters of intrinsically aged skin and young skin were comparable.

Human

Benzophenone-3

The skin penetration of Benzophenone-3 was evaluated in vivo using 6 healthy volunteers (mean age = 37.3 ± 7.7 years) who were free of any dermatological disorders.[28] In the first step, the percentage absorption was measured using an occlusive and difference method. A solution consisting of 0.5 mg of Benzophenone-3 in 10 µl of acetone (2190 nmol) was applied. Following Benzophenone-3 application, any residual formulation was washed off, and the amount removed and analyzed. In the second step, the tape stripping method (a useful procedure for selectively removing the skin's outermost layer, the stratum corneum, and measuring the stratum corneum adsorption) was performed. Benzophenone-3 [1000 nmol in 20 µl of ethylene glycol:triton X100 (90:10 v/v)] was applied to the surface of the skin. The human skin permeation of Benzophenone-3 over a period of 4 h was near 35% of the applied dose with the occlusive method. The amount of topically applied Benzophenone-3 found in the stratum corneum after 30 minutes of exposure using the stripping procedure was evaluated at 4% of the applied dose.

A human study was performed (5 males, 7 females) as a crossover design with sunscreen application to the face or back on day 1, followed by application to the other side on day 8 of the study.[23] A sunscreen lotion with the following composition was applied at a rate of 2 mg/cm^2 to an equal-sized area (112 cm^2) on the face or back of the volunteers: 8% (w/v) homosalate, 7.5% (w/v) octyl methoxycinnamate, 6% (w/v) Benzophenone-3, and 5% (w/v) octyl salicylate. The sunscreen lotion remained occluded for 8 h before it was removed by washing. An area of the skin was immediately tape-stripped using clear tape (3 cm x 1.9 cm). The stratum corneum was sequentially stripped 16 times on the back and 6 times on the face. Sunscreen content in all samples was analyzed. Urine output over 48 h post-application was collected. Blood samples were taken at pre-application baseline and at a suitable steady-state time after application (7.5 h). A substantial amount of all sunscreen chemicals in the stratum corneum of the back was noted after 8 h. Greater amounts of sunscreen were present in the superficial layers (ranging from ~4% to 10% of the applied dose) than in the deeper layers. Approximately 2 to 4 times the amount of sunscreen was present in the superficial stratum corneum layers of the face, when compared to the back. The difference in absorption between the anatomical sites was statistically significant for Benzopeonone-3, octyl salicylate, and homosalate only. The percentage of applied dose in the 6 superficial layers of the stratum corneum was ~10%, 18%, 18%, and 25% for homosalate, octyl methoxycinnamate, Benzophenone-3, and octyl salicylate, respectively. Sunscreens were not detected in the plasma or urine samples.

Benzophenone-4

Benzophenone-4 (in water; 6 mg/ml) was deposited on the skin of each of 21 healthy women (22 to 34 years old; mean age = 25 ± 3 years).[29] Twenty µl of solution were applied. Skin strippings were performed at 1 to 7 h after treatment. The stratum corneum was removed (with transparent adhesive tape) by a series of 6 strippings. After 1 h, and for the first strip, 70% of the Benzophenone-4 remained at the level of the stratum corneum (compared to 40% for PEG-25 PABA [para-aminobenzoic acid]). At 7 h, 40% of the Benzophenone-4 remained at the level of the stratum corneum (compared to 20% for PEG-25 PABA).

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

Benzophenone-2

The fate of Benzophenone-2 was studied in human and zebrafish in vitro cell models.[30] In the human in vitro cell models, Benzophenone-2 was metabolized into a variety of gluco- and sulfo-conjugated metabolites. Similar patterns of Benzophenone-2 biotransformation were observed among zebrafish models (primary hepatocytes, ZFL, and ZELH-zfER cell lines). Metabolic patterns in the zebrafish models and human hepatic cell line HepaRG shared many similarities, while biotransformation rates in the cell lines MELN (human female cancer (invasive ductal carcinoma) cell line) and T47D-KBLuc (human female cancer (mammary gland breast/duct) cell line) were quantitatively low and qualitatively different.

Benzophenone-3

Benzophenone-3 (0.1 µmol) was incubated for 15 min with liver microsomes from untreated Sprague-Dawley rats in the presence of NADPH (1 µmol).[31] 2,5-Dihydroxy-4-methoxybenzophenone, metabolite of Benzophenone-3, was formed. Another metabolite, 2,4-dihydroxybenzophenone (Benzophenone-1, the 4-desmethylated metabolite), was also formed. The amount of 2,5-dihydroxy-4-methoxybenzophenone formed in vitro was approximately the same as 2,4-dihydroxybenzophenone. Data on the specific amount of each metabolite were not included.

The metabolism of Benzophenone-3 by rat and human liver microsomes was studied.[32] When Benzophenone-3 (10 µM) was incubated for 15 min with rat liver microsomes in the presence of NADPH, the following metabolites resulted: 2,4,5-triydroxybenzophenone; 3-hydroxylated benzophenone-3; 5-hydroxylated benzophenone-3; Benzophenone-1; and 2,3,4-trihydroxybenzophenone. Benzophenone-3 was also metabolized by human liver microsomes, yielding Benzophenone-1 and 5-hydroxylated benzophenone-3.

Animal

Dermal

Benzophenone-2

A study was performed, using groups of 10 male Wistar rats, to determine the concentrations of Benzophenone-2 in the rat brain after topical administration.[33] Benzophenone-2 was dissolved in a small amount (volume not stated) of ethanol and olive oil and formulated with Hascobase. The test substance was then applied to shaved skin at a dose of 100 mg/kg twice per day for 4 wk (days per wk not stated). Hascobase, with a small amount of ethanol and olive oil, was applied to the skin of control rats. Blood and tissue Benzophenone-2 concentrations in the frontal cortex and hippocampus were determined. After dermal application (24 h after last dose at 4 wk), the blood level of Benzophenone-2 was ~300 ng/ml. Liver and adipose tissue concentrations were 1354 ng/g wet tissue and 823 ng/g wet tissue, respectively. In the brain structures studied, the Benzophenone-2 concentration ranged from 5 to 19 ng/g tissue. In the hippocampus, the Benzophenone-2 concentration was approximately 3.5-fold lower in the frontal cortex.

To assess the concentration of total Benzophenone-2 (parent compound and its metabolites – glucuronide and sulfate), the liver was homogenized and plasma was mixed with 1 M ammonium acetate buffer. Prior to incubation of the homogenate for 6 h, freshly prepared enzyme mixtures (β-glucuronidase and sulfatase) were added. After hydrolysis with β-glucuronidase and sulfatase, the Benzophenone-2 peak was significantly higher than in the same serum and liver samples before hydrolysis. Calculation of the Benzophenone-2 concentration from the calculated standard curves revealed that the test compound was present in the plasma of treated animals at concentrations ranging from 164 to 648 ng/ml (average = 324 ng/ml; 1.3 µM). After hydrolysis, the Benzophenone-2 concentration was 2218 ng/ml (9 µM). These results indicated that, in the blood, the there was more of the Benzophenone-2 metabolites than the parent compound. Additionally, in the liver, the Benzophenone-2 concentration after hydrolysis was much higher (3758 ng/g) when compared to the free form of the compound (1482 ng/g). Benzophenone-2 concentrations in all examined tissues in control animals were below the detection limit. The authors noted that the results of this study indicate that Benzophenone-2 passes through the blood-brain barrier, but that its concentration in the brain structures is much lower than in the blood.

Benzophenone-3

A study was performed to characterize the skin permeation and tissue disposition of Benzophenone-3 (in ethanol) in rats (groups of 10; 5 males and 5 females per group).[34] The test solution was applied (volume = 100 µl; dose = 5 mg/kg [312.5 µg/cm^2]) topically to a 4 cm^2 area on the back, daily, for 30 d. Two negative control groups received topical applications of 0.9% saline and 70% ethanol solution for 30 d. The positive control group received an intraperitoneal (i.p.) dose (25 mg/kg) of acrylamide for 10 d. Tape stripping was used to recover the application dose that permeated into skin layers. Benzophenone was recovered in appreciable amounts from the application sites. Quantifiable amounts of Benzophenone-3 were detected in plasma samples, indicating systemic absorption from the skin. Benzophenone-3 was also detected in the brain and liver (the only tissues collected). The authors noted that Benzophenone-3 primarily undergoes metabolism in the liver and is subsequently excreted in the urine. The elimination half-life of Benzophenone-3 was estimated to be 7.9 ± 1.7 h. Benzophenone-3 was measurable 24 h after skin application, i.e., at the application site and in the plasma, liver, and brain. The authors concluded that the results of this study indicate that Benzophenone-3 penetrated across the skin after a 30-d topical application, and that systemic absorption was correlative among skin, plasma, and tissue samples.

The percutaneous absorption of Benzophenone-3 and its metabolite (Benzophenone-1) was studied using female Sprague-Dawley rats and their offspring.[35] Benzophenone-3 (10% in cream; dose = 100 mg/kg) was administered dermally (shaved skin on back) twice daily to adult female rats during the prenatal period and adulthood. Control female rats were treated with cream without Benzophenone-3. At 21 d after birth, the offspring (male and female) were divided into groups of 5 males and groups of 5 females. From 43 to 56 d old, the test substance was administered dermally to the male offspring. Cream without Benzophenone-3 was applied to control offspring. The calculation of Benzophenone-3 concentrations from the standard calibration curves revealed that the test substance was present in the plasma of treated animals at a concentration of 215.9 ± 38.5 ng/ml. The concentration of Benzophenone-3 in the liver was 96.81 ± 17.3 ng/g wet tissue. Higher concentrations of Benzophenone-1 (main metabolite, 196.4 ± 67.5 ng/g wet tissue) were also detected in the liver. Only the parent compound was detected in the frontal cortex and hippocampus of the brain at concentrations of 50.6 ± 11.0 and 46.7 ± 14.4 ng/g wet tissue, respectively. In male rats, Benzophenone-3 caused neurodegenerative changes in both the frontal cortex and the hippocampus. The authors noted that these values for tissue levels of Benzophenone-3 were reported for animals treated in the study. The data were not reported as female rats versus male offspring. The authors stated that the results of this experiment showed that Benzophenone-3 is absorbed through rat skin and passes through the blood brain barrier.

Benzophenone-3 (10%), at a dose of 100 mg/kg, was applied to the backs of mated Sprague-Dawley rats (number not stated) twice daily.[36] A cream without Benzophenone-3 was applied to control rats. At 21 d after birth, the offspring were weaned and organized into groups of 5 (males separated from females). From 43 to 56 d of age, the female offspring of test animals received dermal applications of Benzophenone-3 (10%). A cream without Benzophenone-3 was applied to control offspring. At 24 h after the last test substance application, the animals were killed, and the brains and livers were excised. In the plasma of all control rats, the concentration of Benzophenone-3 was below the limit of detection. In the plasma of test

animals, Benzophenone-3 was detected in the range of 70 to 220 ng/ml (average = 169 ng/ml). A much higher concentration of the main Benzophenone-3 metabolite, Benzophenone-1, was detected in the liver (156 ng/g wet tissue), when compared to Benzophenone-3 (25 ng/g wet tissue). After dosing with Benzophenone-3, the concentration in the frontal cortex was 26 ng/g and the hippocampus had a concentration of 40 ng/g. Benzophenone-1 was also detected in the frontal cortex and hippocampus. In the control group, the concentration of Benzophenone-3 in the hippocampus was above the detection limit in only one female rat. Benzophenone-3 was not detected in the frontal cortex and liver.

The metabolism and disposition of [^{14}C]Benzophenone-3 (formulated in different vehicles) was evaluated using Harlan Sprague-Dawley rats (groups of 5) and B6C3F1/N mice (groups of 5).[37] The vehicles used were as follows: paraffin oil, lotion, coconut oil, ethanol:coconut oil, and ethanol. In rats, a single dose of the test substance (0.1, 1, 10, or 15 mg/kg; dose volume = 0.5 to 1 ml/kg) was administered in most of the vehicles. When the lotion (olive oil:emulsifying wax:water formulation) vehicle was used, the dose volume was ≈ 100 µl. Application (using syringe equipped with needle) was made to an area of skin that was not less than 4 cm^2. A foam or steel isolator was used to protect the dermal dosing site. In mice, the dose volume was ≈ 2 ml/kg. Urine and feces were collected for up to 72 h. The following results were at 72-h post-dosing. The absorbed dose varied depending on the vehicle. After application of [^{14}C]Benzophenone-3 to male rats, the percent dose absorbed in all vehicles was high (64 % to 80%), except in the lotion vehicle where absorption was moderate (46%). The % dose absorbed was similar following application of 0.1 mg/kg (73%) or 10 mg/kg (80%) [^{14}C]Benzophenone-3 formulated in paraffin oil. The absorption of [^{14}C]Benzophenone-3 was lower in female rats (30%, 15 mg/kg dose) than in male rats (46%, 10 mg/kg dose) after application of [^{14}C]Benzophenone-3 in the lotion vehicle. The absorbed dose was excreted mainly via the urine (including cage rinse) (18% to 48%) and feces (15% to 22%), with ~3% to 10% of the absorbed dose remaining in the tissues. Urinary metabolites included Benzophenone-3, Benzophenone-3-glucuronide, Benzophenone-1, Benzophenone-1-glucuronide, and Benzophenone-1-sulfates. Novel minor dihydroxy metabolites, including 2,5-dihydroxy-4-methoxybenzophenone, were also detected.

The distribution of [^{14}C]Benzophenone-3 radioactivity in tissues and excreta following dermal application to male mice was similar between the vehicles at 10 mg/kg with the exception of acetone showing higher tissue levels. [^{14}C]Benzophenone-3 absorption in female mice following dermal application at 10 mg/kg lotion or 10 mg/kg ethanol was similar to that seen in males. The unabsorbed dose in female mice was ~41%, with the majority of radioactivity recovered in urine and feces at a 10 mg/kg dose in lotion.

Oral

Benzophenone-3 and Benzophenone-12

An absorption study on Benzophenone-3 involving rats, and absorption studies on Benzophenone-12 involving rats and rabbits were performed.[1] When ingested, absorbed benzophenones were primarily conjugated and excreted in the urine, while the unabsorbed material passed out with the feces.

Benzophenone-2

A dose-response experiment involving 5 doses (10, 33, 100, 333, or 1000 mg/kg) of Benzophenone-2 was performed using female Sprague-Dawley adult, ovariectomized rats (groups of 5).[38] Doses were administered (by gavage) once per day for 5 d. Blood and urine samples were collected at different time points (every 30 min) after test substance administration. Additionally, the time-dependent metabolism and excretion of Benzophenone-2 were analyzed in a kinetic experiment, for further identification of metabolites. In this kinetic experiment, urine and serum samples were analyzed after *Helix pomatia* glucuronidase-/sulfatase (HPG) hydrolysis. Serum concentrations of Benzophenone-2 after dosing ranged from 0.1 µg/ml (after 10 mg/kg dose) to 1.1 µg/ml (after 1000 mg/kg dose). After hydrolysis with HPG, the serum concentrations of total Benzophenone-2 ranged from 1 to 62 µg/ml. Benzophenone-2 was metabolized to glucuronide- and sulfate-conjugates. In the serum, the maximum concentrations of Benzophenone-2, benzophenone-2-glucuronide, and benzophenone-2-sulfate were observed at 30 min post-dosing. The highest concentrations of Benzophenone-2 and its metabolites in the urine were measured at 120 min post-dosing. It was suggested that this biotransformation occurs via a first-pass effect in the gut wall or the liver.

Benzophenone-3

The toxicokinetics and metabolism of Benzophenone-3 was evaluated using groups of 7 male Sprague-Dawley rats.[39] Benzophenone-3 (in corn oil) was administered orally at a dose of 100 mg/kg (dose volume = 4 ml/kg). Blood samples were collected at various time points up to 24 h after dosing. Benzophenone-3 was converted into Benzophenone-1, which was formed via *o*-demethylation. Benzophenone-1 was subsequently converted to 2,3,4-trihydroxybenzophenone. Benzophenone-3 was also metabolized to 2,2'-dihydroxy-4-methoxybenzophenone, which was formed via the aromatic hydroxylation of Benzophenone-3. After a single oral dose of Benzophenone-3, the toxicokinetics curve showed a peak concentration (C_{max}) of 21.21 ± 11.61 µg/ml within 3 h (T_{max}), and then declined rapidly. The concentrations of the metabolites in rat blood decreased much more slowly over time, when compared to the parent compound.

Groups (5 animals per group) of mated female Sprague-Dawley rats were fed the following Benzophenone-3 concentrations (in low-phytoestrogen chow) from gestation day (GD) 6 until weaning on postnatal day 23: 1000; 3000;

10,000; 25,000; or 50,000 ppm.[40] Serum concentrations of Benzophenone-3 and its metabolites were measured on GD 10, 15, and 20, and on postnatal day 23. The limit of detection for Benzophenone-3, Benzophenone-1, and Benzophenone-8 was < 0.005 µg/ml. The limit of detection for 2,3,4-trihydroxybenzophenone was < 0.1 µg/ml or 0.05 µg/ml. Both Benzophenone-8 and 2,3,4-trihydroxybenzone were below the limits of detection. Therefore, only serum concentrations of Benzophenone-3 and Benzophenone-1 (metabolite) were reported. In the 1000 ppm group, the mean values (on postnatal day 23) for Benzophenone-3 and Benzophenone-1 were 0.0072 ± 0.0008 µg/ml and 0.0382 ± 0.0122 µg/ml, respectively. In the 50,000 ppm group, the mean values (on postnatal day 23) for Benzophenone-3 and Benzophenone-1 were 0.6886 ± 0.2447 µg/ml and 1.0066 ± 0.3874 µg/ml, respectively.

The metabolism and disposition of [^{14}C]Benzophenone-3 were evaluated using Harlan Sprague-Dawley rats (groups of 5) and B6C3F1/N mice (groups of 5).[37] A mixture of Benzophenone-3 and [^{14}C]Benzophenone-3 (in corn oil) was administered orally (by gavage) at a single target doses of 10, 100, or 500 mg/kg (male mice and rats), and a single target dose of 100 mg/kg (female mice and rats). The dose volume was 5 ml/kg in rats and 10 ml/kg in mice. The animals were killed and the following tissues and organs were collected for analysis: adrenals, brain, lung, heart, spleen, pancreas, kidneys, testes or uterus and ovaries, liver, thyroid, thymus, small intestine, cecum, large intestine, urinary bladder, and adipose and muscle samples. The distribution of radioactivity (at up to 24 h and 72 h) was reported for tissues/organs collectively, and individually for the rat liver and kidney. In male rats, the radioactivity in tissues increased with the increasing dose. In general, the male rat livers had a higher tissue:blood ratio (2.27 to 4.93) when compared to the kidney (1.26 to 3.53) at 72 h post-dosing. Values for total radioactivity in the tissues of male rats at 2 h, 24 h, and 72 h after dosing with 100 mg/kg were 27.5%, 3.1%, and < 0.5%, respectively. These results suggest that Benzophenone-3 was distributed to the tissues, but was not retained in the tissues. No sex differences (rats) in the disposition of [^{14}C]Benzophenone-3 following oral administration were apparent. The total dose of [^{14}C]Benzophenone-3 recovered in male and female rats was > 94%. After dosing with [^{14}C]Benzophenone-3 (100 and 500 mg/kg) in male mice, it was excreted mainly in the urine (40 to 41%, including cage rinse) and feces (24 to 39%) within 72 h. The tissues with the most radioactivity in male mice were the thymus and thyroid in both 100 and 500 mg/kg dose groups. The disposition was similar in female mice 72 h following a single 100 mg/kg gavage administration of [^{14}C]Benzophenone-3, with ~34% and ~24% in the urine and feces, respectively. The total radioactivity recovered in the 500 mg/kg dose group for male mice was lower (~69%) than in 100 mg/kg dose groups for male mice (~89%) and female mice (~76%).

Overall, [^{14}C]Benzophenone-3 was well absorbed and excreted mainly in the urine (39% to 57%) and feces (24 % to 42%) in male and female rats and mice. The distribution of Benzophenone-3 in tissues was minimal in rats (0.36%) and mice (< 0.55%). In male and female rats and mice, urinary metabolites included Benzophenone-3, Benzophenone-3-glucuronide, Benzophenone-1, Benzophenone-1-glucuronide, and Benzophenone-1-sulfates. Novel minor dihydroxy metabolites, including 2,5-dihydroxy-4-methoxybenzophenone, were also detected.

Benzophenone-12

Groups of 6 male rats (Carworth Farms Elias strain) were fed Benzophenone-12 at dietary concentrations of 1.25% and 5% daily for 35 d, in accordance with Organization for Economic Co-operation and Development (OECD) Test Guideline (TG) 417.[5] Results indicated that Benzophenone-12 had low absorption after oral feeding. The daily recovery of unchanged material from the feces was ~90%. The conjugation and urinary excretion of the test substance (metabolized to glucuronide conjugate) in rats fed both dietary levels was ~10% of the dose over the 35-d test period. The authors concluded that Benzophenone-12 did not have any bioaccumulation potential in this study.

Human

Dermal

Benzophenone-3

Solid-phase microextraction, combined with gas chromatography-quadrupole ion trap mass spectrometry, was used to identify Benzophenone-3 and its metabolites in human urine.[41] A urine specimen was collected from a subject after a sunscreen containing Benzophenone-3 (~ 8 ml) was applied topically to the body. The results indicated the presence of Benzophenone-3 and its metabolite, 2,4-dihydroxybenzophenone.

Eleven subjects applied a sun protecting lotion containing 4% Benzophenone-3 (40 g for average body area of 2 m^2).[42] The lotion was applied to most of the body, and the subjects were instructed to shower only once (i.e., after 12 h during the 48-h period). During the 48 h after application, urine samples were collected. The data indicated that application of the lotion resulted in excretion of Benzophenone-3 for as long as 48 h post-application. The average total amount of Benzophenone-3 excreted was 11 mg (median = 9.8 mg), which is approximately 0.4% of the amount applied.

The systemic absorption of Benzophenone-3 was evaluated in a 2-wk, single-blinded study involving 32 healthy volunteers (15 males, 17 postmenopausal females).[43] The subjects served as their own control. During the first week, a basic cream formulation without Benzophenone-3 was applied topically (whole-body application, 2 mg per cm^2) daily for 4 d. This dose corresponded to 40 g for an average body area of 2 m^2. The protocol for the second week was the same, and involved topical application of 10% Benzophenone (in cream). Benzophenone-3 was absorbed through the skin and detected

in the urine. The maximum concentration of Benzophenone-3 in the urine was 200 ng/ml in women (at 3 to 4 h after application) and 300 ng/ml in men (at 3 h after application). Results from this study also indicated that serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) in both men and women were unchanged, but statistically significant differences in testosterone levels (decreased) were observed in men and women during the 2 wk study. Minor differences in serum 17-β-estradiol (E2) and inhibin B levels were observed in men only. It was determined that the differences in hormone levels observed were unrelated to Benzophenone-3 exposure.

Twenty-five subjects applied a sunscreen containing Benzophenone-3 (4%), morning and night, for 5 d.[44] The 25 subjects were randomly divided into 2 groups (Groups A and B). The sunscreen was applied to most of the body (in both groups), and the subjects were allowed to shower once per day (prior to next application). Unlike Group A, the application sites of Group B subjects were irradiated after test substance application (time varied between 9 h and 15 h). From days 1 to 5, the UVA doses ranged from 60 J/cm^2 to 100 J/cm^2. The 60 J/cm^2 irradiation was for 34 min and 17 min on each side of the body. The total dose of UVA varied among participants, i.e., between 400 and 707 J/cm^2. For UVB irradiation, the sites of subjects were irradiated according to Fitzpatrick skin type (types I to III). The UVB dose was ~ 195 mJ/cm^2 for 90 s, and the total UVB dose varied among participants from 0.46 to 2 J/cm^2. In both groups, urine samples were obtained daily for 5 d and for 5 d after the last application. After 10 d, the subjects excreted 1.2% to 8.7% (mean = 3.7%) of the total amount of Benzophenone-3 applied, and there was no statistically significant difference between the 2 groups. The authors concluded that a large amount of Benzophenone-3 was absorbed, and that Benzophenone-3 accumulated in the body as the subjects excreted Benzophenone-3 five d after the last application.

A sunscreen cream containing Benzophenone-3 was applied (2 mg/cm^2) to 32 subjects (15 males and 17 females), and this amount corresponded to 40 g over an average body area of 2 m^2.[45] Application of the sunscreen formulation was described as a daily whole-body topical application of 10% (w/w) Benzophenone-3 for 4 d. Showering, bathing, and swimming were not allowed until 4 h after the daily application. Blood concentrations were measured at 0, 1, 2, 3, 4, 24, and 96 h. Urine concentrations were measured at 0, 24, 48, 72, and 96 h. Prior to the first application, the sunscreen was not detected in the plasma or urine. The maximum median plasma concentration of Benzophenone-3 was 187 ng/ml in females, and 238 ng/ml in males. The level of Benzophenone-3 in the urine of females was 44 ng/ml, and was 81 ng/ml in the urine of males.

Serum samples were obtained from 2 volunteers after topical application of a sunscreen cream containing 5% Benzophenone-3.[46] The cream (20 g) was applied to 1 volunteer, and the other volunteer received a 30 g application. Each volunteer applied the cream all over the body. Blood samples were collected before and after application at different time intervals for a period of 24 h. After application, the amount of Benzophenone-3 in the serum increased significantly and reached a maximum concentration ranging between 6 h (200 µg/l, after 20 g dose) and 9 h (304 µg/l, after 30 g dose). The amount of Benzophenone-3 in the serum then decreased slowly. At 24 h after cream application, high amounts of Benzophenone-3 were present in the serum (84 µg/l, after 20 g dose; 206 µg/l after 30 g dose). Formation of the Benzophenone-8 metabolite occurred at a very small extent. The Benzophenone-1 metabolite was detectable from the first hour after cream application, and the increase was slightly more pronounced during the first 6 h. At 24 h post-application, the amount of Benzophenone-1 in the serum was 34 µg/l (after 20 g dose) and 102 µg/l (after 30 g dose).

A dermal absorption study on a sunscreen containing 5% Benzophenone-3 was performed using 9 adult subjects.[47] The sunscreen was applied (8 g) to the skin (arms and legs) using a glue bottle with a cotton gauze head. Urine was collected within the next 12 h after application. Urine samples were mixed with acetate buffer solution containing β-glucuronidase. Using HPLC, Benzophenone-3 and the following 3 metabolites were detected in the urine: Benzophenone-1; 2,3,4-tri-hydroxybenzophenone; and 2,2'-dihydroxymethoxybenzophenone. The limits of detection for Benzophenone-3 and its metabolites were 0.5 to 1 µg/ml in urine sample solution and, except for the baseline samples, the concentrations in all samples were far above the limits.

A study was performed to determine whether active sunscreen ingredients are absorbed into the systemic circulation.[48] The study involved groups of 6 subjects (1 group per product). Study participants were enrolled from July to August of 2018. None of the participants were using any of the sunscreen products tested in the study or products containing any of the listed active ingredients. The sunscreen formulations containing Benzophenone-3 applied were: spray product #1 (6% Benzophenone-3), spray product #2 (5% Benzophenone-3), and lotion (4% Benzophenone-3). Each product was applied (2 mg/cm^2) to 75% of the body surface area 4 times per day for 4 d. The subjects remained in the clinic for up to 7 d, during which time they were not exposed to direct sunlight. In each group, 30 blood samples per person were collected over 7 d. The application of each product containing Benzophenone-3 resulted in plasma Benzophenone-3 concentrations that exceeded 20 ng/ml on day 7. For all participants, plasma concentrations exceeded 0.5 ng/ml within 2 h after a single application on day 1. Geometric mean maximum plasma concentrations of Benzophenone-3 reported following product application were as follows: 209.6 ng/ml (for 6% Benzophenone spray product), 194.9 ng/ml (for 5% Benzophenone-3 spray product), and 169.3 ng/ml (for 4% Benzophenone-3 lotion). The relationship between recent, self-reported personal care product use and ingredient (Benzophenone-3 included) concentrations in the urine was evaluated in 100 adolescent girls.[49] Study participants were recruited in May to July of 2013. The use of sunscreen was associated with 57.8% higher urinary concentrations of Benzophenone-3.

The systemic absorption and pharmacokinetics of Benzophenone-3 in sunscreen products were studied using 38 healthy participants.[50] The study was conducted between January and February of 2019. The protocol and product types were similar to that in the preceding study. Product application was described as 2 mg/cm^2 to 75% of the body surface area at 0 h on day 1, and 4 times on day 2 through day 4 at 2-h intervals. Thirty-four blood samples were collected from each participant over 21 d. The maximum plasma concentrations of Benzophenone-3 were 258.1 ng/ml (from 4% Benzophenone-3 sunscreen lotion) and 180.1 ng/ml (from 6% Benzophenone-3 aerosol spray). The authors concluded that Benzophenone-3 was systemically absorbed.

The dermal uptake of Benzophenone-3 from clothing was studied using 3 subjects.[51] Cotton shirts (purchased in May of 2016) were exposed to Benzophenone-3 at an elevated concentration (final concentration = 4.4 µg/m^3 for 32 d). The 3 subjects wore the exposed shirts for 3 h. After the exposure period, they wore their usual clothing during the collection of urine samples for 48 h. The rate of urinary excretion of the sum of Benzophenone -3 and Benzophenone-1 (metabolite of Benzophenone-3) increased for all 3 subjects during and following the 3-h exposure. The summed mass of Benzophenone-1 and Benzophenone-3 that was excreted during the first 24 h (attributable to wearing the exposed t-shirts) were 12, 9.9, and 82 µg for the first, second, and third participant, respectively. The authors noted that the analysis of these results, taken together with predictions of steady-state models, suggest that dermal uptake of Benzophenone-3 from clothing could meaningfully contribute to overall body burden.

Benzophenone-3 absorption (over a 4-h period) after application of a sunscreen containing 6% Benzophenone-3 was calculated.[52] The calculation appears below:

> 60 g (amount of product applied/4h) x 0.06 (6% Benzophenone-3 in product) /75 kg (average weight of women) = 0.048 g/kg or 48 mg/kg or 48 ppm/exposure
>
> 48 ppm/exposure x 0.08 (8% Benzophenone-3 absorbed topically) = 3.84 ppm or 3840 ppb absorbed over 4 h (i.e., 1 day's exposure).

The ratio of fetal to maternal blood levels after just 2 applications over a 4-h period of the sunscreen was 384 ppb/3840 ppb (at 10% fetal exposure) and 2880 ppb/3840 ppb (at 75% fetal exposure).

Biomonitoring Studies

Details relating to the following biomonitoring study summaries are presented in Table 5.

Human

Benzophenone-1, Benzophenone-2, and Benzophenone-3

Benzophenone-1, Benzophenone-2, Benzophenone-3, and 4-hydroxybenzophenone (metabolite of Benzophenone-1 and Benzophenone-3) were detected in urine samples obtained from 20 male subjects.[53] The authors noted that there seemed to have been a relationship between the presence of Benzophenone-3 and Benzophenone-1 because, in all samples in which Benzophenone-3 was present, Benzophenone-1 was also present. Furthermore, they noted that this observation is suggestive of the possible conversion of Benzophenonoe-3 to Benzophenone-1 and that the content of Benzophenone-1 may be due to human metabolism to and not direct exposure. Spot urine samples (157 total) obtained from a segment of the general German population (59 females, 39 males, and 59 children) were analyzed.[54] Benzophenone-1 and Benzophenone-3 had high detection rates (26%). A study was performed to investigate the exposure of human embryos and fetuses to UV filters.[55] Placentas (12) from volunteer mothers in Spain were collected at delivery. Benzophenone-1 was detected in all samples. 4-Hydroxybenzophenone, metabolite of Benzophenone-1 and Benzophenone-3, was detected in 3 of the 12 placental samples. Urinary concentrations of benzophenones were measured in 34 Tunisian women.[56] Benzophenone-1 and Benzophenone-3 were found in 91.2% and 64.7% of the analyzed samples, respectively.

Benzophenone-1 and Benzophenone-3 were detected in the urine of reproductive-aged women.[57] A total of 143 women provided 509 spot urine samples collected across 2 months of study (3 to 5 samples per woman). Geometric mean urinary concentrations of Benzophenone-3 and Benzophenone-1 were 4.3 µg/l and 3.3 µg/l, respectively. A prospective study involving 200 pregnant women was performed.[58] The women appeared to have been most exposed to Benzophenone-3. The following benzophenones were all detectable in amniotic fluid and cord blood, and, except for 4-hydroxybenzophenone, also in fetal blood: Benzophenone-1, Benzophenone-3, 4-methylbenzophenone, and 4-hydroxybenzophenone. Benzophenone-1 and Benzophenone-3 were only detectable in the fetal circulation in cases of high maternal exposure. 4-Methoxybenzophenone appeared to pass into fetal and cord blood more freely.

Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-4
Benzophenone-6, and Benzophenone-8

Urinary concentrations of the following benzophenone derivatives were evaluated in a national sample of the South Korean population (1576 subjects): Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-4, Benzophenone-8, and 4-hydroxybenzophenone.[59] The detection rates for Benzophenone-1 and 4-hydroxybenzophenone were 56% and 88%, respectively. The detection rate for the following benzophenones was below 25%: Benzophenone-2, Benzophenone-3, Benzophenone-4, and Benzophenone-8. Benzophenones have been identified in human menstrual blood of 25 subjects in

Southern Spain.[60] Benzophenone-3 was detected most frequently (in 24 of 25 subjects), followed by Benzophenone-6 (in 17 of 25 subjects), and Benzophenone-1 (in 11 of 25 subjects). A study with data on benzophenones in the urine was performed using 441 adult pre-menopausal females in South Korea.[61] The detection frequencies of benzophenones in urine samples were: Benzophenone-1 (98.4%), Benzophenone-3 (74.6%), and Benzophenone-8 (22.9%).

Benzophenone-1 and Benzophenone-3

The presence of UV filters in semen, serum, and the urine was studied using 300 men.[62] Only 6 of the men had used sunscreen during the 48 h preceding sample collection. Benzophenone-1 and Benzophenone-3 were detected in 19% and 27% of the seminal fluid samples, respectively.

Benzophenone-3

Urine samples (166 total) were collected from children and adults in the US and China.[63] Benzophenone-3 was detected in practically all urine samples. The concentrations of Benzophenone-3 in children (geometric mean = 9.97 ng/ml) and adults (geometric mean: 15.7 ng/ml) from the US were statistically significantly higher when compared to children (geometric mean = 0.622 ng/ml) and adults (geometric mean = 0.099 ng/ml) from China. The urinary excretion of ingredients in personal care products over a 6-d period was studied using 8 subjects.[64] A total of 352 individual urine samples was collected over a 6-d period. Benzophenone-3 was frequently detected, i.e., in 70% of the total urine samples. Human adipose fat samples were collected from 20 subjects.[65] High concentrations of Benzophenone-3 (maximum of 4940 ng/g wet weight) were detected. Postmortem brain material (hypothalamus and white-matter tissue) obtained from up to 24 individuals was analyzed for the presence of Benzophenone-3.[66] In the hypothalamus, the mean amount (n = 24) of Benzophenone-3 was below the limit of detection. In the white-matter, the mean amount (n = 10) of Benzophenone was 0.32 ng/g. A study on human UV filters in human breast milk was performed, and involved 79 breast milk samples from mothers in Spain.[67] The percentage of samples that contained UV filters was 24%, and two of the major contributors were Benzophenone-3 (779.9 ng/g milk) and its metabolite, 4,4'-dihydroxybenzophenone (73.3 ng/g milk). Concentrations of UV filters in breast tissue (3 serial locations within) from 40 women undergoing mastectomy for breast cancer were measured.[68] Benzophenone-3 was measured in 83 of 120 (69%) tissue samples and at least 1 breast region for 33 of 40 women (range: 0 to 26 ng/g tissue).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute toxicity studies summarized below are described in Table 6. (Data from the previous benzophenones reports are not included in the table.)

Dermal

In studies on Benzophenones-3, -4, -8, and -12 involving rabbits, there was no toxicity at doses of 5 g/kg and greater .[1] The highest dose administered in these studies was 16 g/kg.

A sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated for dermal toxicity in a study involving 24 Wistar albino rats (12 males, 12 females).[69] The authors concluded that the acute dermal LD_{50} of the sunscreen formulation was greater than 2000 mg/kg in male and female rats. The same sunscreen formulation (0.6% to 0.9% Benzophenone-3) was applied to the skin of 6 male New Zealand rabbits.[69] Systemic toxicity was not observed. The acute dermal toxicity of Benzophenone-12 was evaluated using 5 albino rabbits.[5] The LD_{50} was > 10,000 mg/kg.

Oral

Benzophenones-1, -2, -3, -4, -6, -8, -9, -11, and -12 were practically nontoxic to slightly toxic (Benzophenones-2, -4, and -11) when administered orally to rats at doses up to 16 g/kg.[1] Benzophenone-4 was evaluated for acute oral toxicity using 20 rats (strain not stated).[1] Doses of the test substance (in agar/tween) ranging from 1250 to 10,000 mg/kg were administered orally by gavage. Dosing was followed by a 7- to 14-d observation period. Clinical signs (ataxia) were observed. An LD_{50} of 3530 mg/kg was reported.

The acute oral toxicity of Benzophenone-1 was evaluated using rats (number and strain not stated).[6] The LD_{50} was 8600 mg/kg. The acute oral toxicity of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated using 10 female Wistar albino rats.[69] The LD_{50} for the sunscreen formulation was > 2000 mg/kg. The acute oral toxicity of Benzophenone-8 was evaluated using 6 female Wistar rats of the CLR:(WI) strain.[8] The LD_{50} was > 2000 mg/kg. Benzophenone-12 (20% suspension) was evaluated for acute oral toxicity using 10 male rats of the CF Nelson strain.[5] The LD_{50} was > 10,000 mg/kg.

Short-Term Toxicity Studies

Repeated dose toxicity studies (short-term and subchronic toxicity) summarized below are described in Table 7. (Data from the previous benzophenones reports are not included in the table.)

Dermal

In a 2-wk dermal toxicity study, B6C3F$_1$ mice (5 males and 5 females per group) received topical applications in amounts of 0.5 to 8 mg of Benzophenone-3 in an acetone or lotion vehicle.[70] The only effects noted were minimal, variable increases in liver and kidney weights. In another 2-wk dermal toxicity study, F344/N rats (5 males and 5 females per group) received topical applications of 1.25 to 20 mg of Benzophenone-3 in an acetone or lotion vehicle.[70] The only effects noted were small and variable increases in liver and kidney weights. Benzophenone-3 (in ointment base) was applied to the skin of male Sprague-Dawley rats (groups of 4 to 6 animals; weights = 300 g) at a dose of 100 mg/kg, twice daily for 4 wk.[71] The results of this study suggest that Benzophenone-3 is not toxic to rats when applied dermally at a dose of 100 mg/kg for 4 wk. The short-term dermal toxicity of Benzophenone-3 was studied using female Sprague-Dawley rats and their offspring.[35] Benzophenone-3 (10% in cream; dose = 100 mg/kg) was administered dermally (shaved skin on back) twice daily to adult female rats from the first to the last day of pregnancy (~22 to 23 days). At 21 d after birth, the offspring (male and female) were divided into groups of 5 males and groups of 5 females. From 43 to 56 d, the test substance was administered dermally to the male offspring. The dosing of adult pregnant females did not significantly alter their body weight (bw) or cause any apparent adverse effects, when compared to control rats. No significant differences in bw and sex-ratio were observed in the offspring.

Oral

When rats were fed Benzophenone-3 at concentrations up to 1% in the diet for 27 d, no toxic effects were observed.[1] A no-effect-level of 2.5% was reported in a study in which rats were fed Benzophenone-8 in the diet at concentrations up to 10% for 36 d. Groups of mice received Benzophenone-8 (in corn oil, 50 to 5000 mg/kg) by gavage daily for 2 d.[2] No toxic signs or deaths were observed after dosing with 50 mg/kg. Signs of toxicity were observed at doses of 166 to 5000 mg/kg. At doses of 1666.6 and 5000 mg/kg, abnormal gait and a very low mortality incidence were reported. In another experiment, groups of mice were dosed, by gavage, with 1500 mg/kg Benzophenone-8 (2 doses, 24 h apart). Body drop, decreased activity, and abnormal gait were observed.

In a 2-wk oral toxicity study, B6C3F$_1$ mice (5 males and 5 females per group) received feed containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] The no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for mice. There was a dose-related increase in liver weight associated with hepatocyte cytoplasmic vacuolization, up to and including the highest dietary concentration. In another 2-wk oral toxicity study, F344/N rats (5 males and 5 females per group) received diets containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] The NOAEL for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for rats. Liver and kidney weights were increased in dosed rats. Enlarged livers were associated with a marked hepatocyte cytoplasmic vacuolization in rats received diets containing ≥ 6250 ppm. Renal lesions consisting of dilated tubules and regeneration of tubular epithelial cells were observed primarily in high dose rats.

A short-term oral toxicity study on Benzophenone-4 was performed using groups of 26 Wistar rats (13 males, 13 females/group).[7] The test substance was administered orally (in corn oil, by gavage) at doses of 750, 1000, and 1250 mg/kg/d. Male rats were treated 2 wk before mating and thereafter for a total of 48 dosing days. Female rats were treated 2 wk before mating, and during mating, gestation, and lactation, for a total of approximately 63 d of dosing. The NOAEL (systemic toxicity) for Benzophenone-4 in this study was established at 1250 mg/kg/d for male and female rats. Groups of 6 male rats (Carworth Farms Elias strain) were fed Benzophenone-12 at dietary concentrations of 1.25% and 5% daily for 35 d, in accordance with OECD TG 417.[5] There were no lesions of the liver or kidneys at histological examination. The repeated dose toxicity of Benzophenone-12 was evaluated in Wistar rats.[5] The test substance (in 0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80) was administered by gavage to groups of Wistar rats (F_0 animals: 12 males, 12 females/group) at doses of 100, 300, and 1000 mg/kg/d. The duration of treatment was described as follows: 10-wk premating period (males), 2-wk premating period (females), 2-wk mating period (both sexes), ~2 d post-mating (males), entire gestation period, up to 30 d of lactation (corresponding to 21 d of lactation and up to 9 d post-weaning), and 35 d post-mating (for sperm-negative females). Pups from the F_1 litter were selected (F_1 rearing animals) for specific post-weaning examinations. An NOAEL of 1000 mg/kg/d for general, systemic toxicity was determined. (Developmental and reproductive toxicity data are included in that section of this safety assessment.)

Subchronic Toxicity Studies

Oral

In subchronic (90-d) oral toxicity studies, Benzophenones-3 and -12, at 1% and 1.8% in the diet, respectively, were nontoxic to rats.[1] Benzophenones-1 and -12 elicited toxic effects (liver and kidney lesions) in rats at 0.6 and 1.9 g/kg, respectively, when fed for 90 d. In the same time period, Benzophenone-3, fed at 0.5% in the diet, and Benzophenone-8, fed at 5%, produced toxic effects (degenerative nephrosis). In a 120-d feeding study, Benzophenone-12 was nontoxic to dogs at a concentration of 0.6% in the diet.

The subchronic oral toxicity of Benzophenone-1 was evaluated in a 90-d study involving male and female rats (number and strain not stated).[6] The NOAEL was 236 mg/kg bw/d. In a 13-wk oral toxicity study, B6C3F$_1$ mice (10 males and 10 females per group) received feed containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] The NOAEL

for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for mice. Mild increases in liver weights were observed in mice of both sexes, and kidney weights were increased variably in dosed females. Microscopic lesions were observed only in the kidneys of male mice that received 50,000 ppm. In another 13-wk oral toxicity study, F344/N rats (10 males and 10 females per group) received diets containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] The NOAEL for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for rats. Liver and kidney weights were increased in dosed rats. Kidney lesions progressed to include papillary degeneration or necrosis. The liver lesion appeared to regress; liver enzymes in serum remained elevated. Results on subchronic oral toxicity are included in an NTP oral carcinogenicity study on Benzophenone-3 involving male and female Sprague-Dawley rats.[72] Groups of 10 male and 10 female rats were exposed to 0 or 10,000 ppm Benzophenone-3 in the diet for 14 wk. In males, the absolute and relative liver and right kidney weights were increased in the 10,000 ppm group compared to the control group. In females, the absolute kidney weight was significantly decreased, and the relative liver weight was significantly increased relative to the control group.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies summarized below are described in Table 8.

Embryo/Ovary Cultures

The embryotoxicity of Benzophenone-3 was evaluated in the fish embryotoxicity test using zebrafish embryos.[73] The applied number of zebrafish embryos was 40 at each concentration in 4 replicates. The experiment was continued until 120 h post-fertilization. The range of Benzophenone-3 (in dimethyl sulfoxide (DMSO)) concentrations tested was 4.38 µM to 110 µM. Benzophenone-3 decreased the number of hatched embryos after 96 h post-fertilization. The EC_{50} value was 54.3 µM. Other malformations were observed, but their frequency was not concentration-dependent. These included pericardial and yolk sac edema, deformed jaw and ventricle or dilated gut, and jaw deformity. The effect of Benzophenone-3 (in DMSO) on follicular assembly was studied using whole ovary cultures collected from Wistar rats.[74] Ovaries (n = 120) were collected from rats at birth (postnatal day 0). Pups from the same litters were randomly assigned to different treatment groups so that each group contained ovaries of different pups from different litters. The ovary cultures were treated for 7 d with the following Benzophenone-3 concentrations (in (DMSO): 0.0058 µM, 0.276 µM, 0.576 µM, and 0.876 µM. Even at the lowest concentration of Benzophenone-3 (0.0058 µM), stimulation of the process of germ cell nest breakdown and a decrease in the reserve of total oocytes were observed.

Animal

Dermal

In a 13-wk dermal dosing study, B6C3F_1 mice (10 males and 10 females per group) received topical doses of 22.75 to 364 mg/kg Benzophenone-3 in acetone.[75] Epididymal sperm density was decreased (whether or not statistically significant not stated) at all 3 dose levels evaluated (22.75, 91.0, and 364.0 mg/kg). In female mice, there was no significant difference in estrous cycle length between the control group and each dose group. A study was performed to analyze whether dermal exposure to Benzophenone-3 during pregnancy affects the outcome of a second pregnancy in mice.[76] Pregnant mice (number not stated) were exposed dermally to Benzophenone-3 (50 mg/kg/d) from GD 0 to 6. Dermal exposure to Benzophenone-3 during early pregnancy resulted in an intrauterine growth restriction (IUGR) phenotype, disturbed sex ratio, and alterations in the growth curve of the offspring in the mouse model.

Oral

The reproductive toxicity of Benzophenone-1 was evaluated using female rats (number and strain not stated).[6] The test substance was administered orally for 3 d. An NOAEL of 100 mg/kg/d was reported. The developmental toxicity of Benzophenone-2 (in 10% ethanol/90% corn oil vehicle) was evaluated using groups of 5 timed pregnant C57BL/6NCr mice.[77] Benzophenone-2 (6.25 mg) was administered via gavage on GD 12 through 17. In the test group, 8 of 57 male fetuses had hypospadias ($p = 0.0064$, when compared to controls). The co-administration of Benzophenone-2 with an estrogen receptor antagonist (10 µg in vehicle (subcutaneously (s.c.)) during gestation, yielded normal genital tubercles; i.e., no hypospadias in 26 of 26 mice. The authors concluded that Benzophenone-2 may cause hypospadias via signaling through the estrogen receptor. Benzophenone-3 (administered in feed) was tested for its effects on fertility and reproduction in Swiss CD-1 mice, according to the continuous breeding protocol.[78] Based on the results of a dose-finding study, 1.25%, 2.5%, and 5.0% (w/w) were chosen to investigate effects on fertility and reproduction. Male and female mice were continuously exposed for a 7-d precohabitation and a 98-d cohabitation period. In the 2.5% and 5.0% dose groups, feed consumption was consistently higher, but F_0 bw were consistently lower. The authors noted that these findings suggest that Benzophenone-3 may have been adversely affecting metabolism or the digestive process. It was concluded that Benzophenone-3 caused systemic toxicity, but had minimal effects on fertility and reproduction. In a 13-wk oral dosing study, B6C3F_1 mice (10 males and 10 females per group) received feed containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] Mice in the highest dose group (50,000 ppm in feed) exhibited a decrease in epididymal sperm density and an increase in length of the estrous cycle.

The effects of oral exposure to Benzophenone-3 on growth and morphology of the mammary gland and anogenital distance was evaluated using 3 groups of mated BALB/c female mice.[79] From pregnancy day 0 until the day before weaning

(lactational day 21), the females were dosed orally with Benzophenone-3 (in tocopherol-stripped corn oil). The following doses were administered: 0.03 mg/kg/d, 0.212 mg/kg/d, and 3 mg/kg/d. In males, the anogenital index was reduced after exposure to 30 and 212 µg/kg/d at postnatal day 21 and in puberty. In adult males, no differences in anogenital distance were observed. In females, the anogenital index was unaffected at postnatal day 21, but decreased (at 212 µg/kg/d) when measured at puberty. No effects on female anogenital index were observed in adulthood. In a 13-wk oral dosing study, F344/N rats (10 males and 10 females per group) received diets containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3.[70] Rats receiving a diet with 50,000 ppm Benzophenone-3 showed markedly lower epididymal sperm density and an increase in the length of the estrous cycle at the end of the study. A study was performed to determine the effects of maternal and lactational exposure to Benzophenone-3 on the development of offspring.[40] Groups (7 to 8 animals per group) of mated female Sprague-Dawley rats were fed the following Benzophenone-3 concentrations (in low-phytoestrogen chow) from GD 6 until weaning on postnatal day 23: 1000; 3000; 10,000; 25,000; or 50,000 ppm. There were no statistically significant differences in the mean number of implantation sites/litter, mean resorptions per litter, % litters with resorptions, number and weights of live fetuses, or sex ratios between the control and Benzophenone-3 dose groups.

Groups of 25 pregnant Sprague-Dawley rats were fed low-phytoestrogen chow containing 3000 or 30,000 ppm Benzophenone-3 from GD 6 until postnatal day 21.[80] The male offspring evaluated in this study were weaned on postnatal day 28, and then dosed with the same concentrations of Benzophenone-3 (in chow and milk). The animals were killed on postnatal day 30. Rats exposed perinatally to 30,000 ppm Benzophenone-3 had statistically significantly lower weights of the paired-testis, paired-epididymis, and prostate. Results relating to developmental toxicity are included in an NTP oral carcinogenicity study on Benzophenone-3 involving male and female Sprague-Dawley rats.[72] On GD 6, groups of 42, 35, 35, and 43 F_0 time-mated female rats were fed diets containing 0, 1000, 3000, and 10,000 ppm Benzophenone-3, respectively, for 39 d. Dietary concentrations of 1000, 3000, and 10,000 ppm Benzophenone-3 resulted in average daily doses of approximately 70, 206, and 660 mg Benzophenone-3/kg bw/d during gestation, and 157, 478, and 1609 mg/kg/d over lactation days 1 – 14. Groups of 50 (1000 and 3000 ppm) or 60 (0 and 10,000 ppm) F_1 rats per sex continued on study after weaning, and were fed diets containing the same exposure concentrations for 105 wk; 10 F_1 rats per sex from the 0 and 10,000 ppm groups were evaluated at 14 wk. The administration of Benzophenone-3 had no effects on the percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups. Benzophenone-3 was evaluated for developmental toxicity in accordance with OECD TG 414, using groups of 25 mated Wistar rats of the Crl:WI (Han) strain.[9] Benzophenone-3 (in corn oil) was administered at doses of 40, 200, and 1000 mg/kg/d (once daily, by gavage) on days 6 through 19 post-coitum. The NOAEL for Benzophenone-3 was 200 mg/kg/d. In a study involving groups of 26 Wistar rats (13 males, 13 females/group), Benzophenone-4 was administered orally (in corn oil, by gavage) at doses of 750, 1000, and 1250 mg/kg/d.[7] Male rats were treated 2 wk before mating and thereafter for a total of 48 d of dosing. Female rats were treated 2 wk before mating, during mating, during gestation and during lactation, for a total of approximately 63 d of dosing. The NOAEL (reproductive toxicity) for Benzophenone-4 in this study was established at 1250 mg/kg/d.

The developmental toxicity of Benzophenone-12 (in 0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80) was evaluated using groups of 50 Wistar rats (25 males for mating, 25 females).[5] The test substance was administered by gavage to mated females at doses of 100, 300, and 1000 mg/kg/d. The groups were dosed daily, from implantation to one day prior to the expected day of parturition (GD 6 to 19). The NOAEL for maternal and prenatal developmental toxicity was 1000 mg/kg/d. Benzophenone-12 (in 0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80) was administered by gavage to groups of Wistar rats (F_0 animals: 12 males, 12 females/group) at doses of 100, 300, and 1000 mg/kg/d.[5] The duration of treatment was described as follows: 10-wk premating period (males), 2-wk premating period (females), 2-wk mating period (both sexes), ~2 d post-mating (males), entire gestation period, 21 d of lactation and up to 9 d post-weaning, and 35 d post-mating (for sperm-negative females). The NOAEL for reproductive performance and fertility of the F_0 parental rats and developmental toxicity in the offspring was 1000 mg/kg/d.

GENOTOXICITY STUDIES

Genotoxicity studies (in vitro and in vivo) summarized below are described in Table 9. (Data from the previous benzophenones reports are not included in the table.)

In Vitro

Benzophenone-2 (up to 10,000 µg/plate), Benzophenone-6 (up to 1000 µg/plate), and Benzophenone-8 (up to 700 µg/plate) were reported to be weakly mutagenic with metabolic activation in the Ames test.[1] Benzophenones-6 and -8 were mutagenic in one of the Salmonella typhimurium strains (TA1537) tested. Benzophenone-2 was weakly mutagenic in the mouse lymphoma forward mutation assay (at doses of 24 and 32 µg/plate) and in a cytogenic assay evaluating sister chromatid exchanges and chromosome aberrations (at doses of 100 and 200 µg/plate). These effects in L5178Y mouse lymphoma cells were observed at the high end of the range of doses tested. Benzophenones-1, -3, -4, -9, and -11 were non-mutagenic both with and without metabolic activation in the Ames test.

In a modified Ames test, Benzophenone-2 and Benzophenone-6 (concentrations up to 1000 µg/ml) were not genotoxic to Salmonella typhimurium strains with or without metabolic activation.[2] Benzophenone-2 and Benzophenone-6 did not induce unscheduled DNA synthesis in rat hepatocytes at concentrations up to 1000 nmol/ml. In a sister chromosome exchange

assay, Benzophenone-8 was tested using Chinese hamster ovary cell cultures. Without metabolic activation, there was no significant increase in sister chromatid exchanges at concentrations ranging from 333 ng/ml to 10 µg/ml, but a slight increase was noted at 10 µg/ml. With metabolic activation, there was no increase in sister chromatid exchanges at concentrations ranging from 3.1 to 50 µg/ml. Benzophenone-8 was not genotoxic in a forward mutation assay involving Chinese hamster ovary cells, at concentrations ranging from 2.2 to 66.6 µg/ml with or without metabolic activation.

The photo-genotoxicity of Benzophenone-1 (1 to 25 µg/ml, in culture medium) and apoptotic parameters were evaluated using human keratinocytes (HaCaT cells).[81] Results indicated that Benzophenone-1 photosensitized and generated intracellular reactive oxygen species (2.02 folds) under sunlight/UV radiation. Decrease in cell viability was recorded as 80.06%, 60.98%, and 56.24% under sunlight, UVA, and UVB, respectively. In the same study, the genotoxicity potential of Benzophenone-1 (5 to 25 µg/ml, in culture medium) was confirmed through photo-micronuclei and cyclobutene pyrimidine dimers (CPDs) formation. HaCaT cells treated with Benzophenone-1 in the presence of UVB (1.08 J/cm^2) caused cyclobutane CPD formation. Micronuclei formation was detected in HaCaT cells treated with Benzophenone-1 (10 µg/ml) in the presence of UVB (1.08 J/cm^2). Cells exposed to different concentrations of Benzophenone-1 in the presence of UVA (2.7 J/cm^2) exhibited statistically significant ($p > 0.01$) DNA damage when compared to control cells. The genotoxicity of Benzophenone-1, Benzophenone-3, Benzophenone-6, and Benzophenone-8 (doses up to 10 µg/well) was evaluated in the luminescent *umu*-test, using *Salmonella typhimurium* strain TL210.[82] Results indicated positive results for Benzophenone-3 and "pseudo-positive" (not defined) results for Benzophenone-1 and Benzophenone-8. In the same study, the genotoxicity of Benzophenone-1 (doses up to 600 µg/plate), Benzophenone-3 (up to 200 µg/plate), Benzophenone-6 (up to 2000 µg/plate), and Benzophenone-8 (up to 300 µg/plate) was evaluated in the Ames test using *S. typhimurium* strains TA98 and TA100 (with and without metabolic activation).[82] None of the test substances produced clear positive results with or without metabolic activation.

The genotoxicity of Benzophenone-3 and Benzophenone-8 (each in seawater) was evaluated at doses of 4 to 10 µl per plate using *S. typhimurium* strain TA98 (without metabolic activation).[83] Neither ingredient was genotoxic. The genotoxicity of Benzophenone-3 and Benzophenone-8, each in chlorinated bromide-rich water (artificial seawater), was also evaluated in the Ames test using *S. typhimurium* strain TA98 without metabolic activation. Only Benzophenone-8 (1:10) had clear genotoxic activity that was dose-related (doses of 4, 6, 8, and 10 µl). A sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated for genotoxicity using the following *S. typhimurium* strains: TA 98, TA100, TA1535 and TA1538.[69] The formulation was tested at a dose of 5000 µg/plate with and without metabolic activation. The sunscreen formulation was not genotoxic. The cytogenetic effect of Benzophenone-3 on human peripheral lymphocytes was evaluated using in vitro chromosomal aberrations and micronuclei assays.[84] Lymphocyte cultures were exposed to the following 5 concentrations of Benzophenone-3: 0.20 µg/ml, 0.10 µg/ml, 0.05 µg/ml, 0.025 µg/ml, and 0.0125 µg/ml. A statistically significant increase in chromosomal aberrations and aberrant cell frequencies was observed at all test concentrations, when compared to the solvent (DMSO) control. In the micronuclei test, Benzophenone-3 caused a statistically significant increase in micronuclei formation at all test concentrations. The effect of Benzophenone-3 on DNA damage was studied using human breast epithelial cells.[85] Concentrations of 1 µM and 5 µM Benzophenone-3 increased DNA damage in a manner that was similar to that of treatment with E2, and in an estrogen-receptor alpha (Erα)-dependent manner. Benzophenone-3 was evaluated for genotoxicity using *S. typhimurium* strains TA98 and TA100, and *Escherichia coli* strain *uvr*A pKM101.[72] The test substance was evaluated at doses up to 6000 µg/plate with and without metabolic activation. Benzophenone-3 was non-genotoxic.

A bacterial reverse mutation assay was used to evaluate the genotoxicity of Benzophenone-8 (in DMSO), using *S. typhimurium* strain TA100 and *E. coli* (*E. coli*) strain WP2vurA.[8] Strain TA100 was selected for testing at doses up to 1500 µg/plate, and strain WP2vurA was selected for testing at doses up to 5000 µg/plate. Benzophenone-8 was negative for genotoxicity in this assay, with and without metabolic activation. The mutagenicity of Benzophenone-8 (in ethanol) was evaluated in the *Salmonella*/mammalian microsome mutagenicity assay using the following *S. typhimurium* tester strains: TA98, TA100, TA1535, TA1537, and TA1538.[86] Benzophenone-8 test concentrations ranged from 0.008 to 700 µg/plate. Benzophenone-8 caused a weak, but reproducibly significant increase in the number of TA1537 revertants per plate. Benzophenone-8 (in ethanol) was tested in the L5178Y TK+/- mouse lymphoma mutagenesis assay (with and without metabolic activation) at concentrations ranging from 13 to 56 µg/ml.[87] Cultures treated in the presence of metabolic activation exhibited a significant increase in the mutant frequencies, and a dose response was evident. Benzophenone-8 was genotoxic in this assay. The genotoxicity of Benzophenone-12 in the mammalian cell gene mutation assay (mouse lymphoma L5178Y cells) was evaluated.[5] Benzophenone-12 (in DMSO) was tested at doses up to 50 µg/ml (with metabolic activation) and up to 52 µg/ml (without metabolic activation). Benzophenone-12 was non-genotoxic without metabolic activation, and results were ambiguous with metabolic activation.

In Vivo

The genotoxicity of Benzophenone-1 was evaluated in the micronucleus assay (OECD TG 474) using mouse erythrocytes.[6] The doses tested were not stated. Genotoxicity results were classified as inconclusive. Benzophenone-2 and Benzophenone-6 did not induce sister chromatid exchanges in Chinese hamster bone marrow cells from animals dosed orally (doses up to 500 mg/kg).[2] In the micronucleus test, the oral dosing of mice with Benzophenone-8 (1500 mg/kg) did not cause a significant increase in the number of bone marrow micronuclei.

The genotoxicity of Benzophenone-3 was evaluated using the Drosophila somatic mutation and recombination test (SMART) and the in vivo cytogenetics assay using rat bone marrow cells.[88] In the SMART assay, larva from a mating of "multiple wing hair" (mwh) females with heterozygous "flare" (flr) males were exposed to 0, 3000, or 3500 ppm Benzophenone-3. None of the Benzophenone-3-treated larva produced flies with significantly more single or multiple wing spots than controls. An in vivo cytogenetic assay in rat bone marrow cells was conducted to evaluate the clastogenicity of Benzophenone-3.[88] Sprague-Dawley rats were treated by gavage with a single administration of 0, 500, 1670, or 5000 mg/kg Benzophenone-3, or a dose of 5000 mg/kg/d Benzophenone-3 for 5 consecutive days. None of the Benzophenone-3 doses caused any significant increase in chromosomal aberrations. The genotoxicity of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated in the mammalian erythrocyte micronucleus test using groups of 10 Wistar albino rats.[69] Doses of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg were administered dermally for 2 consecutive days. The sunscreen formulation was non-genotoxic. The same sunscreen formulation (0.6% to 0.9% Benzophenone-3) was evaluated for genotoxicity in the mammalian bone marrow chromosome aberration test using groups of 10 Wistar albino rats.[69] Doses of the sunscreen, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg, were administered dermally for 2 consecutive days. The sunscreen formulation was non-genotoxic. Ovariectomized mice (Balb/c female mice) were exposed to Benzophenone-3 at 10 d after the surgical procedure.[85] Eight mice were dosed orally with E2 and 12 mice were dosed orally with Benzophenone-3 daily for 4 d. Each mouse was administered 1 µl of tocopherol-stripped corn oil per gram of bw to deliver E2 (0.25 mg/kg/d) or Benzophenone-3 (3 mg/kg/d). Results indicated that R-loops and DNA damage were detected in mammary epithelial cells of mice treated with Benzophenone-3.

CARCINOGENICITY STUDIES

In Vitro

Benzophenone-1

Effects of Benzophenone-1 on the proliferation and metastasis of MCF-7 human breast cancer cells expressing estrogen receptors were studied.[89] The underlying mechanisms for these effects was also studied, including the study of alterations in transcriptional and translational levels of proliferation and metastasis-related markers (cyclin D1, p21, and cathepsin D). Treatment of the cells with Benzophenone-1 (0.1 to 10 µM) promoted the proliferation of MCF-7 cells in a manner that was similar to the positive control (E2). The addition of Benzophenone-1 also markedly induced the migration of MCF-7 cells in a manner that was similar to E2. Regarding underlying mechanisms of action, an increase in the expression of cyclin D1 and cathepsin D, and a decrease in p21 (at both transcriptional and translational levels) were reported. The authors concluded that Benzophenone-1 may accelerate the growth of MCF-7 breast cancer cells by regulating cell cycle-related genes and promote cancer metastasis through amplification of cathepsin D.

A wound healing assay and western blot assay were performed to show the effect of Benzophenone-1 on the migration of BG-1 ovarian cancer cells and the protein expression of epithelial-mesenchymal transition (EMT)-related genes.[90] The EMT process is associated with cell migration. Benzophenone-1 (1 µM) statistically significantly enhanced the migration capability of BG-1 cells by reducing the wounded area in the cell monolayer relative to the control, i.e., in a manner that was similar to E2 (0.001 µM). The authors stated that the results of this study indicate that Benzophenone-1 may have the ability to induce ovarian cancer metastasis via regulation of the expression of EMT markers and migration of estrogen receptor-expressing BG-1 ovarian cancer cells.

Benzophenone-3

The effect of Benzophenone-3 (concentrations up to 150 µg/l) on cancer cell growth was studied using NCI-H460 lung cancer cells.[91] At concentrations of 50 µg/l, 100 µg/l, and 150 µg/l, Benzophenone-3 statistically significantly increased colony formation of the NCI-460 cells, in both number and size. These observations indicate that Benzophenone-3 has a cancer potentiating effect by enhancing anchorage-independent survival and growth of lung cancer cells.

Animal

Oral

Benzophenone-3

The oral carcinogenicity of Benzophenone-3 was evaluated in an NTP study using male and female B6C3F1/N mice and male and female Sprague-Dawley rats.[72] Groups of 50 male and 50 female mice were fed diets containing 0, 1000, 3000, or 10,000 ppm Benzophenone-3 in the diet (equivalent to average daily doses of approximately 0, 113, 339, and 1207 mg Benzophenone-3/kg bw, respectively, for male mice and 0, 109, 320, and 1278 mg/kg, respectively, for female mice) for 104 (female mice) or 105 (male mice) wk. Survival of all exposed groups of male and female mice was not statistically significantly different from that of the control groups. Mean bw of 1000 and 3000 ppm males and females were within 10% of those of the control groups throughout the study. Mean bw of 10,000 ppm male and female mice were at least 10% lower than those of the control groups, generally after wk 17 and 12, respectively. Feed consumption by exposed groups of male and female mice was not statistically significantly different from that of the control groups.

The incidences of pigment in the bone marrow were statistically significantly increased in 10,000 ppm male and female mice. The incidences of pigment in the spleen were statistically significantly increased in 10,000 ppm male mice and 3000 ppm and 10,000 ppm female mice. In the liver, the incidence of hepatocyte syncytial alteration was statistically significantly increased in all exposed groups of male mice. In the kidney, the incidence of renal tubule cytoplasmic alteration was statistically significantly increased in 10,000 ppm male mice. The incidence of osseous metaplasia was statistically significantly increased in 10,000 ppm female mice, when compared to the control group. The authors concluded that there was no evidence of carcinogenic activity in male or female B6C3F1/N mice at exposure concentrations of 1000, 3000, and 10,000 ppm.

In the same NTP carcinogenicity study, on GD 6, groups of 42, 35, 35, and 43 F_0 time-mated female rats were fed diets containing 0, 1000, 3000, and 10,000 ppm Benzophenone-3, respectively, for 39 d. Groups of 50 (1000 and 3000 ppm) or 60 (0 and 10,000 ppm) F_1 rats per sex continued on study after weaning and were fed diets containing the same exposure concentrations for 105 wk; 10 F_1 rats per sex from the 0 and 10,000 ppm groups were evaluated at 14 wk. Dietary concentrations of 1000, 3000, and 10,000 ppm resulted in average daily doses of approximately 58, 168, and 585 mg Benzophenone-3/kg bw, respectively, for males and 60, 180, and 632 mg/kg body weight, respectively, for females. Survival of all exposed groups of F_1 male and female rats was not statistically significantly different from that of the control groups. Over the course of the study, mean bw of F_1 male rats and females in the 10,000 ppm exposure groups were 10 – 25% lower than those of the control groups. After wk 77, F_1 female rat mean bw in the 3000 ppm exposure group were 10% lower than those of the control group. Feed consumption by exposed groups of F_1 males and females was generally similar to that of the control group throughout the study.

In the brain, the occurrence of malignant meningiomas in male rats at the end of the 2-year study was 0/50 (control group), 1/50 (1000 ppm group), 3/50 (3000 ppm group), and 0/50 (10,000 ppm group). One male rat in the 3000 ppm group had a malignant meningioma in the spinal cord. In the thyroid gland, the incidence of C-cell adenoma in 3000 ppm female rats was statistically significantly greater than that in the control group at the end of the 2-year study. Only one female rat, in the 10,000 ppm group, had bilateral C-cell adenomas; the rest were unilateral lesions. One animal in the 1000 ppm group had both a C-cell adenoma and a C-cell carcinoma (in the opposite gland). There was no significant exposure concentration-related difference in the incidence of C-cell adenomas in male rats (0 ppm (7/50); 1000 ppm (10/50); 3000 ppm (8/50); and 10,000 ppm (8/50)) when compared to the control group.

In the uterus, the incidence of stromal polyps in 3000 ppm females was statistically significantly increased. A statistically significantly increased incidence of atypical endometrium hyperplasia of the uterus also occurred at 3000 ppm; however, the incidence of adenocarcinoma was statistically significantly decreased in this group. In the adrenal cortex, the incidences of focal hypertrophy were statistically significantly increased in 1000 and 3000 ppm female rats at the end of the 2-year study. In the testes, the incidence of interstitial cell hyperplasia showed a statistically significant positive trend, but there were no statistically significant pairwise comparisons of the exposed groups to the control group. The incidence of fibrinoid necrosis of the arterioles was statistically significantly increased in 10,000 ppm males when compared to the control group. In the pancreas, the incidence of chronic active inflammation affecting the arterioles was statistically significantly increased in 1000 ppm males, when compared to the control group at the end of the 2-year study. The incidences of mammary gland fibroadenoma and carcinoma were statistically significantly decreased, relative to the control group, in 10,000 ppm females at the end of the 2-year study (fibroadenomas: 32/50 (control), 30/50 (1000 ppm), 27/50 (3000 ppm), and 18/50 (10,000 ppm); carcinomas: 7/50 (control), 5/50 (1000 ppm), 7/50 (3000 ppm), and 1/50 (10,000 ppm)).

The authors concluded that, under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of Benzophenone-3 exposure in male Hsd:Sprague Dawley SD rats, based on the occurrence of malignant meningiomas in the brain. There was equivocal evidence of carcinogenic activity in female Hsd:Sprague Dawley SD rats, based on the increased incidence of thyroid C-cell adenomas and the increased incidence of uterine stromal polyps. There was no evidence of carcinogenic activity in male or female B6C3F1/N mice at exposure concentrations of 1000, 3000, and 10,000 ppm. It was noted that increases in the incidences of non-neoplastic lesions of the testis in male rats and of the uterus and adrenal cortex in female rats occurred with exposure to Benzophenone-3. Increases in the incidences of non-neoplastic lesions of the bone marrow (males and females), spleen (males and females), kidney (males and females), and liver (males) in mice occurred with exposure to Benzophenone-3.

Combined Challenge of Benzophenone-3 and Estradiol

Benzophenone-3

Groups of female BALB/c mice (number per group not stated) were fed diets with and without Benzophenone-3 (70 mg/kg bw and then were injected daily for 5 d with saline control or E2 (1 µg/injection).[92] Benzophenone-3 was compounded into the diets at 0.75 g/kg chow for pubertal animals and 1.5 g/kg chow for adult animals; each dosage yielded consumption of approximately 70 mg/kg bw/d. Both pubertal and adult BALB/c mice were placed on low fat diet (LFD; 10% kcal fat) or high fat diet (HFD; 60% kcal fat) with and without Benzophenone-3, ovariectomized, allowed time for recovery and clearance of endogenous hormones, and then treated with E2 or control for 5 d. While no Benzophenone-3 effects were seen in the adult mice (data unavailable), the pubertal mice fed HFD plus Benzophenone-3 showed higher mammary gland proliferation (mammary epithelial proliferation) in response to E2 than did mice fed HFD alone. No

Benzophenone-3 effects were observed in the absence of E2, and no Benzophenone-3 effects were observed in mice fed LFD.

Additionally, the effects of Benzophenone-3 (doses of 0.7 mg/kg bw, 7 mg/kg bw, and 70 mg/kg bw) in an acute exposure regimen were evaluated, with and without co-treatment with E2, in ovariectomized, pubertal BALB/c mice (number not stated) fed HFD.[92] After 1 week the mice were ovariectomized. Recovery was allowed for 3 weeks after ovariectomy before Benzophenone-3 and E2 treatments. The mice were injected daily for 5 d with saline control or E2 (1 µg/injection) and/or given Benzophenone-3 by gavage in vegetable oil (0.7 mg/kg bw, 7 mg/kg bw, or 70 mg/kg bw). Benzophenone-3 by itself had no effects at any dose. However, Benzophenone-3 augmented the proliferative response to E2 in both mammary ducts and mammary duct ends at 70 mg/kg bw/day, and in mammary duct ends at 7 mg/kg bw/day.

For tumorigenesis promotion experiments, female Trp53-null transplanted mice (number not stated) generated from BALB/c Trp53+/– breeding mice) were randomly assigned into various dietary groups.[92] In the Trp53-null mouse model, fragments of donor mammary epithelium were collected from female BALB/c Trp53-null mice at 8 weeks of age, and transplanted into the cleared inguinal mammary fat pads of 3-week-old female wild type BALB/c mice. For the continuous LFD group, the diet was initiated after transplantation at 3 weeks of age and maintained throughout the studies. For the HFD-LFD and LFD-HFD groups, mice were initially fed one diet from 3 weeks until 10 weeks of age, and then switched to the other diet thereafter. For diets containing Benzophenone-3, Benzophenone-3 was compounded into the diets at 0.75 g/kg chow for pubertal animals (3 to 10 weeks of age) and 1.5 g/kg chow for adult animals; each dosage yielded consumption of approximately 70 mg/kg bw/d. All mice were killed at estrus; 5-bromo-2′-deoxyuridine (BrdU) (70 µg/g bw) was administered via i.p. injection 2 h before the animals were killed for analysis of cellular proliferation. Benzophenone-3 was protective for epithelial tumorigenesis in mice fed lifelong LFD, while promotional for epithelial tumorigenesis in mice fed an adult HFD. Benzophenone-3 increased tumor cell proliferation, decreased tumor cell apoptosis, and increased tumor vascularity dependent on specific dietary regimen and tumor histopathology. Although Benzophenone-3 seemed protective on LFD, spindle cell tumors arising in these mice showed increased proliferation and decreased apoptosis.

In Vitro Cell Transformation Studies

Benzophenone-1

The xenoestrogenic effect of Benzophenone-1 on BG-1 human ovarian cancer cells expressing estrogen receptors and relevant xenografted animal models, when compared to E2, was evaluated.[93] In the in vitro cell viability assay, Benzophenone-1 (0.01 to 10 µM) statistically significantly increased BG-1 cell growth, as did E2. The mechanism underlying BG-1 cell proliferation induced by Benzophenone-1 was shown to be related to the up-regulation of cyclin D1, a cell cycle progressor. Both Benzophenone-1 and E2 induced cell growth and up-regulation of cyclin D1 were reversed by co-treatment with an ER antagonist, suggesting that Benzophenone-1 may, similar to E2, mediate the cancer cell proliferation via an estrogen receptor-dependent pathway. However, the expression of p21 (regulator of cell cycle progression at G_1 phase) was not altered by Benzophenone-1, though it was down-regulated by E2.

In a second experiment, BG-1 cells (5×10^6) were injected s.c. into the backs of groups of 6 female mice of the BALB/*c nu*/*nu* strain. The mice were monitored for tumor growth. Once the tumors reached a volume of 50 mm^3, the mice were surgically ovariectomized. One week after surgery, 6 mice were injected s.c. with E2 (0.02 mg/kg) every 2 d for 8 wk, and another group of 6 mice was dosed s.c. with Benzophenone-1 (200 mg/kg). The vehicle control group was dosed with corn oil. Benzophenone-1 or E2 treatment statistically significantly increased the tumor mass formation (compared to corn oil vehicle) within 8 wk. At histopathological examination, the tumor sections of the E2 or Benzophenone-1 group displayed extensive cell formations with high density and disordered arrangement. These results were supported by the increased number of BrdUrd positive nuclei and the over-expression of cyclin D1 protein. The authors noted that the results of this study suggest that Benzophenone-1 is an endocrine disrupting chemical that exerts xenoestrogenic effects (in manner similar to E2) by stimulating the proliferation of BG-1 ovarian cancer via the estrogen receptor signaling pathway associated with the cell cycle.

A study was performed to evaluate the effects of Benzophenone-1 on prostate cancer progression, including cell proliferation and migration.[94] Additionally, the alterations in protein expressions of cell cycle related genes, as well as cathepsin D gene as a metastasis marker by Benzophenone-1, were investigated in an effort to explain the underlying mechanism. To evaluate the effect on cell proliferation, the 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (n = 3) was performed using LNCaP prostate cancer cell cultures. This cell line was originally isolated from the lymph node of a patient with metastatic prostate cancer. LNCaP cells were treated with Benzophenone-1 (0.01 to 10 µM) for 4 d. The incubation medium was described as phenol-free Dulbecco's modified eagle medium (DMEM) supplemented with 1% DMSO. To demonstrate the connection between Benzophenone-1 and the androgen receptor signaling pathway, LNCaP cells were co-treated with Benzophenone-1 (1 µM) and biclutamide (0.001 µM, androgen receptor antagonist; n = 3). In the migration assay, LNCaP cells were treated with 10% charcoal/dextran-treated fetal bovine serum (FBS) containing 1 µM Benzophenone-1 for 5 d (n =4). The Western blot analysis was used to measure protein expressions for c-fos, cyclin E, p321, and cathepsin D. LNCaP cells were cultured with Benzophenone-1 for a fixed period of time. After treatment, whole cell lysates of LNCaP cells were prepared (in buffer solution) in a time-dependent manner (0, 24, and 48 h). The proteins were transferred to a polyvinylidene difluoride membrane, and the membranes were incubated overnight with the following

antibodies: rabbit polyclonal anti-cyclin E, anti-c-fos antibody, anti-cathepsin D antibody, mouse monoclonal anti-p21, and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This experiment was repeated (n = 3).

Benzophenone-1 increased the viability of LNCaP cells at concentrations of 1 μM and 0.1 μM. In the MTT assay, when the cells were co-treated with Benzophenone-1 (1 μM) and biclutamide (0.001 μM), the cell viability that was increased by Benzophenone-1 alone was statistically significantly reduced. These results suggest that the proliferative effects of Benzophenone-1 on LNCaP cells was mediated by the androgen receptor signaling pathway. In the experiments relating to cell mobility, Benzophenone-1 (1 μM) increased cell migration when compared to the DMSO control. In parallel with the changes in cell viability levels, the migration activity of LNCaP cells increased by Benzophenone-1 was statistically significantly reduced by co-treatment with biclutamide (0.001 μM). These results indicate that the stimulatory effects on LNCaP cell migration induced by Benzophenone-1 were mediated via the androgen receptor signaling pathway.

Protein expression of cyclin E, one of the proteins required for cell cycle progression, was enhanced by Benzophenone-1 at 24 and 48 h. The protein expression level of p21 (regulator of cell cycle progression) was statistically significantly reduced by Benzophenone-1 at 24 h, when compared to DMSO. Protein expression of c-fos was not statistically significantly induced by Benzophenone-1. For cathepsin D (metastasis marker), its protein expression levels were statistically significantly increased by Benzophenone-1 (100 μM) at 24 and 48 h, when compared to the control. To determine whether or not the effects of Benzophenone-1 on the expressions of cyclin E, p21, and cathepsin D were mediated by the androgen receptor signaling pathway, a Western blot analysis was performed on protein samples isolated from LNCaP cells treated with Benzophenone-1 (1 μM) in the presence of biclutamide. The protein levels of cyclin E, p21, and cathepsin D were not changed at 24 and 48 h. These results may suggest that the protein expressions of these genes are induced by Benzophenone-1 via the androgen receptor signaling pathway. The authors concluded that the results of this study indicate that Benzophenone-1 may enhance the progression of prostate cancer by regulating cell cycle and metastasis-related genes via the androgen receptor signaling pathway.

Benzophenone-1, Benzophenone-3, Benzophenone-6, and Benzophenone-8

The second process in carcinogenesis, promotion, was studied using the Bhas promotion assay.[82] This is a test that is used to detect the formation of transformation foci, using Bhas 42 cells established from BALB/3T3 cells. Benzophenone-1, Benzophenone-3, Benzophenone-6, and Benzophenone-8 were tested, and each was evaluated at concentrations ranging from 2 to 100 μg/ml. On day 21 of incubation, the cells were fixed with methanol and dyed. After air-drying, the number of transformation foci was counted using a stereoscopic microscope. The transformation foci were identified using the following 5 criteria: (1) more than 50 cells in a focus area, (2) cells in the focus area are spindle-shaped and different from surrounding cells, (3) cells in the focus area across each other in a random sequence, (4) cells grow in a stacked manner, and (5) the cytoplasm is intensely dyed by basicity. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) served as the positive control.

Dosing (all doses) with Benzophenone-3 and Benzophenone-6 did not result in any statistically significant increase in the number of foci relative to the solvent controls, indicating negative promotion activity. Particularly, Benzophenone-6 and Benzophenone-8 produced less foci than the solvent controls at concentrations of 5 μg/ml and above 20 μg/ml, respectively. Cell survival rates declined at the concentrations at which the number of foci decreased. Thus, the effect of cytotoxicity was believed to have been the cause of the decrease in the number of foci. For testing with Benzophenone-1, there was no increase in the number of foci at concentrations below 5 μg/μl. However, at 10 μg/ml, there was a statistically significant increase to 6 ± 2.4 foci/well. This increase was more than twice that of the number of foci in the solvent controls (2.2 ± 1.5 foci/well). However, the increase was 1.5% per gram when compared to the number of foci in the positive controls (50 ng/ml TPA, 20.2 ± 5.2 foci/well). At a concentration of 20 μg/ml, the number of foci was comparable to that of the solvent controls, but the cell survival rate was lower (31%), suggesting toxicity of the test substance. Benzophenone-1 was believed to have been a tumor promoter (at 10 μg/ml), based on results indicating that it caused a statistically significant increase to more than twice that of the controls. However, the tumor promotion potential of Benzophenone-1 was apparently weak when compared to the level in the positive controls. The authors noted that the results of this study indicate that none of the test substances resulted in a statistically significant increase in the number of foci (relative to the solvent controls DMSO and methanol) over the range of concentrations tested, indicating negative promotion activity.

ANTI-CARCINOGENICITY STUDIES

Benzophenone-8 and Benzophenone-12

The in vivo anti-tumor activity of Benzophenone-8 and Benzophenononone-12 was evaluated using a two-stage mouse skin carcinogenesis model.[95] In this model, (±)-*E*-4-methyl-2-[-*E*-hydroxyamino]-5-nitro-6-methoxy-3-hexamide (NOR-1) served as the inducer and TPA as the promoter. Groups of 15 pathogen-free, female hairless mice of the HOS:HR-1 strain were used. Skin tumors were induced by a single dose of NOR-1 (390 nmol in 100 μl of acetone). At 1-wk post-dosing, TPA (1.7 nmol in 100 μl of acetone) was applied to the skin twice weekly for 20 wk as a tumor promoter. Each test substance was administered at a concentration of 0.0025% to mice through drinking water (ad libitum), beginning at 1 week prior to tumor initiation and ending at 1 week after tumor initiation. All animals were examined weekly for the development of skin papillomas. When compared to the positive control (NOR-1) group, the following observations were made for both test substances: 2-wk delay in tumor appearance, statistically significant inhibition ($p < 0.001$) of tumor incidence (60% for

Benzophenone-8; 50% for Benzophenone-12), and statistically significant inhibition of tumor burden (papilloma inhibition per mouse: 70% for Benzophenone-8 and 50% for Benzophenone-12). Benzophenone-8 was a more potent inhibitor of skin tumors than Benzophenone-12.

OTHER RELEVANT STUDIES

Effect on Gene Expression

Benzophenone-3

A study was performed to determine whether Benzophenone-3 exposure alters gene expression profiling in the prostate and testis.[80] Groups of 25 pregnant Sprague-Dawley rats were fed low-phytoestrogen chow containing 3000 or 30,000 ppm Benzophenonoe-3 from GD 6 until postnatal day 21. The offspring were killed on postnatal day 30 and tissue samples were collected. RNA samples from the prostate and testis (1 male pup per litter; 5 litters per group) were extracted. Microarray gene expression profiling was performed on the tissue samples. Results indicated that gene expression profiles of the prostate and testis were differentially affected by Benzophenone-3 dose and duration of exposure. Tissue-specific alterations were also indicated. Microarray analyses of prostate gene expression patterns of rats exposed perinatally to Benzophenone-3 identified significant expression of 334 and 689 genes in the 3000 and 30,000 ppm exposure groups, respectively, when compared to the controls ($p < 0.05$; fold change > 1.5). Seventy-six genes overlapped between the 2 Benzophenone-3 exposure groups in the prostate. Microarray analyses of testis-gene expression patterns identified 239 and 1159 genes that were significantly altered in the testis in animals of the 3000 ppm and 30,000 ppm Benzophenone-3 perinatally exposed groups, respectively. Between the 2 Benzophenone-3 exposure groups, 220 genes overlapped in expression profile in the testis. The authors noted that the gene expression changes observed in this study were only observed at concentrations that exceed typical human exposure to Benzophenone-3.

Effect on Melanogenesis

Benzophenone-2

The dual action of Benzophenone-2 in the biosynthetic pathway of melanin has been identified.[96] It has been observed to act as a weak competitive inhibitor of tyrosinase (inhibition constant (K_i) = 2020 ± 90 µM; half maximal inhibitory concentration (IC_{50}) = 3820 ± 390 µM). Both forms of Benzophenone-2 (protonated and deprotonated) interact with tyrosinase, the enzyme that catalyzes the production of melanin from tyrosine. Benzophenone-2 (at 250 and 500 µM) also accelerated the conversion of dopachrome (intermediate in melanin biosynthesis) to melanin.

Neurotoxicity

Benzophenone-2

A study was performed, using groups of 10 male Wistar rats, to determine apoptosis and oxidative stress markers in the rat brain after topical administration of Benzophenone-2.[33] The markers studied were: active form of caspase-3, pro-apoptotic protein (Bax), and anti-apoptotic protein (Bcl-2). The effect of dosing on these markers was studied to determine whether Benzophenone-2 may be involved in the induction or exacerbation of neurodegenerative changes. Benzophenone-2 was dissolved in a small amount (volume not stated) of ethanol and olive oil, and formulated with Hascobase. The test substance was then applied to shaved skin at a dose of 100 mg/kg for 4 wk. Hascobase with a small amount of ethanol and olive oil was applied to the skin of control rats. In the hippocampus, where the Benzophenone-2 concentration was ~3.5-fold lower than in the frontal cortex, no statistically significant changes in oxidative stress and apoptosis markers were observed. In the frontal cortex, there was no change in apoptosis markers, but, unexpectedly, the oxidative stress markers were reduced. The authors concluded that Benzophenone-2 did not exacerbate oxidative stress and apoptosis markers in the hippocampus and frontal cortex. However, it did lower oxidative stress in the frontal cortex.

Benzophenone-2 and Benzophenone-3

The effect of Benzophenone-2 and Benzophenone-3 on the neuroblastoma (SH-SY5Y) cell line was evaluated by studying effects on cell viability and caspase-3 (main executive enzyme in programmed cell death) activity).[97] The MTT reduction test and LDH release activity assay were used. After a 72-h incubation period, both Benzophenone-2 and Benzophenone-3 produced a statistically significant cytotoxic effect at concentrations of 10 µM and 100 µM in both assays. Additionally, both Benzophenone-2 and Benzophenone-3 caused an increase in caspase-3 activity at much lower concentrations (from 0.01 µM to 0.1 µM). The authors noted that the results of this study indicate that Benzophenone-2 and Benzophenone-3 adversely affected the viability of nerve cells, most likely by enhancing the process of apoptosis.

Benzophenone-3

The toxicity of Benzophenone-3 to primary cortical neurons and primary cortical astrocytes (cultured from E17 and E19 rat fetuses) was studied.[34] Cultures were treated with the following 3 concentrations at culture durations of 24 h, 48 h, and 7 d: 0.1 µg/ml, 1 µg/ml, and 10 µg/ml. Cell viability was analyzed using the standard MTT assay. The experiments were performed in triplicates on a minimum of 3 independent cultures. Untreated cultures served as controls. No significant

differences in astrocyte viability were observed for a 24-h or 48-h exposure when compared to the control group. A 36% decrease in neuron viability was observed when cultures were exposed to Benzophenone-3 (10 µg/ml) for 7 d.

A study was performed to determine the effects of Benzophenone-3 on apoptosis and the expression of estrogen, androgen, and arylhydrocarbon receptors (AhR) in the rat frontal cortex and hippocampus.[98] The test substance was administered dermally to pregnant female Sprague-Dawley rats and to their male offspring through 6 and 7 wk of age. Benzophenone-3 (in a cream) was applied to a 25 cm^2 (5 cm x 5 cm) area on the back, at a dose of 100 mg/kg, twice daily. After birth, the offspring were observed for any abnormalities daily. The animals were killed at 24 h after the last dose of Benzophenone-3. Brain structures (hippocampus and frontal cortex) were removed. Benzophenone-3 in the frontal cortex induced the mitochondrial apoptosis pathway by increasing the active forms of caspase-3 and caspase-9, thereby inducing the pro-apoptotic proteins Bax and Bak and increasing the number of cells with apoptotic DNA fragmentation. In the hippocampus, an increase in caspase-9 and a downward trend in the level of anti-apoptotic proteins were observed. In both regions of the brain, the contents of estrogen receptor beta (Erβ) in the nuclear fraction and G protein-coupled receptor 30 (GPR30) in the membrane fraction were statistically significantly reduced. Benzophenone-3 statistically significantly increased AhR in the cytosol of the frontal cortex, but had no effect on the content of this receptor in the hippocampus. The authors noted that the results of this study indicate that exposure to Benzophenone-3 induces the mitochondrial apoptosis pathway in the rat frontal cortex.

Mouse neuronal cells (from neocortical and hippocampal tissues prepared from Swiss mouse embryos) were used to evaluate the neurotoxicity of Benzophenone-3 (in DMSO).[99] Primary neuronal cell cultures were exposed to Benzophenone-3 (1 to 100 µM) for 24 h. A continuous 24-h exposure of neocortical cultures to Benzophenone-3 (25 to 100 µM) induced apoptosis in mouse neuronal cells. Hippocampal cells exhibited weaker vulnerability.

The neurotoxicity of Benzophenone-3 and its metabolite (Benzophenone-1) was studied using female Sprague-Dawley rats and their offspring.[35] Benzophenone-3 (10% in cream; dose = 100 mg/kg) was administered dermally (shaved skin on back) twice daily to adult female rats (number not stated) during the prenatal period and adulthood. Control female rats were treated with cream without Benzophenone-3. At 21 d after birth, the offspring (male and female) were divided into groups of 5 males and groups of 5 females. From 43 to 56 d of age, the test substance was administered dermally to the male offspring. Cream without Benzophenone-3 was applied to control offspring. In brain structures, selected markers of brain damage were measured. Though the neurotoxicity of Benzophenone-3 and its metabolite (Benzophenone-1) were to have been studied, it was noted that Benzophenone-1 was not identified in structures of the brain. Thus, results relating to neurotoxicity are reported for Benzophenone-3 only. In structures of the brain, results indicated that dosing with Benzophenone-3 raised oxidative stress and induced apoptosis in the brain. Benzophenone-3 increased the concentration of extracellular glutamate in examined brain structures and changed the expression of glutamate transporters. The results of this study indicated that dermal Benzophenone-3 exposure may cause damage to neurons that might be associated with the increase in the level of extracellular glutamate. The authors noted that this increase is most likely evoked by changes in expression of the glutamate transporters, glutamate transporter-1 (GLT-1) and cystine/glutamate antiporter (xCT).

Behavioral Toxicity

Benzophenone-3

In a study performed to characterize the skin permeation and tissue disposition of Benzophenone-3 (in ethanol) in rats (groups of 10; 5 males and 5 females per group)., behavioral toxicity was also assessed[34] The test solution was applied (volume = 100 µl; dose = 5 mg/kg (312.5 µg/cm^2)) topically to a 4 cm^2 area on the back, daily for 30 d. (Results relating to skin permeation and tissue distribution are included in the section on Skin Penetration.) In this study, various behavioral testing protocols were used to assess the arousal (open field tests), locomotion (open field and ladder test), habituation (open field test), and motor coordination (open field and ladder test) of the animals over the study duration. Each rat was tested individually, 4 h after dosing on day 29, to assess behavioral changes from the topical applications. Except for positive controls, all animals (test and negative (saline and vehicle (70% ethanol solution) control groups) passed the 29-d study period without significant adverse effects. Visible impairment was observed in the positive control (acrylamide) group.

Immunomodulatory Effects

Benzophenone-2

The in vitro effect of Benzophenone-2 on the production of interferon (IFN)-γ and interleukin (IL)-10 was studied.[100] IFN-γ and IL-10 are two cytokines representing the Th-1 lymphocyte and Th-2 lymphocyte response, respectively, by activated murine splenocytes. Splenocytes were cultured in the presence of different concentrations of Benzophenone-2 (0.01 to 10 µM). Benzophenone-2 (10 µM) shifted the Th1/Th2 balance toward a Th2 response (lower IFN-γ production and higher IL-10).

Benzophenone-2 (in ethanol) was administered dermally (100 mg/kg), twice daily for 4 wk, to 10 male Wistar rats.[101] Immunological parameters were assayed 24 h after the last administration. Dosing with Benzophenone-2 did not change relative weights of the spleen and thymus, and was not toxic to splenocytes and thymocytes. However, dosing did increase the proliferative activity of splenocytes, and also enhanced the metabolic activity and viability of splenocytes and thymocytes.

Benzophenone-4

The immunosuppressive activity of Benzophenone-4 (0.01%) was evaluated using human dendritic cells (e.g., CD14+ human monocytes).[102] Cytokines can be released by dendritic cells and regulate the activation of T cells. The culturing of monocytes with Benzophenone-4 (0.01%) did not induce significant morphological changes and did not impair monocyte differentiation. The monocytic marker CD14 was unchanged. The effect of Benzophenone-4 (0.01%) on the expression of surface molecules that are critical for dendritic cell function was also investigated. Immature and mature dendritic cells were cultured with Benzophenone-4 (0.01%). Immature dendritic cells generated with or without the test substance showed a similar expression profile. In mature dendritic cells, treatment with the test substance led to down-regulation of HLA-DR (major histocompatibility complex (MHC) molecule) and CD40 (cell surface receptor that belongs to tumor necrosis factor receptor family) expression. Benzophenone-4 treatment also slightly decreased the secretion of IL-12, but this did not reach statistical significance. Treatment with Benzophenone-4 did not impair the proliferation of lymphocytes. Thus, in this study, Benzophenone-4 modulated the phenotype and function of monocyte-derived dendritic cells. CD40 expression was reduced by Benzophenone-4. All of these features suggest that the treatment of dendritic cells with Benzophenone-4 favors an immature activation status that can regulate T cell responses.

Endocrine Activation

Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-4, and Benzophenone-8

A study was performed to investigate the thyroid-activation potential of benzophenones, using a rat pituitary carcinoma cell line (GH3 cell line) and a rat thyroid follicular cell line (FRTL-5 cell line).[103] Also, zebrafish (*Danio rerio*) embryo exposure (up to day 6 post-fertilization) involved the benzophenones (Benzophenones-1, -2, -3, -4, and -8) that were identified based on the transcriptional changes that were observed in the cells. The test concentrations in GH3 cells were as follows: Benzophenone-1 (up to 6.9 mg/l (32 µM)), Benzophenone-2 (up to 2.5 mg/l (10 µM)), Benzophenone-3 (up to 22.8 mg/l (100 µM)), Benzopheone-4 (up to 98.7 mg/l (320 µM), and Benzopenone-8 (up to 24.4 mg/l (100 µM)). In FRTL-5 cells, the test concentrations were: Benzophenone-1 (up to 68.6 mg/l), Benzophenone-2 (up to 78.8 mg/l), Benzophenone-3 (up to 73 mg/l), Benzophenone-4 (up to 98.7 mg/l), and Benzophenone-8 (up to 24.4 mg/l). The test concentrations in zebrafish embryos were: Benzophenone-1 (up to 1000 µg/l), Benzophenone-3 (up to 320 µg/l), and Benzophenone-8 (up to 320 µg/l). Results indicated that, in GH3 cells, Benzophenone-1 (1 to 32 µM), Benzophenone-2 (0.32 to 10 µM), Benzophenone-3 (at doses around 32 µM), and Benzophenone-8 (at doses around 32 µM), but not Benzophenone-4, statistically significantly down-regulated the *Tshβ*, *Trhr*, and *Trβ* genes. For Benzophenone-4 (concentration not stated), slight but significant down-regulation was observed only for the *Trβ* gene. Additionally, some of the benzophenones (Benzophenones -1, -2, -3, and -4 (10 to 320 µM; Benzophenone-8 (3.2 to 100 µM) statistically significantly upregulated the *Nis* and *Tg* genes, while down-regulating the *Tpo* gene in the FRTL-5 cells. Zebrafish larvae treated with Benzopheonone-3 and Benzophenone-4 had a statistically significant decrease in triiodothyronine (T3) levels, but not thyroxine (T4) levels, at test concentrations as low as 32 µg/l. However, Benzophenone-1 statistically significantly decreased both T3 and T4 levels in fish larvae at 320 and 1000 µg/l. The up-regulation of the *dio1* and *ugtr1ab* genes in the zebrafish suggests that decreased thyroid hormones are caused by changing metabolism of the hormones. The results of this study indicate that benzophenones can alter thyroid hormone balances by influencing the central regulation and metabolism of hormones.

Benzophenone-2

The endocrine activation potential of Benzophenone-2 was evaluated using groups of 11 ovariectomized adult Sprague-Dawley rats.[104] The test groups were dosed orally (by gavage) with 250 mg/kg and 1000 mg/kg Benzophenone-2 (1 ml) daily for 5 d. Another group was dosed with E2 valerate (600 µg/kg) according to the same procedure. Control animals were dosed with olive oil. Dosing was initiated 14 d after ovariectomy. Average food intake was significantly reduced during the treatment period. However, there were no differences in liver, spleen, nor adrenal weights between test and control groups. Dosing with E2 valerate resulted in significantly increased uterine weight. Both doses of Benzophenone-2 also had this effect on the uterus. Blood luteinizing hormone levels were statistically significantly reduced after dosing with E2 valerate and 1000 mg/kg Benzophenone-2. There was no evidence of changes in mRNA levels of gonadotropin releasing hormone in the preoptic area of the hypothalamus. A dose-dependent suppression of T4 concentration by Benzophenone-2 was observed. T3 levels were also reduced.

A dose-response experiment involving 5 doses (10, 33, 100, 333, or 1000 mg/kg) of Benzophenone-2 was performed using female Sprague-Dawley adult, ovariectomized rats (groups of 5).[38] Doses were administered (by gavage) once per day for 5 d. Free levels of Benzophenone-2 in rat serum were sufficient to induce an unequivocal estrogen-like effect in the uterus. When compared to the vehicle (olive oil) control group, mean uterine weight was increased statistically significantly in the 333 mg/kg and 1000 mg/kg dose groups. A similar study (groups of 12; same doses and protocol) involved ovariectomized rats of the same strain.[105] E2 valerate served as the positive control. None of the animals showed clinical signs of toxicity. Benzophenone-2 exerted an estrogenic effect on the following uterine parameters at the administered doses: wet weight, complement protein 3 (C3), insulin-like growth factor (IGF1), and estrogen receptor β (Erβ) gene expression. According to results from another study, Benzophenone-2 acts as a n Erα and Erβ agonist mimicking the effects of E2 benzoate.[106]

Benzophenone-2 was evaluated for its effect on the hypothalamic-pituitary-thyroid (HPT) axis.[101] The test substance was dissolved in a small amount (volume not stated) of ethanol and olive oil and formulated with Hascobase. The test substance was then applied to shaved skin of 10 male Wistar rats at a dose of 100 mg/kg for 4 wk. HPT activity was increased, i.e., the level of thyroid-stimulating hormone (TSH) was reduced and the free fraction of T3 and T4 in the blood was increased.

Benzophenone interference with the thyroid hormone axis was studied.[107] Whether or not Benzophenone-2 inhibits key reactions of thyroid hormone biosynthesis catalyzed by thyroid peroxidase was examined in this study. A novel in vitro assay, based on human recombinant thyroid peroxidase stably transfected into the human follicular thyroid carcinoma cell line FTD-238, was used. Benzophenone-2 (300 nmol/l) combined with the thyroid peroxidase substrate hydrogen peroxide (10 µmol/l) inactivated human recombinant thyroid peroxidase.

Benzophenone-2 interference with thyroid function was also studied in vivo.[107] Groups of 12 adult female Sprague-Dawley rats were bilaterally ovariectomized and fed a soy-free diet containing iodide ad libitum. At 14 d after ovariectomy, groups of 12 rats were dosed orally (by gavage, once per day) with Benzophenone-2 at the following doses (dose volume = 1 ml): 10 mg/kg, 33 mg/kg, 100 mg/kg, 333 mg/kg, and 1000 mg/kg. The animals were killed at day 5, and thyroid glands were excised. A dose-dependent decrease in total serum T4 levels was observed, with statistically significant alterations at doses of 333 mg/kg and 1000 mg/kg. The small decrease in total T3 was not statistically significant. TSH levels were increased at doses of 333 mg/kg and 1000 mg/kg, and this increase was statistically significant at both doses. Thyroid peroxidase activities in the thyroid glands of treated animals were measured ex vivo, but no statistically significant dose-dependent changes were observed. In the livers of animals treated with 1000 mg/kg Benzophenone-2, type I 5'-deiodinase activity was decreased, and this decrease was statistically significant. However, an increase in type I 5'-deiodinase activity was observed at a dose of 33 mg/kg.

Benzophenone-3

The effect of a sunscreen containing Benzophenone-3 (10%) on thyroid function was studied using 32 subjects (15 men and 17 women).[108] The product was applied daily as a whole-body topical application (2 mg/cm^2) in 1 week. The daily amount of cream applied over 4 d was 40 ± 3 g (mean value for men) and 35 ±3 g (mean value for women). Hormone levels were measured by commercially available automated immunoassay systems. No biologically significant effects on hormone levels were observed. This indicates that absorbed Benzophenone-3 was not capable of disturbing the homeostasis of thyroid hormones in adult humans. There was no effect on TSH levels, and there was no increase in the level of T4 or T3 in males or females.

The estrogenic activity of Benzophenone-3 was evaluated in a reporter gene assay using the human cervical epithelioid HeLa cell line as the host cell line for the generation of stable reporter cells for screening substances that act via human estrogen-receptor-alpha (hERα) and β (hERβ).[109] The following 3 reporter cell lines (all estrogen receptor cell lines) were used: HELN, HELN Erα, and HELN Erβ. HELN Erα and HELN Erβ cell lines exhibited transactivation of luciferase gene expression by E2. Luciferase (served as the reporter) assays were performed at concentrations between 0.1 µM and 10 µM. Cells were incubated with Benzophenone-3 for 16 h. Benzophenone-3 activated Erα moderately and had almost no effect on Erβ. Benzophenone-3 was not considered estrogenic at 10 µM.

The effect of Benzophenone-3 on the secretory and proliferative activity of rat (adult female Wistar rats) adrenocortical cells was investigated in vitro.[110] Within 120 min of culture, Benzophenone-3 (1 x 10^{-6} µM to 0.01 µM) stimulated basal corticosterone production from dispersed adrenocortical cells. The chronic, 24-h exposure to Benzophenone-3 (0.0001 µM) increased basal corticosterone secretion from cultured adrenocortical cells. The proliferative activity of the cultured adrenocortical cells was unaffected by treatment with Benzophenone-3.

Benzophenone-3 was evaluated for estrogenic potential, both in vivo and in vitro.[111] In MCF-7 breast cancer cells incubated for 6 d, Benzophenone-3 increased cell proliferation, with a median effective concentration (EC_{50}) between 1.56 and 3.73 µM. In the uterotrophic assay, immature Long-Evans rats (ages not stated) received Benzophenone-3 in powdered feed for 4 d. An increase in uterine weight (weak effect, active at dose of 1525 mg/kg/d) was reported.

The estrogen/antiestrogen and androgen/antiandrogen effects of Benzophenone-3 were evaluated using *Saccharomyces cerevisiae* strains BLYES and BLYAS.[73] Concentration-response curves were fitted by nonlinear regression. In the estrogen assay, an EC_{50} (half maximal effective concentration) value of 6.44 µM was reported for Benzophenone-3. In the androgen assays, Benzophenone-3 did not increase the bioluminescence of the BLYAS strain. Thus, the androgenicity of Benzophenone-3 was not proven. In anti-estrogen assays, Benzophenone-3 showed a sigmoidal concentration-response curve. In antiandrogen assays, the EC_{50} value for Benzophenone-3 was 10.2 µM. The results of this study indicate that Benzophenone-3 has estrogenic and anti-androgenic potential.

Effect on Hematological Parameters

Benzophenone-2

Benzophenone-2 was also evaluated for its effect on hematological parameters.[101] The test substance was dissolved in a small amount (volume not stated) of ethanol and olive oil and formulated with Hascobase. Benzophenone-2 was then applied

to shaved skin at a dose of 100 mg/kg for 4 wk. Dosing with Benzophenone-2 had no effect on the following: leukocyte count, erythrocyte count, platelet count, erythrocyte morphology, and erythrocyte hemoglobin content.

Cytotoxicity

Benzophenone-3

The cytotoxicity of a sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3 was evaluated using the L929 fibroblast (murine) cell line.[112] The nanocapsules contained poly(Ɛ-caprolactone), carrot oil, and a non-ionic surfactant. Cell viability was studied using the MTT assay for the assessment of cell metabolic activity. The nanocapsules were seeded at a concentration of 30 µg/ml. Non-loaded (blank) and Benzophenone-3-loaded nanocapsules did not exhibit metabolic changes or cell death in the cell culture. Cell viability above 70 wt % was recorded (91.12 wt % for non-loaded and 89.45% for Benzophenone-3-loaded nanocapsules). It was noted that these data indicate that the sunscreen formulation was non-cytotoxic.

Photoprotective Effect

Benzophenone-3

The photoprotective effect of Benzophenone-3 (in vehicle consisting of isopropyl myristate and SD alcohol) against UVA radiation was evaluated using 30 female Hartley albino guinea pigs.[113] Applications were made to the dorsal lumbar area (depilated skin). The erythema grade increased with increasing concentrations of Benzophenone-3. At the vehicle control site, a mean erythema grade of 1.5 ± 0.11 was reported. Concentrations of 0.1% and 0.3% produced erythema grades greater than 1+, and provided very little photoprotection. Significant photoprotection was noted after the application of 1%, 3%, and 6% solutions ($p \leq 0.01$, 0.001, and 0.001, respectively), with erythema grades less than 1+ for the latter two treatments. The 6% solution resulted in greater photoprotection than the 3% solution ($p \leq 0.001$).

Phototoxicity Mechanism

Benzophenone-3 and Benzophenone-8

Benzophenone-3 (10 µM) significantly increased phosphodiesterase 4B (PDE4B) expression UVB (20 mJ/cm^2)-irradiated normal human keratinocytes (from neonatal foreskins) in vitro.[114] PDE4B has a well-established role in inflammatory responses in immune cells. Additionally, upon UVB irradiation, Benzophenone-3 upregulated the expression of pro-inflammatory factors such as prostaglandin endoperoxide synthase 2, tumor necrosis factor α, IL-8, and S100A7. Benzophenone-3 downregulated the level of cornified envelope associated proteins, which are important in the development of the epidermal permeability barrier. Benzophenone-8 (10 µM), which shares the 2-hydroxy-methoxyphenyl methanone moiety with Benzophenone-3, also upregulated PDE4B expression in normal human keratinocytes. The Benzophenone-3 and UVB co-stimulation-induced PDE4B upregulation and its association with the upregulation of pro-inflammatory mediators and the downregulation of epidermal differentiation markers were confirmed in a reconstituted three-dimensional human epidermis model. The authors concluded that PDE4B has a role in the mechanism of Benzophenone-3-induced phototoxicity.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies summarized below are described in Table 10. (Data from the previous benzophenones reports are not included in the table.)

Irritation

In Vitro

The hen's egg-chorioallantoic membrane test (HET-CAM) was used to evaluate the irritation potential of a sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3.[112] The nanocapsules contained poly(Ɛ-caprolactone), carrot oil, a non-ionic surfactant, and Benzophenone-3 (0.005 wt%). The formulation was non-irritating to the embryonated hen's egg membrane. The dermal corrosion potential of Benzophenone-4 was determined using a three-dimensional human epidermis model,.[7] Approximately 25 mg of solid test article was evenly applied to the apical surface of each tissue. The exposure period for the test article was up to 60 min, and the MTT assay was performed on exposed tissue samples. Benzophenone-4 was considered corrosive to the skin.

Animal

At concentrations up to 16%, Benzophenones-1, -4, and -6 were non- to minimally irritating, and Benzophenone-11 was non-irritating, to rabbit skin.[1] Benzophenone-2 and Benzophenone-3 (both at 100%) were non-irritating to rabbit skin. Benzophenone-9 was non-irritating to rabbit skin at concentrations up to 10.72%. Results from a cumulative skin irritation test indicated that Benzophenone-4 was capable of causing minimal irritation in rabbits at a concentration of 10%.

A sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was tested in a study involving 24 Wistar albino rats (12 males, 12 females).[69] The formulation (2000 mg/kg) was applied to a 2" x 2", 4-ply gauze pad, and the patch was applied to hairless, dorsal skin for 24 h. There were no signs of erythema or edema. The skin irritation potential of this

sunscreen formulation (0.6% to 0.9% Benzophenone-3) was also evaluated using 6 male New Zealand rabbits [69] The formulation was applied for 72 h to a 25 cm^2 area of dorsal skin, using a 2" x 3", 4-ply gauze pad. There was no evidence of erythema or edema. Benzophenone-8 was evaluated for skin irritation potential using 3 New Zealand white rabbits.[8] The test substance (0.5 g in water (0.5 ml)) was applied to the skin for 4 h using a semi-occlusive patch. Benzophenone-8 was classified as a non-irritant. A skin irritation test on Benzophenone-12 (ground to fine powder) was performed using 3 male New Zealand white rabbits.[5] The test substance was applied (0.5 g, abraded and intact skin of back) for 4 h under an occlusive patch. Benzophenone-12 was classified as non-irritating to the skin.

Human

Benzophenones-1, -2, -3, and -6 were nonirritating to the skin of human subjects at concentrations up to 16%.[1] Benzophenone-1 and Benzophenone-6 were also nonirritating at a much higher concentration of 100%. Benzophenone-4 was irritating at a concentration of 16% in one test, but nonirritating at concentrations of 5% and 25% in other tests. Benzophenone-11 was also irritating at a concentration of 16%, but nonirritating at 4%, 8%, or 20%. Benzophenone-3 and Benzophenone-12 were nonirritating at a concentration of 25%, but mild to no irritation was observed at a lower concentration of 3% Benzophenone-3. Benzophenone-8 was irritating at a concentration of 25%, but nonirritating at 2%. Benzophenone-9 was non-irritating at concentrations up to 10.72%.

The frequency of irritant reactions to Benzophenone-4 was studied using 80 subjects.[115] Benzophenone-4 was tested on each subject at concentrations of 2%, 5%, and 10% in petrolatum. Each test concentration of Benzophenone-4 (20 µl) was applied to an 8-mm diameter Finn chamber. Patches were applied for 2 d to the upper back. Benzophenone-4 (5% in petrolatum) induced skin irritation in 4 subjects. Benzophenone-4 (10% in petrolatum) induced skin irritation in 6 subjects.

Sensitization

In Vitro

The in vitro antioxidant response element (ARE)-nuclear erythroid 2-related factor 2 (Nrf2) Luciferase test method was used to evaluate the skin sensitization potential of Benzophenone-8.[8] The test substance was evaluated at concentrations up to 200 mM in DMSO using the KeratinoSens cell line. Benzophenone-8 was classified as positive in the KeratinoSens assay. The authors stated that further testing is required, having noted that this test is part of a tiered strategy for the evaluation of skin sensitization potential.

Animal

Benzophenone-3 was evaluated for skin sensitization potential using the Kligman guinea pig maximization test.[1] Induction involved intradermal injection of 5% Benzophenone-3 in corn oil or 50% Benzophenone-3 in aqueous Freund's Adjuvant. This was followed by challenge with 2.5% Benzophenone-3 in petrolatum. Results were negative.

The skin sensitization potential of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was tested in a study involving 10 adult male guinea pigs.[69] The formulation was loaded on a 2 cm x 4 cm filter paper that was secured with an occlusive dressing. Observations relating to challenge reactions were assessed after 24 h of the induction, and reactions were scored. The sunscreen formulation was classified as a non-sensitizer. The local lymph node assay was used to evaluate the sensitization potential of Benzophenone-3.[9] Groups of 4 female mice of the CBA strain were used, and the test substance was applied at concentrations of 12.5%, 25%, and 50%. Applications were made to the dorsum of each ear lobe on 3 consecutive days. Benzophenone-3 was classified as a non-sensitizer. The maximization test was used to assess the cutaneous allergenic potential of Benzophenone-12.[116] Ten female albino guinea pigs were tested. The intradermal induction of sensitization in the test group was performed with a 15% dilution of Benzophenone-12. The epidermal induction of sensitization was conducted for 48 h under occlusion with the test substance (at 40% in PEG 300). Two weeks after epidermal injection, the control and test animals were challenged (24 h) with Benzophenone-12 (at 40% in PEG 300). Seven of 9 surviving test animals had sensitization reactions. Benzophenone-12 was evaluated for skin sensitization potential in the maximization test using 20 guinea pigs (10 males, 10 females) of the Pirbright white (Tif:DHP) strain.[5] Benzophenone-12 was applied at a concentration of 5% during the first week of induction, and at a concentration of 30% during the second week. The challenge phase (week 5; i.e., 2 wk after induction) consisted of a single, 24-h application of Benzophenone-12 (20% in petrolatum (w/w)). Results indicated that 65% and 60% of the animals were sensitized to Benzophenone-12 at 24 h and 48 h after challenge, respectively.

Human

Benzophenone-1 was non-sensitizing at a concentration of 1% in human subjects.[1] Evidence of fatiguing, possible sensitization at 5%, and no sensitization at 2.5% were noted after testing with Benzophenone-2. Benzophenone-3, Benzophenone-4, and Benzophenone-11 were non-sensitizing at a concentration of 10%. Benzophenone-3 was also non-sensitizing at 3% in one test, but minimum sensitization at this concentration was observed in another test. Benzophenone -4 also did not induce sensitization at a concentration of 5%. Benzophenone-11 was a non-sensitizer at a higher concentration of 20%. Benzophenone-8 induced skin sensitization at a concentration of 10%, but not at 2%. At a concentration of 100%, Benzophenone-6 did not induce sensitization.

Photosensitization/Phototoxicity

Animal

Benzophenone-8 (3%) and Benzophenone-3 (6%) were non-phototoxic in guinea pigs and rabbits, respectively.[1]

Human

Benzophenone-2, Benzophenone-3, and Benzophenone-4

Cosmetic products containing Benzophenones-2, -3, or -4 (0.l% to 3.5%) were evaluated for phototoxicity using human subjects.[1] *Products containing Benzophenones-2, -3, and -4 were non-phototoxic in all studies; however, a number of subjects experienced slight irritation (usually a 1 + response) to the test material. Cosmetic products containing up to 3.5% Benzophenone-3 were tested for photoallergenicity potential in human subjects. The products were non-photoallergenic in all studies; however, a number of subjects experienced irritation or sensitization to the test material.*

OCULAR IRRITATION STUDIES

Ocular irritation studies summarized below are described in Table 11. (Data from the previous benzophenones reports are not included in the table.)

In Vitro

The ocular irritation potential of Benzophenone-4 was evaluated using the MatTek EpiOcular™ model (normal human-derived keratinocytes in the 3-dimensional human tissue model).[7] Tissues were exposed to Benzophenone-4 (solid, 50 mg) for ~ 6 h, and Benzophenone-4 was classified as irritating to the human eye. An ocular irritation study on Benzophenone-8 was performed using the bovine corneal opacity and permeability test.[8] Corneas from 3 animals were exposed to the test substance (20% w/v in paraffin oil) for 4 h, and Benzophenone-8 was not a severe irritant or corrosive agent.

Animal

Most of the ocular irritation tests indicated that Benzophenones-1, -2, -3, -6, -9, -11, and -12 were non-irritating to the eyes of rabbits.[1] *Some studies indicated that Benzophenones-1, -2, and -4 were slightly to moderately irritating at 100% concentration; however, Benzophenones-1 and -2 were nonirritating when tested at 16% in dimethyl phthalate (DMP) or petrolatum. Although Benzophenone-4 was irritating at concentrations of 8% and 16% in DMP or petrolatum, it was nonirritating when tested as a 5% solution in water. Whereas one study indicated that Benzophenone-11 (5% in DMP) was slightly irritating, another revealed that 16% Benzophenone-11 in DMP was nonirritating.*

The ocular irritation potential of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was studied using 3 adult New Zealand albino rabbits.[69] There were no signs of gross toxicity or adverse effects, and the formulation was classified as practically non-irritating to the eye. Benzophenone-12 (undiluted) was evaluated for ocular irritation potential using 6 New Zealand white rabbits.[5] Test substance (0.1 g) instillation yielded no evidence of ocular irritation.

CLINICAL STUDIES

Retrospective and Multicenter Studies

The retrospective and multicenter studies summarized below are described in Table 12.

Benzophenone-3

Patients (age range: 3 to 96 yr) with suspected allergic contact dermatitis were evaluated and then patch tested by 12 North American Contact Dermatitis Group (NACDG) dermatologists with a screening series of 50 allergens.[117] Of the 4094 patients patch tested with 3% Benzophenone-3, 0.5% had allergic reactions. An NACDG study that was performed involved 5800 patients who were patch tested with Benzophenone-3 (3% in petrolatum).[118] The incidence of positive reactions was 0.6%. The relevance of this incidence of positive reactions was classified as follows: 20.6% (definite relevance), 50% (possible relevance) and 2.9% (past relevance). Data from 64 allergenicity studies (between 1992 and 2006) were aggregated and analyzed.[119] This was done in order to evaluate the irritation and sensitization potential of sunscreen products containing Benzophenone-3 at concentrations between 1% and 6%. The mean rate of contact allergy to Benzophenone-3 was 0.07%. A cross-sectional analysis of patients patch tested by the NACDG between 2001 and 2010 was performed.[120] A frequent allergen in sunscreens was Benzophenone-3, whereby 70.2% of the patients (26 of 37 patients patch tested) had an allergic reaction to 10% Benzophenone-3 (in petrolatum) and 64.4% of the patients (56 of 87 patients patch tested) had an allergic reaction to 3% Benzophenone-3 (in petrolatum). NACDG patch testing results from January of 2007 to December of 2008 were reported.[121] Standardized patch testing was used at 13 centers in North America. A total of 5085 patients was tested. Possible allergic reactions to Benzophenone-3 (3% in petrolatum) were observed in 0.9% of the patients.

A 3% aqueous solution of Benzophenone-3 was applied to the midback of 4 patients, using Finn chambers.[122] All four patients were photoallergic to Benzophenone-3. Over a 6-year period, 187 patients (76 males, 111 females) with a history of photosensitivity were photopatch tested with 18 allergens, using standard techniques.[123] Testing revealed a total of 37 (20%) photocontact reactions. Careful history taking resulted in a diagnosis of clinically relevant photoallergic contact dermatitis in

54% of the 37 patients or 11% (20) of the total tested. Nine of the relevant responses were due to Benzophenone-3 (2% in petrolatum). Patients with positive photopatch tests to sunscreen agents were retrospectively selected from the database of the contact dermatitis clinic at the Skin and Cancer Foundation in Australia.[124] Nine patients had a positive photopatch test reaction to Benzophenone-3 (10% in white petrolatum). Two patients had positive reactions at non-irradiated sites. A study involving 35 patients (11 men, 24 women) was performed in Argentina to determine the proportion of photosensitive patients with photoallergic contact dermatitis to Benzophenone-3.[125] Patients were patch tested with Benzophenone-3 (10% in petroleum jelly). Photoallergic contact dermatitis was identified in 6 patients (17.14%). Five of these patients (14.28%) had at least one positive reaction to Benzophenone-3 in the photocontact test. Over a 7-year period, 355 consecutive patients with suspected photosensitivity were tested at Swedish dermatology clinics.[126] In 28 of the patients (7.9%), a total of 42 allergic reactions was found. The most common allergen was Benzophenone-3 (2% in petrolatum), with 15 photocontact allergic reactions and 1 contact allergic reaction.

Benzophenone-4

In a study by the NACDG, 4857 patients were patch tested (years 2013 through 2014), and the positive reaction rate for Benzophenone-4 (10% in petrolatum) was 2.1% (100 allergic reactions).[127] The phototoxicity of Benzophenone-4 was studied using 80 subjects.[115] Benzophenone-4 was tested at concentrations of 2%, 5%, and 10% in petrolatum. One subject had a weak positive reaction (+ reaction), with no concomitant erythema score, to Benzophenone-4 (10% in petrolatum) at the irradiated site.

Benzophenone-2, Benzophenone-3, and Benzophenone-4

Twenty-seven patients reported reactions due to sunscreen allergy (itchy bumps and burning).[128] Of these, 11 (10 women, 1 man) patients agreed to photopatch testing. One patient had a delayed-type hypersensitivity photopatch test reaction to Benzophenone-2 (1% in petrolatum), and another patient had a photopatch test reaction to Benzophenone-3 (10% in petrolatum). A retrospective analysis was performed, and involved the reviewing of 1527 charts in the University of British Columbia Contact Dermatitis Clinic patch test database from January of 2009 to July of 2012.[129] Twenty-three of the patients were tested with the sunscreen series at the clinic. Also, all 1527 patients were patch tested with 70 allergens on the NACDG screening series. Benzophenone-3 and Benzophenone-4 were tested at a concentration of 10% in petrolatum. Of the 23 patients tested, 2 had positive reactions (allergic contact dermatitis) to Benzophenone-3 and 1 had a positive reaction to Benzophenone-4. Of the 1527 patients screened (no specific history of sunscreen allergy), 8 patients reacted to Benzophenone-3 in the NACDG series. A total of 5592 patients was patch tested with Benzophenone-4 (10% in petrolatum) in an NADCG study (years 2015 through 2016).[130] Values for the clinical relevance of allergic reactions to 10% Benzophenone-4 (in petrolatum) were: definite relevance (3 of 93 patients (3.2%)), probable relevance (12 of 93 patients (12.9%)), possible relevance (45 of 93 patients (48.4%)), and past relevance (8 of 93 patients (8.6%)). In the same report, 5595 patients were patch tested with Benzophenone-3 (10% in petrolatum). Of the patients patch tested, 24 had an allergic reaction. The British Society for Cutaneous Allergy (BSCA) retrospectively reviewed the results from their facial patch test series over a 2-year period.[131] Of the 1390 patients patch tested with Benzophenone-4 (2% in petrolatum), 0.79% had allergic reactions. Of 4224 patients patch tested with Benzophenone-3 (10% in petrolatum), 0.17% had allergic reactions.

Fifteen patients (4 males, 11 females; mean age = 47.7 years) reacted to sunscreens.[132] Positive patch test (procedure not stated) results were as follows: 4 allergic contact dermatitis reactions to Benzophenone-4, and 2 allergic contact dermatitis and 5 photoallergic contact dermatitis reactions to Benzophenone-3. Four-hundred-two patients (ages not stated) with suspected clinical photosensitivity were patch and photopatch tested with Benzophenone-3 and Benzophenone-4 (each at 10% in petrolatum).[133] Of the 402 patients, there were 3 allergic and 9 photoallergic reactions to Benzophenone-3 and no photoallergic or allergic reactions to Benzophenone-4. Twelve patients with a history of acute eruption on photoexposed areas, induced by ketoprofen or tiaprofenic acid, were patch tested.[134] Photopatch test results were positive for Benzophenone-3 (in 3 of 12 patients) and negative for Benzophenone-4. Photopatch testing (over 2-year period) of Benzophenone-3 and Benzophenone-4 was performed using 1155 patients from 17 centers across the United Kingdom, Ireland, and the Netherlands.[135] Benzophenone-3 (10% in white paraffin) caused photoallergic contact reactions in 27 patients. Benzophenone-4 (5% in white paraffin) and Benzophenone-4 (10% in white paraffin) caused photoallergic contact reactions in 2 and 5 patients, respectively. The following allergic reactions were also reported: 5% Benzophenone-4 (2 patients), 10% Benzophenone-3 (9 patients), and 10% Benzophenone-4 (9 patients). The irritation reactions observed included: 5% Benzophenone-4 (2 patients), 10% Benzophenone-3 (2 patients), and 10% Benzophenone-4 (4 patients).

A study was performed to identify the photoallergens that caused photoallergic contact dermatitis in the population attending an outpatient clinic in Columbia.[136] The study involved 82 patients with a clinical diagnosis of photoallergic contact dermatitis. Benzophenone-3 (concentration not stated) was photoallergenic in 22 of 82 patients (26.8%), and Benzophenone-4 (concentration not stated) was photoallergenic in 2 of 82 patients (2.4%). An investigation of photoallergic contact dermatitis frequency was performed using 347 patients from centers across 12 European countries.[137] Benzophenone-4 (2% in petrolatum) elicited photoallergic contact dermatitis in 3 patients. Allergic contact dermatitis reactions to Benzophenone-3 (10% in petrolatum) were observed in 6 patients. In a retrospective chart review, 160 patients (37 male, 123 female) underwent photopatch testing in Canada between January of 2001 and December of 2010.[138] Benzophenone-3 induced photoallergic reactions in 12 patients, allergic reactions in 17 patients, and both allergic and

photoallergic reactions in 6 patients. Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions. A prospective study was performed to evaluate the frequency and causes of photoallergic contact dermatitis among dermatology outpatients.[139] The study involved 1000 consecutive dermatology outpatients in Poland. Photoallergic contact dermatitis was ultimately confirmed in 15 patients: 7 females and 8 males. Of these, 2 patients had a positive reaction to Benzophenone-3 (10% in petrolatum). One patient had a positive reaction to Benzophenone-4 (2% in petrolatum). The photopatch testing of sunscreens was performed in a study involving 157 children (69 male, 88 female).[140] Tests were performed in a single photo-investigation center during years 2000 to 2011. Benzophenone-3 induced photoallergy in 33% of the children.

Benzophenone-3, Benzophenone-4, and Benzophenone-10

From 1989 to 1991, 214 patients were patch tested to a sunscreen series.[141] Benzophenone-3 and Benzophenone-10 accounted for 27 and 8 positive patch tests, respectively. Over a period of 3 years, 553 patients were patch tested (Finn chambers) with 10% Benzophenone-3, 10% Benzophenone-4, and 10% Benzophenone-10.[142] Thirteen patients and 1 patient had positive reactions to 10% Benzophenone-3 and 10% Benzophenone-10, respectively. Thirteen patients had positive reactions to 10% Benzophenone-4. One patient had a positive reaction to both Benzophenone-3 and Benzophenone-4. A retrospective analysis of positive photopatch test episodes (years 1993 through 1998 in London) was undertaken using results retrieved from the environmental dermatology database, and further verified with the original archived patch test documentation for each individual patient.[143] In 111 patients with positive reactions (4.1%), there were 155 allergic contact or photoallergic reactions to allergens in the photopatch series. The most common UV filter photoallergen was Benzophenone-3 (14 positive results), followed by Benzophenone-10 (9 positive results). Forty-nine patients (1.8%) had a total of 75 allergic contact reactions, 51 due to UV filters. Benzophenone-10 accounted for 13 allergic contact reactions, and Benzophenone-3 accounted for 8 allergic contact reactions.

A study was conducted to determine the threshold UVA elicitation dose in photopatch testing. Twenty-three patients with a variety of photosensitive disorders were patch and photopatch tested.[144] Benzophenone-3 and Benzophenone-10 produced positive responses. Seven patients with ketoprofen-induced photodermatitis were patch tested and photopatch tested with Benzophenone-3, Benzophenone-4, and Benzophenone-10 (test concentrations not stated).[145] All non-irradiated patch test results for the three benzophenones were negative. Four and 2 patients had positive UVA photopatch tests to Benzophenone-3 and Benzophenone-10, respectively. Photopatch test results for Benzophenone-4 were negative. From February 1985 to March 1987, 280 patients with photosensitivity and other patients suspected of sunscreen dermatitis were patch and photopatch tested with a series of contact allergens and photoallergens (test concentration = 2% in petrolatum).[146] During the first 16 months of the study period (February 1985 to May 1986), there were 2 patients who were allergic to Benzophenone-10. In the remaining 10 months, 4 patients were allergic to Benzophenone-10. Photopatch results for Benzophenone-10 were negative.

Case Reports

The case reports summarized below are described in Table 13.

Benzophenone-2

Epicutaneous tests were performed on two patients (both with itching erythema) who had been using nail varnish and nail varnish remover and one patient who had artificial nails (itching erythema at perionychium of several fingers; also marked erythema and edema).[147] The three patients had sensitization reactions to Benzophenone-2, an allergen in nail varnish remover. Symptoms and skin changes disappeared when use of this product was discontinued. A male patient presented with subacute chest and arm eczema after use of a toilet water product.[148] Patch testing with an ingredient of the product, Benzophenone-2 (2% in petrolatum), yielded a positive reaction (++). Reactions were not observed in 15 control subjects. Severe dermatitis (worsened after sun exposure) was observed in a female patient.[149] Patch test results for Benzophenone-2 (1% in petrolatum) were positive (+++ reaction).

Benzophenone-3

Erythema and blistering (at application) were observed after a female patient applied ketoprofen gel topically to the right popliteal fossa and right shoulder.[150] Patch test results for Benzophenone-3 were negative; however, a positive photopatch test reaction to Benzophenone-3 (+++) was reported on day 4. A female patient who applied a sunscreen experienced itching and a burning sensation of the nose, cheeks, and dorsa of the hands after 3 h of direct sun exposure.[151] Patch testing with an ingredient of the sunscreen, Benzophenone-3 (2% in petrolatum), yielded a +++ reaction. Anaphylaxis (with generalized cutaneous wheal and flare reaction) was observed in a female patient after widespread application of a sunscreen to the skin.[152] Blinded patch testing with Benzophenone-3 (sunscreen ingredient, concentration not stated) induced a wheal and flare reaction after 15 min. Non-blinded patch tests for Benzophenone-3 in 2 control subjects yielded negative results. In prick tests, results for the sunscreen and Benzophenone-3 were positive.

An acute, itchy rash was observed on a female patient (face, trunk, and limbs) after application of a sunscreen to her daughter's skin.[153] The patient was patch tested with Benzophenone-3 (concentration not stated), and an acute urticarial wheal and flare reaction was observed. Patch test results for Benzophenone-3 in 5 control subjects were negative. In another

case report, a male patient with dermatitis was patch tested with Benzophenone-3 (3% in petrolatum).[154] The patient had a strong patch test reaction at 48 h and 96 h. A female patient experienced an anaphylactic reaction 15 min after applying a sunscreen all over her body.[155] Generalized wheals were observed. Patch testing with Benzophenone-3 (10% in petrolatum) resulted in an urticarial reaction at the test site. The results of an assay for detection of IgE to Benzophenone-3 were negative.

Benzophenone-3 and Benzophenone-4

Persisting erythema on light-exposed skin was reported in the history of a male patient who had applied sunscreen on several occasions.[156] Photopatch test results were negative for Benzophenone-4. Photopatch test results for Benzophenone-3 were positive (+++) at 72 h. Hand dermatitis was observed in a female hairdresser over a 2-year period.[157] Patch testing with Benzophenone-4 (10% in petrolatum) yielded a positive reaction, but patch test results for Benzophenone-3 were negative. A female patient presented with a 2- to 3-year history of intermittent burning and pruritic facial eczema.[158] Test results were significant for a 2+ photocontact reaction to Benzophenone-3. A questionable photocontact reaction to Benzophenone-4 was reported. There was no reaction to Benzophenone-3 when the site was not irradiated. Immediately after irradiation, urticaria at the Benzophenone-3 photopatch test site was observed. Another case involved a female patient with a 1-year history of perioral itching and erythema, and a 3-d history of erythematous swelling over her face and front of her neck.[158] The patient had a 1+ reaction to Benzophenone-3 at both patch and photopatch test sites. A case of acute facial swelling in a diver has been reported.[159] Subsequent patch testing (standard test) for contact dermatitis yielded a positive reaction to Benzophenone-4 (concentration not stated).

Benzophenone-3, Benzophenone-4, Benzophenone-8, and Benzophenone-10

A female patient presented with eyelid dermatitis for 1 year and facial dermatitis for two months.[160] Patch test were as follows: Benzophenone-3 (++), Benzophenone-4 (+), and Benzophenone-10 (negative results). Face eczema developed in a female patient after use of a cosmetic cream.[161] For Benzophenone-3, positive patch test (++ reaction) and photopatch test (+++ reaction) reactions were reported. For Benzophenone-10, patch test results were negative, but photopatch test results were positive (+++ reaction). A female patient was referred for phototesting and patch testing after recurrent episodes of dermatitis and systemic symptoms.[162] Urticarial reactions to Benzophenone-3, Benzophenone-8, and Benzophenone-10 at test sites were observed. Because of the severe reactions, UVA irradiation was not completed.

Other Clinical Reports

Other clinical reports summarized below are described in Table 14.

Benzophenone-3 (2% to 10%), Benzophenone-4 (1 % to l0%), Benzophenone-8 (2% to 10%), and Benzophenone-10 (0.5% to 10%) have been tested for sunscreen efficacy in large populations of human subjects, and under various sources of UV radiation.[1] In all tests combined, there were no reports of irritancy or phototoxic reaction to these ingredients.

A study was performed to identify association between exposure to potentially endocrine-activating chemicals and the age of menarche in adolescent girls.[163] Data from 1598 participants who had completed the reproductive health questionnaire and laboratory examination for the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) for years 2003 to 2008 were used. The weighted mean age of menarche was 12 years of age, indicating that exposure to Benzophenone-3 was not significantly associated with the age of menarche. The association of Benzophenone-3 with serum total testosterone levels was examined using child and adolescent participants (588 total) in NHANES (2011–2012).[164] Multivariable linear regression was performed to estimate associations between natural log–transformed serum testosterone and quartiles of urinary Benzophenone-3 in male and female children and adolescents. The values for urinary Benzophenone-3 (free or total not specified) in the quartiles were: male children (15.57 ng/ml), male adolescents (20.03 ng/ml), female children (18.31 ng/ml), and female adolescents (35.59 ng/ml). There were no significant associations between testosterone and Benzophenone-3 in male or female children, and no evidence of consistent trends with increasing quartiles of exposure. The influence of Benzophenone-3 and other chemicals on the age of menarche in 200 girls was studied.[165] A log w/v increase in childhood (pre-pubertal) urinary levels of Benzophenone-3 was associated with decreased time to menarche. The influence of Benzophenone-3 and other chemicals on the age of menarche in 200 girls was studied.[165] A log w/v increase in childhood (pre-pubertal) urinary levels of Benzophenone-3 was associated with decreased time to menarche.

The association between maternal urinary phenol concentrations during pregnancy and fetal growth was studied in a population of 476 mothers wo had participated in a birth cohort between 2006 and 2008.[166] An association between urinary Benzophenone-3 and lower abdominal circumference in males was made. A study (cohort of 922 pregnant women) was performed to study the association between prenatal exposure to Benzophenone-3 and gestation age and birth weight.[167] Average Benzophenone-3 urinary concentrations were associated with an increase in gestational age. A study (338 children) for determining an association between urinary phthalates, parabens, and phenols found in personal care products with pubertal timing in girls and boys was performed.[168] No such association relating to urinary Benzophenone-3 was found. Placental weights and birth weights were available for 473 mother-son pairs in a cohort for whom Benzophenone-3 was measured in spot urine samples.[169] A positive association between urinary Benzophenone-3 and both placental weight and child birth weight was observed. A study was performed to examine whether maternal and paternal preconception urinary

concentrations of Benzophenone-3 (e.g., from dietary and personal care product exposure) and other chemicals were associated with the risk of preterm birth among couples attending fertility care.[170] This study included 417 female and 229 male participants in the Environmental and Reproductive Health (EARTH) study who gave birth to 418 singleton infants between 2005 and 2018. No consistent pattern of association was observed for Benzophenone-3 in either parent.

The photoallergenicity of Benzophenone-4 (10% in petrolatum) and Benzophenone-10 (10% in petrolatum) was evaluated using 15 eczematous dermatitis patients.[171] There were no positive reactions to Benzophenone-4 (10% in petrolatum). Three subjects had positive reactions to Benzophenone-10 (10% in petrolatum).

EPIDEMIOLOGICAL STUDIES

The epidemiological studies summarized below are described in Table 15.

A case-control study on idiopathic male infertility and exposure to phenols in the environment was performed.[172] The study involved 877 idiopathic infertile men and 713 fertile controls. There was no evidence for an association between exposure to Benzophenone-3 and idiopathic male infertility. Urinary levels of Benzophenone-3 and the incidence of Hirschsprung's disease were investigated using a total of 423 patients in China.[173] Results indicated a positive association between women identified with medium to high levels of Benzophenone-3 (maximum detection level = 22,800 ppb) in the urine and the incidence of Hirschsprung's disease. A calculation relating to the concentration of Benzophenone-3 in the blood after a 4-h application of a sunscreen product containing 6% Benzophenone-3 is presented at the end of the section on Absorption, Distribution, Metabolism, and Excretion – Human.[52] In this publication, the authors noted that since the embryonic period of neural crest cell migration associated with Hirschsprung's disease does not occur until weeks 5-12 of pregnancy, women can unintentionally expose their fetus to extremely high levels of Benzophenone-3 over time. They noted that the analysis of human exposure levels to Benzophenone-3 from sunscreen use, under normal conditions, demonstrates that enough Benzophenone-3 can cross into the mother's blood, making it available to the fetus at high enough levels that can inhibit migration of neural crest cells during critical embryonic development.

A total of 413 men provided urine and semen samples (years 2005 to 2009), and the relationship between urinary concentrations of benzophenones and semen quality was studied.[174] The following benzophenones were quantified in the urine: Benzophenone-1, Benzophenone-2, Benzophenone-3, and Benzophenone-8. Benzophenone-2 and Benzophenone-8 were associated with changes in semen endpoints, including sperm concentration, sperm viability, motility, sperm head, and morphology. No associations were observed for Benzophenone-1 or Benzophenone-3. A study (215 university students) was performed to examine associations between urinary concentrations of benzophenone-type UV filters and semen quality and reproductive hormone levels.[175] Urinary concentrations of the following benzophenones were measured: Benzophenone-1, Benzophenone-2, Benzophenone-3, and Benzophenone-8. Results were as follows: statistically significant positive association between urinary Benzophenone-1 and Benzophenone-3 concentrations and serum follicle stimulating hormone (FSH) levels; urinary Benzophenone-1 concentration statistically significantly positively associated with testosterone(T)/E2; and urinary Benzophenone-1 concentration negatively associated with inhibin B/FSH ratio.

RISK ASSESSMENT

Dermal

Benzophenone-3

Results from a risk assessment on Benzophenone-3 exposure indicated margin of safety (MOS) values of 42 for whole body sunscreen treatment twice per day over 6 h, and 1307 for face sunscreen treatment twice per day over 6 h.[24] The MOS values are based on an NOAEL of 200 mg/kg/day from a rat oral teratogenicity study.[9] The authors noted that a MOS of >100 is considered acceptable. Regarding the lower MOS value, the authors noted that if personal care products containing Benzophenone-3 at the maximum concentration authorized in the European Union and Australia (10%) would be applied on the total area of the human body (0.5 mg/cm^2 twice daily for 6 h), the MOS value of 42 indicates a possible health risk.

The daily systemic exposure dose and MOS for UV filters was estimated by in vitro permeation studies for the 6-h skin exposure of the face or the whole body in humans to a sunscreen, defined as a silicone-based oil-in-water emulsion containing 10% Benzophenone-3 and 5% ethylhexyl triazone [176] Three in vitro experiments were performed using a full-thickness porcine-ear skin mimicking in-use conditions. Ear skin was obtained from pigs that were approximately 6 months old, and the skin disc was mounted in the diffusion cell. In the first experiment, the sunscreen was spread uniformly onto the diffusion area (2 cm^2), and the exact sunscreen dose was 1 mg/cm^2. This yielded a Benzophenone-3 dose of 100 µg/cm^2 during the 6-h exposure. The receptor chamber was filled with phosphate buffered saline. The second experiment involved a 3-h reapplication (100 µg/cm^2 Benzophenone-3) of the sunscreen to intact skin containing the 100 µg/cm^2 Benzophenone-3 dose (total dose = 200 µg/cm^2 Benzophenone-3). The procedure for the third experiment was the same as in first, except that freshly shaved skin was exposed. The estimated systemic exposure dose of Benzophenone-3 after sunscreen application (at 1 mg/cm^2) for 6 h to the face and whole-body skin was estimated to be 136 mg/cm^2 and 30 mg/cm^2, respectively. Skin shaving increased Benzophenone-3 bioavailability by 1.38-fold. MOS values were estimated according to guidelines applicable for the European Union. For 3 realistic exposure scenarios, MOS values of 48, 34, and 34 for Benzophenone-3 in sunscreen applied to the whole-body indicated some concerns regarding safety for consumers (MOS < 100).

The following safety evaluation (including calculation of the MOS) of Benzophenone-3 was performed by the Scientific Committee on Consumer Products (SCCP).[177]

Benzophenone-3 as a UV-filter in sunscreens up to 6%

Dermal absorption (6% formulation): 9.9% [mean (3.1%) + 2 SD (2 x 3.4%)]
Applied dose (sunscreen): 18 g/d
Typical human bw: 60 kg
NOAEL (oral teratogenicity-rat): 200 mg/kg/d

Systemic exposure dose (SED) = 18.10^3 mg/d x 6/100 x 9.9/100)/60 kg
= 1.78 mg/kg bw/d

MoS = NOAEL/(SED) = 112

Benzophenone-3 as a UV-filter in cosmetics at 0.5% to protect formulations against sunlight

Dermal absorption (2% formulation): 8.0% [mean (4.0%) + 2 SD (2 x 2.0%)]
Applied dose (all cosmetic products): 17.79 g/d
Typical human bw: 60 kg
NOAEL (teratogenicity-rat): 200 mg/kg bw/d

Systemic exposure dose (SED) = ($17.79.10^3$ mg/d x 0.5/100 x 8.0/100)/60 kg
= 0.119 mg/kg bw/d

MoS = NOAEL/SED = 1686

SCCP's opinion on the safety of Benzophenone-3 is stated as follows: SCCP is of the opinion that the use of Benzophenone-3 as a UV-filter up to 6% in cosmetic sunscreen products and up to 0.5% in all types of cosmetic products to protect the formulation does not pose a risk to the health of the consumer, apart from its contact allergenic and photoallergenic potential.

SUMMARY

The safety in cosmetics of Benzophenones-1 to -12 (with the exception of Benzophenone-7) is reviewed in this report; these ingredients are substituted derivatives of a 2-hydroxybenzophenone. These ingredients are reported to function mainly as light stabilizers in cosmetics, but some are also reported to function as sunscreens. In the US, sunscreens are active ingredients in over-the-counter (OTC) drug products, and are not cosmetic ingredients; however, in Europe, sunscreens are classified as cosmetics. All of the benzophenones in this report are soluble in organic solvents. Solubility in water varies from insoluble to soluble (Benzophenone-4).

Substantial changes in ingredient use frequencies are apparent when data from 1983 and 2021 are compared. For example, the use frequency of Benzophenone-2 (299 uses total), which was the highest use frequency reported in the 1983 final report on benzophenones, decreased to a value of 55 in 2021. The use frequency of Benzophenone-4 (240 uses) in the 1983 report increased substantially to a value of 1226 in 2021. Changes in use concentrations are also apparent. Benzophenone-4 had the highest use concentration in both the 1983 report and the current report. In the 1983 report, the maximum use concentration was ≤ 10% in suntan gels, creams and liquids; however, in 2020, Benzophenone-4 was reported as being used at substantially lower concentrations of up to 1.6% in other non-coloring hair preparations (leave-on products).

According to the US FDA proposed rule (no longer in effect) issued in 2007, the following benzophenones were allowed in sunscreens as active ingredients within the concentration specified for each ingredient: Benzophenone-3 (a.k.a. oxybenzone, up to 6%), Benzophenone-4 (a.k.a. sulisobenzone, up to 10%), and Benzophenone-8 (a.k.a. dioxybenzone, up to 3%). In 2019, FDA issued a new proposed rule stating that there are insufficient data for determining that these 3 ingredients are GRASE in OTC sunscreen drug products.

In an in vitro skin penetration study using excised human epidermis, Benzophenone-3 passed through the skin in significant amounts (0.08 g/m^2 or 10% of applied dose). Results from another in vitro study (human skin) indicated that Benzophenone-3 penetrated very quickly in less than 30 min, and that there was no difference in the mean quantity in the stratum corneum at 30 min versus 16 h. For Benzophenone-4, the quantity in the stratum corneum at 30 min was statistically significantly lower at 30 min than at 16 h.

In rats, Benzophenone-2 was detected in the blood, liver, adipose tissue, and in the brain after application to the skin. Metabolism to its sulfate and glucuronide forms was also reported. Results from another rat study indicate that

Benzophenone-3 was also detected in the plasma, liver, and brain after application to the skin, and that Benzophenone-1 was the main metabolite.

After application of Benzophenone-3 (in solution/cream) to the skin of human subjects, it was detected in the stratum corneum and was excreted in the urine. After dermal application of a sunscreen lotion containing Benzophenone-3 to human subjects, Benzophenone-3 was detected in the stratum corneum, but not in the plasma or urine. In another study, a sunscreen containing Benzophenone-3 was applied to human subjects. Some sites were irradiated, whereas others were not. Benzophenone-3 was absorbed and excreted in the urine. Sunscreen application has also resulted in the presence of Benzophenone-3 and the following metabolites in the urine: Benzophenone-1, 2,3,4-trinydroxybenzophenone, and 2,2'-dihydroxymethoxybenzophenone. Other studies have also supported the absorption and excretion of Benzophenone-3 after dermal application. Benzophenone-4 was also detected in the stratum corneum of human subjects after dermal application.

In vitro toxicokinetic studies were performed using human and whole zebrafish embryo cell models. Benzophenone-2 was metabolized into a variety of gluco- and sulfo-conjugated metabolites. When Benzophenone-3 was incubated with rat liver microsomes in the presence of NADPH, the metabolites formed were 2,5-dihydroxy-4-methoxybenzophenone and Benzophenone-1. In a similar experiment, the following Benzophenone-3 metabolites were formed: Benzophenone-1; 2,4,5-trihydroxybenzophenone; 3-hydroxylated benzophenone-3; 5-hydroxylated benzophenone-3; and 2,3,4-trihydroxybenzophenone. In the presence of human liver microsomes and NADPH, Benzophenone-3 was metabolized to Benzophenone-1 and 5-hydroxylated benzophenone-3. Metabolic patterns in the zebrafish models and human hepatic cell line HepaRG shared many similarities, while biotransformation rates in the cell lines MELN (human female cancer (invasive ductal carcinoma) cell line) and T47D-KBLuc (human female cancer (mammary gland breast/duct) cell line) were quantitatively low and qualitatively different.

In a dermal metabolism and disposition study on [^{14}C]Benzophenone-3 involving rats, the absorbed dose was excreted mainly in the urine and feces, with ~3% to 10% of the absorbed dose remaining in the tissues.

When administered orally (gavage) to rats, Benzophenone-2 was metabolized to glucuronide- and sulfate-conjugates. It was suggested that this biotransformation occurs in a first-pass effect in the gut wall or the liver. Following the oral dosing (in corn oil) of rats with Benzophenone-3, it was converted to Benzophenone-1, which was converted to 2,3,4-trihydroxybenzophenone. Benzophenone-3 was also metabolized to 2,2' dihydroxy-4-methoxybenzophenone. In an oral (gavage) metabolism and disposition study involving rats and mice, overall, [^{14}C]Benzophenone-3 was well-absorbed and excreted mainly in the urine. The distribution of Benzophenone-3 in tissues was minimal in rats and mice, and urinary metabolites included: benzophenone-3 glucuronide, Benzophenone-1, benzophenone-1-glucuronide, and benzophenone-1 sulfates. Novel minor dihydroxy metabolites, including 2,5-dihydroxy-4-methoxybenzophenone, were also detected. Results from an oral dosing (dietary) study on Benzophenone-12 involving rats indicated metabolism to its glucuronide conjugate, and that Benzophenone-12 had no bioaccumulation potential.

In human biomonitoring studies, Benzophenones-1, -2, -3, -4, and -8 have been detected in the urine of subjects who had not been dosed with either benzophenone. A metabolite of Benzophenones-1 and -3 (4-hydroxybenzophenone), but not Benzophenone-3, was detected in a study in which human placental samples were evaluated. In other studies, Benzophenone-3 has been detected in amniotic fluid, cord blood, breast milk, adipose tissue, and brain white matter. Furthermore, biomonitoring studies have indicated that Benzophenone-1 is a major metabolite of Benzophenone-3, and that the presence of Benzophenone-3 derivatives in the urine suggests that demethylation was the major route of Benzophenone-3 metabolism.

In an acute dermal toxicity study (rats) on a sunscreen formulation containing 0.6% to 0.9% Benzophenone-3, and LD_{50} of > 2000 mg/kg was reported. In a similar study on Benzophenone-12 involving rabbits, the LD_{50} was > 10,000 mg/kg.

After oral dosing (method not stated), Benzophenone-1 was classified as practically non-toxic (LD_{50} = 8600 mg/kg) in rats. The acute oral (gavage) LD_{50} (rats) for a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was > 2000 mg/kg. An acute oral (gavage) LD_{50} of 3530 mg/kg for Benzophenone-4 was reported in a study involving rats. Acute oral (gavage) dosing of rats with Benzophenone-8 resulted in an LD_{50} of > 2000 mg/kg. An LD_{50} of > 10,000 mg/kg was reported for rats dosed orally (in water) with Benzophenone-12.

In a 2-wk dermal toxicity study involving groups of 10 $B6C3F_1$ mice, dosed topically with Benzophenone-3 (0.5 to 8 mg in alcohol or lotion vehicle), minimal effects (variable increases in liver weight) were reported. In another 2-wk study, groups of 10 F344/N rats received topical applications of Benzophenone-3 (1.25 to 20 mg in alcohol or lotion vehicle). Minimal effects (small and variable increases in liver and kidney weights) were observed. The findings reached statistical significance in the higher dose groups.

In a short-term (2 wk) oral (diet) toxicity study involving $B6C3F_1$ mice, the NOAEL for microscopic lesions was 6250 ppm. The same NOAEL for microscopic lesions was reported in a short-term (2 wk) oral toxicity study involving groups of 10 F344/N rats. In a short-term oral (gavage) toxicity study, groups of 26 Wistar rats were dosed orally with Benzophenone-4 at 2 wk prior to mating and 48 d thereafter. Female rats were dosed orally for a total of 66 d. An NOAEL of 1250 mg/kg/bw/d was reported for males and females. Groups of 6 male rats of the Carworth Farms Elias strain were fed Benzophenone-12 in the diet for 35 d. No significant gross lesions were observed. Following repeated oral (gavage) dosing

of groups of 24 Wistar rats with Benzophenone-12 (0.5% carboxymethylcellulose suspension in drinking water) during a pre-mating period (10 wk for males; 2 wk for females), a 2-wk mating period, and up to 30 d of lactation, an NOAEL of 1000 mg/kg bw/d for general systemic toxicity was determined. Benzophenone-3 (in ointment base, 100 mg/kg) was non-toxic when applied to the skin of groups of 4 to 6 male Sprague-Dawley rats twice daily for 4 wk. In another study, mated female Sprague-Dawley rats received dermal applications of Benzophenone-3 (10% in cream; dose = 100 mg/kg) during the prenatal period and adulthood. Their male offspring subsequently received dermal applications from 43 to 56 d of age. No adverse effects on pregnant females or on the offspring were noted.

In a 90-d oral study (dosing method not stated) involving rats (number and strain not stated), a NOAEL of 236 mg/kg/d was reported for Benzophenone-1. In a 13-wk oral toxicity study involving groups of 20 $B6C3F_1$ mice, a NOAEL of 6250 ppm was reported for Benzophenone-3. When groups of 20 F344/N rats were fed Benzophenone-3 in the diet in this study, the same NOAEL was reported. In another study, groups of 20 Sprague-Dawley rats received 10,000 ppm Benzophenone-3 in the diet for 14 wk. In males, the absolute and relative liver and kidney weights were increased relative to the control group. In females, the absolute kidney weight was significantly decreased, but the relative liver weight was significantly increased relative to the control group.

The embryotoxicity of Benzophenone-3 was evaluated in an in vitro test involving zebrafish embryos. Malformation of the somites was observed at concentrations of 52.6 and 78.9 µM. The number of hatched embryos at 96 h post-fertilization was also decreased.

Pregnant mice were exposed dermally to Benzophenone-3 (50 mg/kg/d) from GD 0 to 6. Dermal exposure resulted in an IUGR phenotype, disturbed sex ratio, and alterations in the growth curve of the offspring. In a 13-wk dermal dosing study on Benzophenone-3 involving groups of 20 $B6C3F_1$ mice, it was not possible to establish a NOAEL for decreased epidermal sperm density due to this effect at doses up to the highest dose of 364 mg/kg.

In a developmental toxicity study involving groups of 5 pregnant C57BL/6NCr mice, oral dosing (gavage) with Benzophenone-2 (6.25 mg) on GD 12 through 17, eight of 57 male fetuses had hypospadias ($p = 0.0064$). In a continuous breeding study involving Swiss CD-1 mice, the animals were fed Benzophenone-3 at concentrations up to 5% during a 7-d precohabitation period and a 98-d cohabitation period. Minimal effects on fertility and reproduction were observed. From pregnancy (day 0) to the day before weaning (lactational day 21), mated BALB/c female mice were dosed orally with Benzophenone-3 (in tocopherol-stripped corn oil) at doses of 0.03, 0.212, and 3 mg/kg/d. The offspring (no less than 9 litters per dose) were exposed in utero and during the first 21 d of postnatal life. Study results suggested that even low doses of Benzophenone-3 can disrupt hormone sensitive organs during critical windows of development.

In an oral dosing study (method not stated), the reproductive toxicity of Benzophenone-1 was evaluated using female rats (number and strain not stated). After 3 d of dosing, a NOAEL of 100 mg/kg/d was reported. The oral dosing of groups of 5 Sprague-Dawley rats with Benzophenone-2 for 5 d caused a statistically significant increase in mean uterine weight at the 2 highest doses of 333 mg/kg and 1000 mg/kg. The same effect for Benzophenone-2 was observed in a study (same protocol) involving groups of ovariectomized rats of the same strain. In groups of 25 mated Wistar rats of the Crl:WI (Han) strain, Benzophenone-3 (in corn oil) was administered orally at doses of 40, 200, and 1000 mg/kg/d on days 6 through 19 post-coitum. The NOAEL for Benzophenone-3 was 200 mg/kg/d. Groups of 25 pregnant Sprague-Dawley rats were fed low-phytoestrogen chow containing 3000 or 30,000 ppm Benzophenone-3 from GD 6 until postnatal day 21. The higher dose caused statistically significantly lower weights of the paired-testis, paired-epididymis, and prostate. There were no changes in the relative weights of the paired epididymis and prostate in either exposure group. There also were no differences in seminal vesicle weight. Groups (7 to 8 animals per group) of mated female Sprague-Dawley rats were fed dietary concentrations up to 50,000 ppm Benzophenone-3 (in low-phytoestrogen chow) from GD 6 until weaning on postnatal day 23. There were no statistically significant differences in the following: mean number of implantation sites/litter, mean resorptions per litter, % litters with resorptions, number and weights of live fetuses, or sex ratios between the control and Benzophenone-3 dose groups.

On GD 6, groups of 42, 35, 35, and 43 F_0 time-mated female rats were fed diets containing 0, 1,000, 3,000, and 10,000 ppm Benzophenone-3, respectively, for 39 d. Dietary concentrations of 1000, 3000, and 10,000 ppm Benzophenone-3 resulted in average daily doses of approximately 70, 206, and 660 mg Benzophenone-3/kg bw/d during gestation, and 157, 478, and 1609 mg/kg/d over lactation days 1 – 14. Groups of 50 (1,000 and 3,000 ppm) or 60 (0 and 10,000 ppm) F_1 rats per sex continued on study after weaning, and were fed diets containing the same exposure concentrations for 105 wk. Benzophenone-3 had no effects on the percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups. In a 13-wk oral dosing study, F344/N rats (10 males and 10 females per group) received diets containing 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm Benzophenone-3. At 50,000 ppm Benzophenone-3, markedly lower epididymal sperm density and an increase in the length of the estrous cycle were observed.

In a study involving groups of 26 Wistar rats (13 males, 13 females/group), Benzophenone-4 was administered orally (in corn oil, by gavage) at doses of 750, 1000, and 1250 mg/kg/d. The NOAEL (reproductive toxicity) for Benzophenone-4 was 1250 mg/kg/d.

Benzophenone-12 (in 0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80) was administered orally to groups of Wistar rats (F_0 animals: 12 males, 12 females/group) at doses of 100, 300, and 1000 mg/kg/d. The duration of treatment was described as follows: 10-wk premating period (males), 2-wk premating period (females), 2-wk mating period (both sexes), ~2 d post-mating (males), entire gestation period, up to 30 d of lactation (corresponding to 21 d of lactation and up to 9 d post-weaning), and 35 d post-mating (for sperm-negative females). The NOAEL for reproductive performance and fertility of the F_0 parental rats and developmental toxicity in the offspring was 1000 mg/kg/d. The same NOAEL was reported in another study in which Benzophenone-12 (in 0.5% carboxymethyl-cellulose suspension in drinking water + 5 mg/100 ml Tween 80) was administered orally at doses of 100, 300, and 1000 mg/kg/d using groups of 50 Wistar rats (25 males, 25 females). The groups were dosed daily, from implantation to one day prior to the expected day of parturition (GD 6 to 19).

Six pregnant albino Swiss mice were injected s.c. with Benzophenone-3 (in peanut oil, 50 mg/kg) once daily for 10 d (from the 7th to 16th day of gestation). Dosing resulted in severe apoptosis and neurotoxicity in neocortical neurons. Thus, Benzophenone-3 can pass through the placenta and blood-brain barriers.

In an in vitro genotoxicity test, micronuclei formation was detected in human keratinocytes treated with Benzophenone-1 (10 µg/ml) in the presence of UVB. In a photogenotoxicity test involving human keratinocytes, Benzophenone-1 photosensitized and generated reactive oxygen species in the presence of sunlight/UV radiation. The in vitro luminescent *umu* test was used to evaluate the genotoxicity of Benzophenone-1, -3, -6, and -8 (doses up to 10 µg/well) in *Salmonella typhimurium* strain TL210. Positive results were reported for Benzophenone-3.

The genotoxicity of Benzophenone-1 (doses up to 600 µg/plate), Benzophenone-3 (up to 200 µg/plate), Benzophenone-6 (up to 2000 µg/plate), and Benzophenone-8 (up to 300 µg/plate) was evaluated in the Ames test using *S, typhimurium* strains TA98 and TA100 (with and without metabolic activation). Results were negative for each benzophenone tested. The genotoxicity of Benzophenone-3 and Benzophenone-8 (each in seawater, 1:10 or 1:1000) was evaluated at doses of 4 to 10 µl per plate using *S, typhimurium* strain TA98 (without metabolic activation). Only Benzophenone-8 (1:10) had clear genotoxic activity that was dose-related (doses of 4, 6, 8, and 10 µl). In another Ames test, a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was not genotoxic in the following *S, typhimurium* strains at a dose of 5000 µg/plate: TA 98, TA100, TA1535 and TA1538. Benzophenone-3 was also evaluated for genotoxicity at doses up to 6000 µg/plate (with and without metabolic activation) using *S. typhimurium* strains TA98 and TA100, and *E. coli* strain uvrA pKM101. Results were negative with and without metabolic activation.

The cytogenetic effect of Benzophenone-3 on human peripheral lymphocytes was evaluated using in vitro chromosomal aberrations and micronuclei assays. Lymphocyte cultures were exposed to concentrations up to 0.2 µg/ml. A concentration-related, statistically significant increase in chromosomal aberrations and aberrant cell frequencies was observed at all test concentrations. Micronuclei assay results were the same. The effect of Benzophenone-3 on DNA damage was studied using human breast epithelial cells. Concentrations of 1 µM and 5 µM Benzophenone-3 increased DNA damage.

Benzophenone-8 (in ethanol) was evaluated at doses of 0.008 to 700 µg/plate in the *Salmonella*/mammalian microsome mutagenicity assay using the following *S, typhimurium* tester strains: TA98, TA100, TA1535, TA1537, and TA1538. With metabolic activation, Benzophenone-8 caused a weak, but reproducibly significant dose-dependent increase in the number of TA1537 revertants per plate. Benzophenone-8 (in ethanol) was tested in the L5178Y TK+/- mouse lymphoma mutagenesis assay (with and without metabolic activation) at concentrations ranging from 13 to 56 µg/ml. With metabolic activation, a significant dose-related increase in the mutant frequencies was observed. A bacterial reverse mutation assay on Benzophenone-8 (in DMSO) was performed using *S, typhimurium* strain TA100 (doses up to 1500 µg/plate) and *E. coli* strain WP2vurA (doses up to 5000 µg/plate), with and without metabolic activation. Results were negative.

The genotoxicity of Benzophenone-12 (in DMSO) in the mammalian cell gene mutation assay using mouse lymphoma L5178Y cells. Doses up to 50 µg/ml and 52 µg/ml were tested with and without metabolic activation, respectively. Results were negative without metabolic activation and ambiguous with metabolic activation.

The genotoxicity of Benzophenone-3 was evaluated using the *Drosophila* somatic mutation and recombination test (SMART). In the SMART assay, larva from a mating of "multiple wing hair" (mwh) females with heterozygous "flare" (flr) males were exposed to 0, 3000, or 3500 mg/kg Benzophenone-3. None of the Benzophenone-3-treated larva produced flies with significantly more single or multiple wing spots than controls. In the same study, an in vivo cytogenetic assay on Benzophenone-3 using rat bone marrow cells was performed. Sprague-Dawley rats were treated with a single dose of 500, 1670, or 5000 mg/kg Benzophenone-3, or five daily doses of 5000 mg/kg/day. Benzophenone-3 did not cause a significant increase in chromosomal aberrations in this assay.

In the mammalian erythrocyte micronucleus test, the genotoxicity of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated using groups of 10 Wistar albino rats. Doses up to 2000 mg/kg were administered dermally for 2 consecutive days, and Benzophenone-3 was classified as non-genotoxic. The same sunscreen formulation (0.6% to 0.9% Benzophenone-3) was evaluated for genotoxicity in the mammalian bone marrow chromosome aberration test using groups of 10 Wistar albino rats. Identical doses were administered according to the same procedure, and

results were negative. Twelve ovariectomized Balb/c female mice were dosed orally with Benzophenone-3 (3 mg/kg/d) daily for 4 d. DNA damage was detected in mammary epithelial cells.

Effects of Benzophenone-1 on the proliferation and metastasis of MCF-7 human breast cancer cells expressing estrogen receptors were studied. It was concluded that Benzophenone-1 may accelerate the growth of MCF-7 breast cancer cells by regulating cell cycle-related genes and promote cancer metastasis through amplification of cathepsin D. In a wound healing assay, Benzophenone-1 (1 μM) statistically significantly enhanced the migration capability of BG-1 ovarian cells by reducing the wounded area in the cell monolayer. It was noted that the results of this study indicate that Benzophenone-1 may have the ability to induce ovarian cancer metastasis. The effect of Benzophenone-3 (concentrations up to 150 μg/l) on cancer cell growth was studied using NCI-H460 lung cancer cells. Results indicated that Benzophenone-3 had a cancer potentiating effect by enhancing anchorage-independent survival and growth of lung cancer cells.

Groups of 50 male and 50 female mice were fed diets containing 0, 1000, 3000, or 10,000 ppm Benzophenone-3 in the diet (equivalent to average daily doses of approximately 113, 339, and 1207 mg Benzophenone-3/kg bw for male mice and 109, 320, and 1278 mg/kg for female mice) for 104 (female mice) or 105 (male mice) wk. There was no evidence of carcinogenic activity in male or female B6C3F1/N mice at exposure concentrations of 1000, 3000, and 10,000 ppm. The oral carcinogenicity of Benzophenone-3 was evaluated in an NTP study using male and female Sprague-Dawley rats and male and female B6C3F1/N mice. On GD 6, groups of 42, 35, 35, and 43 F_0 time-mated female rats were fed diets containing 0, 1000, 3000, and 10,000 ppm Benzophenonoe-3, respectively, for 39 d. Groups of 50 (1,000 and 3,000 ppm) or 60 (0 and 10,000 ppm) F_1 rats per sex continued on study after weaning and were fed diets containing the same exposure concentrations for 105 wk. There was equivocal evidence of carcinogenic activity of Benzophenone-3 exposure in male Hsd:Sprague Dawley SD rats, based on the occurrence of malignant meningiomas in the brain. There was equivocal evidence of carcinogenic activity in female Hsd:Sprague Dawley SD rats, based on the increased incidence of thyroid C-cell adenomas and the increased incidence of uterine stromal polyps.

Groups of female BALB/c mice (number per group not stated) were fed diets with and without Benzophenone-3 (70 mg/kg bw and then were injected daily for 5 d with saline control or E2 (1 μg/injection). Benzophenone-3 was compounded into the diets at 0.75 g/kg chow for pubertal animals and 1.5 g/kg chow for adult animals; each dosage yielded consumption of approximately 70 mg/kg BW/d. Both pubertal and adult BALB/c mice were placed on low fat diet (LFD; 10% kcal fat) or high fat diet (HFD; 60% kcal fat) with and without Benzophenone-3, ovariectomized, allowed time for recovery and clearance of endogenous hormones, and then treated with E2 or control for 5 d. While no Benzophenone-3 effects were seen in the adult mice (data unavailable), the pubertal mice fed HFD plus Benzophenone-3 showed higher mammary gland proliferation (mammary epithelial proliferation) in response to E2 than did mice fed HFD alone. No Benzophenone-3 effects were observed in the absence of E2, and no Benzophenone-3 effects were observed in mice fed LFD.

Female Trp53-null transplanted mice (generated from BALB/c Trp53+/– breeding mice) were randomly assigned into various dietary groups. In the Trp53-null mouse model, fragments of donor mammary epithelium were collected from female BALB/c Trp53-null mice at 8 wk of age, and transplanted into the cleared inguinal mammary fat pads of 3-wk-old female wild type BALB/c mice. The mice were placed on LFD (10% kcal fat) or HFD (60% kcal fat). Benzophenone-3 was compounded into the diets at 0.75 g/kg chow for pubertal animals (3 to 10 wk of age) and 1.5 g/kg chow for adult animals; each dosage yielded consumption of approximately 70 mg/kg BW/d. Benzophenone-3 was protective for epithelial tumorigenesis in mice fed lifelong LFD, while promotional for epithelial tumorigenesis in mice fed adult HFD.

The xenoestrogenic effect of Benzophenone-1 on BG-1 human ovarian cancer cells expressing estrogen receptors and relevant xenografted animal models, when compared to E2, was evaluated. In the in vitro cell viability assay, Benzophenone-1 (0.01 to 10 μM) statistically significantly increased BG-1 cell growth, as did E2. In a second experiment, BG-1 cells (5×10^6) were injected s.c. into the backs of groups of 6 female mice of the BALB/c nu/nu strain. Study results suggested that Benzophenone-1 is an endocrine disrupting chemical that exerts xenoestrogenic effects by stimulating the proliferation of BG-1 ovarian cancer via the estrogen receptor signaling pathway associated with the cell cycle.

A study was performed to evaluate the effects of Benzophenone-1 on prostate cancer progression. Benzophenone-1 increased the viability of LNCaP prostate cancer cells at concentrations of 1 μM and 0.1 μM. In the MTT assay, when the cells were co-treated with Benzophenone-1 (1 μM) and biclutamide (0.001 μM), the cell viability that was increased by Benzopheonone-1 alone was statistically significantly reduced. These results suggest that the proliferative effects of Benzophenone-1 on LNCaP cells was mediated by the androgen receptor signaling pathway.

Benzophenones-1, -3, -6, and -8 were evaluated in the Bhas promotion assay at concentrations ranging from 2 to 100 μg/ml. Bhas 42 cells established from BALB/3T3 cells were used. Results indicated that none of the test substances caused a statistically significant increase in the number of transformation foci (relative to the solvent controls) over the range of concentrations. Thus, promotion activity was classified as negative.

The in vivo antitumor activity of Benzophenone-8 and Benzophenonone-12 was evaluated using a two-stage mouse skin carcinogenesis model. Groups of 15 pathogen-free, female hairless mice of the HOS:HR-1 strain were used, and skin tumors were induced by a single dose of NOR-1 (390 nmol). Each test substance was administered at a concentration of 0.0025% to

mice through drinking water, beginning at 1 week prior to tumor initiation and ending at 1 week after tumor initiation. Benzophenone-8 was a more potent inhibitor of skin tumors than Benzophenone-12.

Benzophenone-2 was applied (10 mg/kg) to the skin of 10 male Wistar rats for 4 wk. Benzophenone-2 did not exacerbate oxidative stress and apoptosis markers in the hippocampus and frontal cortex; however, it did lower oxidative stress in the frontal cortex.

In the neuroblastoma (SH-SY5Y) cell line, Benzophenone-2 and Benzophenone-3 adversely affected the viability of nerve cells, most likely by enhancing the process of apoptosis. Both test substances produced a statistically significant cytotoxic effect at concentrations of 10 µM and 100 µM. In another study (dermal exposure to male offspring of Sprague-Dawley rats), it was noted that exposure to Benzophenone-3 induces the mitochondrial apoptosis pathway in the rat frontal cortex. A 36% decrease in neuron viability was observed when cultures of rat fetal primary cortical neurons were exposed to Benzophenone-3 (10 µg/ml) for 7 d. The authors noted that the results of this study indicate that exposure to Benzophenone-3 induces the mitochondrial apoptosis pathway in the rat frontal cortex. A continuous 24-h exposure of neocortical and hippocampal cultures (from Swiss mouse embryos) to Benzophenone-3 (25 to 100 µM) induced apoptosis in mouse neuronal cells. Hippocampal cells exhibited weaker vulnerability.

The neurotoxicity of Benzophenone-3 and its metabolite (Benzophenone-1) was studied using female Sprague-Dawley rats and their offspring. Benzophenone-3 (10% in cream; dose = 100 mg/kg) was administered dermally (shaved skin on back) twice daily to adult female rats (number not stated) during the prenatal period and adulthood. Results indicated that dermal Benzophenone-3 exposure may cause damage to neurons that might be associated with the increase in the level of extracellular glutamate.

Benzophenone-2 (at 250 and 500 µM) accelerated the conversion of dopachrome (intermediate in melanin biosynthesis) to melanin.

Benzophenone-3 (in ethanol) was applied (volume = 100 µl; dose = 5 mg/kg [312.5 µg/cm^2]) topically to a 4 cm2 area on the back (10 rats), daily for 30 d. Various behavioral testing protocols were used to assess the arousal (open field tests), locomotion (open field and ladder test), habituation (open field test), and motor coordination (open field and ladder test) of the animals over the study duration. No significant adverse behavioral effects were observed.

Murine splenocytes were cultured in the presence of different concentrations of Benzophenone-2 (0.01 to 10 µM). Benzophenone-2 (10 µM) shifted the Th1/Th2 balance toward a Th2 response (lower IFN-γ production and higher IL-10). It was noted that these results show that Benzophenone-2 at high doses may possess immunomodulatory effects. The dosing of 10 male Wistar rats with Benzophenone-3 (100 mg/kg) dermally for 4 wk did not have a toxic effect on splenocytes and thymocytes, but increased the activity and function of these cells.

The immunosuppressive activity of Benzophenone-4 (0.01%) was evaluated using human dendritic cells (e.g., CD14+ human monocytes). Treatment with Benzophenone-4 did not impair the proliferation of lymphocytes.

In the zebrafish embryo assay on Benzophenone-1, Benzophenone-3, and Benzophenone-8, significant decreases in whole-body T4 and T3 levels were observed at day 6 post-fertilization.

Groups of 11 ovariectomized adult Sprague-Dawley rats were dosed orally (by gavage) with 250 mg/kg and 1000 mg/kg Benzophenone-2 (1 ml) daily for 5 d. A dose-dependent suppression of T4 concentration by Benzophenone-2 was observed. T3 levels were also reduced.

The estrogenic activity of Benzophenone-3 was evaluated (in a reporter gene assay) using the human cervical epithelioid HeLa cell line as the host cell line for the generation of stable reporter cells for screening substances that act via hERα hERβ. Assays were performed at concentrations between 0.1 µM and 10 µM. Benzophenone-3 activated Erα moderately and had almost no effect on Erβ. Benzophenone-3 was not considered estrogenic at 10 µM. Exposure to Benzophenone-3 (0.0001 µM) for 24 h increased basal corticosterone secretion from cultured adrenocortical cells.

Benzophenone-2 was applied to the skin of 10 male Wistar rats at a dose of 100 mg/kg for 4 wk. HPT activity was increased, i.e., the level of TSH was reduced and the free fraction of T3 and T4 in the blood was increased. Benzophenone-2 interference with thyroid function was evaluated in another study. Groups of 12 ovariectomized, female Sprague-Dawley rats received oral doses ranging from 10 to 1000 mg/kg for up to 5 d. A dose-dependent decrease in total serum T4 levels was observed, with statistically significant alterations at doses of 333 mg/kg and 1000 mg/kg. The small decrease in total T3 was not statistically significant.

The dosing of 10 male Wistar rats with Benzophenone-3 (100 mg/kg) dermally for 4 wk had no effect on the following hematological parameters: leukocyte count, erythrocyte count, platelet count, erythrocyte morphology, and erythrocyte hemoglobin content.

The cytotoxicity of a sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3 was evaluated using the L929 fibroblast cell line. The nanocapsules were seeded at a concentration of 30 µg/ml, and the sunscreen formulation was found to be non-cytotoxic. In rat thymocytes, cell mortality increased significantly after 3 h of exposure to 300 µM Benzophenone-3.

In MCF-7 breast cancer cells incubated for 6 d, Benzophenone-3 increased cell proliferation, with an EC_{50} between 1.56 and 3.73 μM. An increase in uterine weight (weak effect, active at dose of 1525 mg/kg/d) was reported in a uterotrophic assay, whereby immature Long-Evans rats were fed Benzophenone-3 in the diet for 4 d. The hormonal activity of Benzophenone-3 was evaluated using *Saccharomyces cerevisiae* strains BLYES and BLYAS. In the estrogen assay, an EC_{50} value of 6.44 μM (estrogenic activity) was reported for Benzophenone-3. In the androgen assays, the androgenicity of Benzophenone-3 was not proven. However, Benzophenone-3 was found to be antiandrogenic (EC_{50} = 10.2 μM).

Benzophenone-8 (10 μM) upregulated PDE4B expression in normal human keratinocytes. Also, Benzophenone-3 and UVB co-stimulation induced PDE4B upregulation. It was concluded that PDE4B has a role in the mechanism of Benzophenone-3-induced phototoxicity.

A sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3 (0.005 wt%) was classified as a non-irritant in the HET-CAM. Benzophenone-4 (25 mg) was considered corrosive to the skin when evaluated using a three-dimensional human epidermis model.

There were no signs of erythema or edema in a group of 24 Wistar albino rats after a 24-h patch application of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%). The same was true in 18 male New Zealand rabbits after a 72-h patch application of the same formulation. When Benzophenone-3 (in isopropyl myristate and SD alcohol vehicle) was applied to the skin of 30 female, Hartley albino guinea pigs, (followed by irradiation with UVA), concentrations of 0.1% and 0.3% produced erythema grades greater than 1+. Solutions containing 3% and 6% Benzophenone-3 produced erythema grades of less than 1+ when applied to the skin of guinea pigs. The authors noted that the erythema grade decreased with increasing concentration because the photoprotection afforded by Benzophenone-3 was concentration-dependent. Benzophenone-8 (0.5 g in water) was evaluated for skin irritation potential using 3 New Zealand white rabbits in a 4-h patch test. Skin irritation was not observed. Benzophenone-12 (0.5 g) was also classified as non-irritating to the skin of rabbits in a 4-h patch test.

In a 48-h patch test involving 80 subjects, Benzophenone-4 (5% in petrolatum) induced skin irritation in 4 subjects. Benzophenone-4 (10% in petrolatum) induced skin irritation in 6 subjects.

Benzophenone-8 was classified as a sensitizer in the in vitro KeratinoSens assay (HaCaT cell line) when tested at concentrations up to 200 mM. The skin sensitization potential of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated in a study involving 30 adult male guinea pigs (3 groups of 10), and results were negative. The local lymph node assay was also used to evaluate the sensitization potential of Benzophenone-3 (12.5%, 25%, and 50%), and results were negative. The maximization test was used to assess the cutaneous allergenic potential of Benzophenone-12, using 10 albino guinea pigs challenged with 40% Benzophenone-12 in PEG 300. Positive reactions were observed in 7 animals. Benzophenone-12 was evaluated for skin sensitization potential in another maximization test using 20 guinea pigs of the Pirbright white (Tif:DHP) strain. Sixty-five percent and 60% of the animals were sensitized to Benzophenone-12 at 24 h and 48 h after challenge, respectively.

In retrospective and multicenter studies, patient patch tests indicated allergic reactions to Benzophenone -3 at concentrations of 3% and 10%. At a test concentration of 3%, study population size ranged from 4094 to 23,908 patients. At a test concentration of 10%, study population size ranged from 157 to 23,908 patients. Allergic reactions to Benzophenone-3 were observed at concentrations as low as 2% within a patient population of 355. At a test concentration of 10% Benzophenone-4, allergic reactions were observed in patient populations ranging from 157 to 4857. Allergic reactions to Benzophenone-4 at lower test concentrations of 2% (in population of 347 patients) and 5% (in population of 1155 patients) were also reported. Benzophenone-10 caused allergic reactions at concentrations of 2% (in population of 280 patients) and 10% (in population of 157 patients). In a study in which 19,570 patients used sunscreens containing 1% to 6% Benzophenone-3, the mean rate for contact allergy to Benzophenone-3 was 0.07%. Photoallergic reactions to Benzophenone-3 were observed at patch test concentrations of 2% (in patient populations of 187 and 355), 3% (in group of 4 patients), and 10% (in patient population of 1000). Benzophenone-4 was photoallergic at a patch test concentration of 2% (in patient population of 1000), but not at 10% (in group of 15 patients). Benzophenone-10 was photoallergic at a patch test concentration of 10% (in group of 15 patients).

The following types of reactions were observed in case reports: sensitization reactions to Benzophenone-2 (at 1% and 2% in petrolatum); contact dermatitis and positive photopatch (2% in petrolatum) test reactions to Benzophenone-3; photoallergic contact urticaria, contact urticaria (at 10% in petrolatum) and anaphylactic reactions (wheal and flare) to Benzophenone-3; contact dermatitis (10% in petrolatum) and negative/questionable photopatch reaction to Benzophenone-4; contact dermatitis and positive photopatch reactions to Benzophenone-10; and anaphylactic reactions to Benzophenone-8 and Benzophenone-10.

In other clinical reports, Benzophenone-3 exposure was not significantly associated with the age of menarche in a population of 1598 participants. However, in another study, (200 girls), urinary levels of Benzophenone-3 were associated with decreased time to menarche. Benzophenone-3 was associated with lower levels of serum testosterone in male adolescents in a study involving male and female children and adolescents (population of 588). In a population of 476 pregnant women, an association between maternal urinary Benzophenone-3 and lower abdominal circumference in males was

made. In a cohort of 922 pregnant women, average Benzophenone-3 urinary concentrations were associated with an increase in gestational age. No association between urinary Benzophenone-3 from personal care products and pubertal timing in girls and boys was found in a population of 338 children. A positive association between urinary Benzophenone-3 in both placental weight and child birth weight was observed in a cohort of 473 mother-son pairs. A study was performed to examine whether maternal and paternal preconception urinary concentrations of Benzophenone-3 (e.g., from dietary and personal care product exposure) were associated with risk of preterm birth. No consistent pattern of association was observed.

In a case-control study on idiopathic male infertility and environmental exposure to phenols, there was no evidence for an association between exposure to Benzophenone-3 and idiopathic male infertility. Urinary levels of Benzophenone-3 and the incidence of Hirschsprung's disease were investigated using a total of 423 patients. Results indicated a positive association between women identified with medium to high levels of Benzophenone-3 in the urine and the incidence of Hirschsprung's disease. The presence of UV filters in semen, serum, and the urine was studied using 300 men. Benzophenones-1 and Benzophenone-3 were detected in urine and seminal fluid. The relationship between urinary concentrations of benzophenones and semen quality was evaluated in a study involving 413 men. Benzophenone-2 and Benzophenone-8 were associated with changes in semen endpoints, including sperm concentration, sperm viability, motility, sperm head, and morphology. No associations were observed for Benzophenone-1 or Benzophenone-3. A study (215 university students) was performed to examine associations between urinary concentrations of benzophenone-type UV filters and semen quality and reproductive hormone levels. A statistically significant positive association between urinary Benzophenone-1 and Benzophenone-3 concentrations and serum FSH levels was noted. Additionally, urinary Benzophenone-1 concentration was statistically significantly positively associated with T/E2, and urinary Benzophenone-1 concentration was negatively associated with inhibin B/FSH ratio.

Results from an SCCP risk assessment using data from a skin penetration study involving full-thickness pig ear skin were used to arrive at a conclusion relating to the safety of Benzophenone-3. A MOS of 1686 was calculated, and the SCCP concluded that the use of Benzophenone-3 as a UV-filter up to 6% in cosmetic sunscreen products and up to 0.5% in all types of cosmetic products to protect the formulation does not pose a risk to the health of the consumer, apart from its contact allergenic and photoallergenic potential. The MOS of 1686 was for use to protect a formulation (0.5%) not use as a sunscreen (6%).

DISCUSSION

The Panel published a safety assessment of benzophenones with the following conclusion in 1983: "On the basis of the available animal data 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." During the same year, the Panel also published an addendum to this existing safety assessment, having concluded that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic and that the published conclusion on Benzophenones-1, -3, -4, -5, -9, and -11 is applicable to these 3 ingredients. In accordance with CIR Procedures & Support to the Expert Panel for Cosmetic Ingredient Safety, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years. Thus, the Panel re-evaluated the conclusion, and in 2005, published re-review summary that stated the Panel determined to not reopen the 1983 published safety assessment until results from NTP carcinogenicity studies on benzophenones were available. An NTP oral carcinogenicity study on Benzophenone-3 was published in May 2020, and results from this study have been reviewed by the Panel, along with other safety test data on benzophenones that have been identified in the published literature since the original safety assessment was published in 1983.

The Panel reviewed a number of systemic toxicity studies on benzophenones. However, the Panel noted that these studies were performed at high concentrations that are not relevant to cosmetic exposure. The NTP oral carcinogenicity study on Benzophenone-3 reviewed by the Panel involved rats and mice. Results indicated equivocal evidence of carcinogenicity, i.e., male rats with benign thyroid tumors and malignant meningiomas in the absence of a dose response, and no evidence of carcinogenicity in mice. Based on these results, the Panel did not express any concern over the carcinogenic potential of benzophenones in cosmetic products.

The issue of incidental inhalation exposure from the use of Benzopheone-3 and Benzophenone-4 in cosmetic products was discussed by the Panel. Benzophenone-3 is being used in aerosol hair spray (maximum concentration of 0.014%), pump hair spray (maximum concentration of 0.05%), and in pump deodorant spray (at maximum concentration of 0.08%). Benzophenone-4 is also being used in aerosol hair spray (maximum concentration of 0.015%) and pump hair spray (maximum concentrations of 0.001% to 0.1%). The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of Benzophenone-3 or Benzophenone-4. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following benzophenone ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Benzophenone-1	Benzophenone-5	Benzophenone-10*
Benzophenone-2	Benzophenone-6*	Benzophenone-11*
Benzophenone-3	Benzophenone-8*	Benzophenone-12*
Benzophenone-4	Benzophenone-9	

** Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group,*

TABLES

Table 1. Definitions, idealized structures, and reported functions of the ingredients in this safety assessment. [4,CIR Staff]

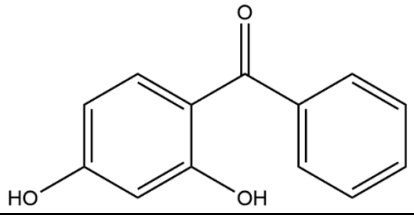
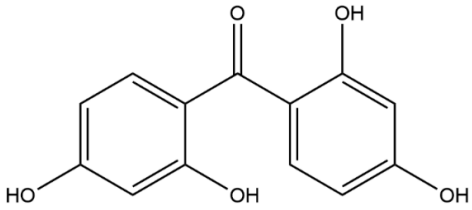
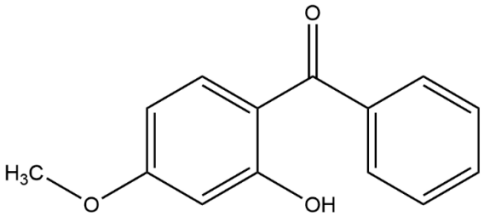
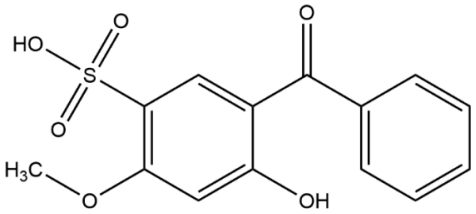
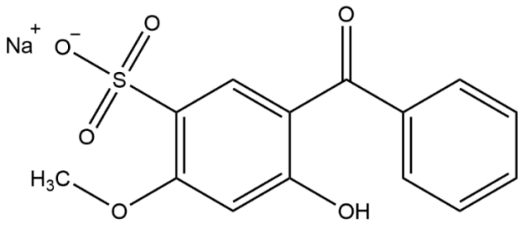
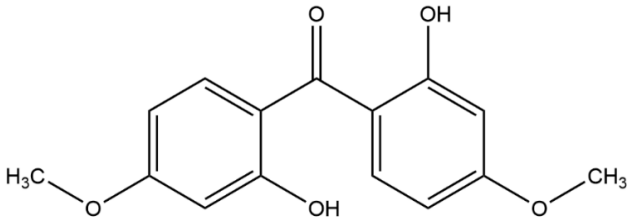
Ingredient /CAS No.	Definition & Structures	Function(s)
Benzophenone-1 131-56-6	Benzophenone-1 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-2 131-55-5	Benzophenone-2 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-3 131-57-7	Benzophenone-3 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers; Sunscreen Agents
Benzophenone-4 4065-45-6	Benzophenone-4 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers; Sunscreen Agents
Benzophenone-5 6628-37-1	Benzophenone-5 is the sodium salt of Benzophenone-4 and conforms to the structure: 	Light Stabilizers
Benzophenone-6 131-54-4	Benzophenone-6 is a benzophenone derivative that conforms to the structure: 	Fragrance Ingredients; Light Stabilizers

Table 1. Definitions, idealized structures, and reported functions of the ingredients in this safety assessment. [4,CIR Staff]

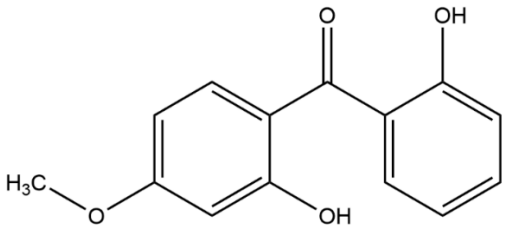
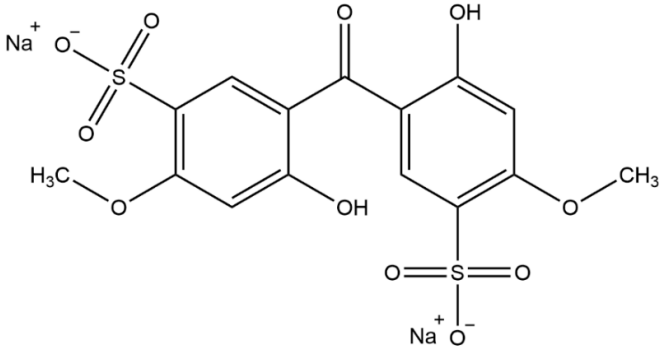
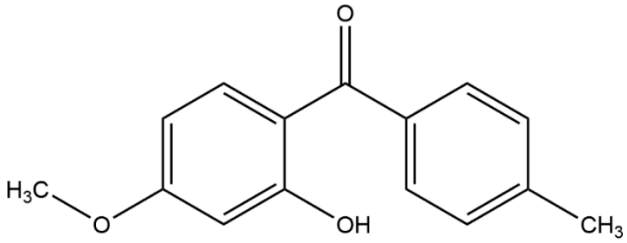
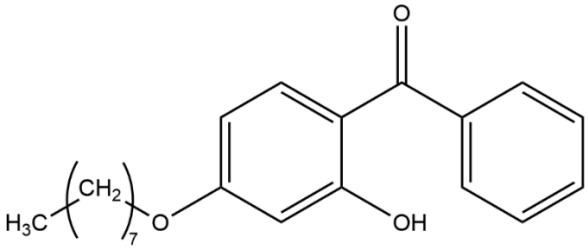
Ingredient /CAS No.	Definition & Structures	Function(s)
Benzophenone-8 131-53-3	Benzophenone-8 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers; Sunscreen Agents
Benzophenone-9 76656-36-5	Benzophenone-9 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-10 1641-17-4	Benzophenone-10 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-11 1341-54-4	Benzophenone-11 is a mixture of Benzophenone-6, Benzophenone-2, and other tetra-substituted benzophenone materials.	Light Stabilizers
Benzophenone-12 1843-05-6	Benzophenone-12 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers

Table 2. Chemical properties

Property	Value/Results	Reference
Benzophenone-1		
Form	Light-yellow powder	[1]
Molecular weight (g/mol)	214.21	[1]
Specific gravity (g/ml)	1.27	[1]
Solubility	Soluble in methanol, ethanol, ethyl acetate, methyl ethyl ketone, acetone, ether, and acetic acid; slightly soluble in benzene; insoluble in water	[1]
Melting point (ºC)	144	[1]
log K_{ow}	2.96 (estimated)	[10]
UV absorption λ_{max} (nm)	290	[1]
Benzophenone-2		
Form	Yellow crystalline solid	[1]
Molecular weight (g/mol)	302.33	[1]
Solubility	Soluble in methanol, ethanol, methyl ethyl ketone; slightly soluble in water	[1]
Melting point (ºC)	195	[1]
log K_{ow}	2.78 (estimated)	[10]
UV absorption λ_{max} (nm)	283	[1]
Benzophenone-3		
Form	Light, cream-colored powder	[1]
Molecular weight (g/mol)	228.26	[1]
Solubility	Soluble in most organic solvents; insoluble in water	[1]
Melting point (ºC)	66	[1]
log K_{ow}	3.79 (estimated)	[10]
UV absorption λ_{max} (nm)	289	[1]
Benzophenone-4		
Form	Pale, ivory-colored powder	[1]
Molecular weight (g/mol)	318.39	[1]
Solubility	Soluble in water, methanol, and ethanol	[1]
Melting point (ºC)	147	[1]
log K_{ow}	0.37 (estimated)	[10]
UV absorption λ_{max} (nm)	288	[1]
Benzophenone-5		
Formula weight (g/mol)	330.29 (sodium cation is 22.99)	[1]
log K_{ow}	-1.42 (estimated)	[10]
Benzophenone-6		
Form	Light yellow solid	[1]
Molecular weight (g/mol)	274.26	[1]
Specific gravity (g/ml)	1.34	[1]
Solubility	Soluble in methanol, ethanol, ethyl acetate, methyl ethyl ketone, and toluene; insoluble in water	[1]
Melting point (ºC)	124	[1]
log K_{ow}	3.90 (estimated)	[10]
UV absorption λ_{max} (nm)	281	[1]
Benzophenone-8		
Form	Yellow crystalline solid	[1]
Molecular weight (g/mol)	244.24	[1]
Solubility	Soluble in methanol, ethanol, ethyl acetate, isopropanol, ether, and acetone; slightly soluble in water	[1]
Boiling point (ºC @ 1 mm Hg)	164-166	[1]
Melting point (ºC)	73.5-74.5	[1]
log K_{ow}	3.82 (estimated)	[10]
UV absorption λ_{max} (nm)	285	[1]
Benzophenone-9		
Form	Light yellow powder	[1]
Formula weight (g/mol)	478.35 (2 sodium cations are 45.97)	[1]
Solubility	Soluble in methanol and ethanol; insoluble in ethyl acetate and benzene	[1]
Melting point (ºC)	350	[1]
log K_{ow}	-2.78 (estimated)	[10]
UV absorption λ_{max} (nm)	284	[1]
Benzophenone-10		
Molecular weight (g/mol)	242.27	[1]
log K_{ow}	4.07 (estimated)	[10]
UV absorption λ_{max} (nm)	300	[1]
Benzophenone-11		
Form	Yellow or tan powder	[1]
Specific gravity (g/ml)	1.38	[1]
Solubility	Soluble in methanol, ethanol, ethyl acetate, and methyl ethyl ketone; insoluble in water	[1]
Melting range (ºC)	85-105	[1]
UV absorption λ_{max} (nm)	285	[1]
Benzophenone-12		
Molecular weight (g/mol)	326.44	[1]
log K_{ow}	6.96 (estimated)	[10]

Table 3. Current and historical frequency and concentration of use of benzophenones according to duration and exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Benzophenone-1				**Benzophenone-2**			
	2021[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2021[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**168**	**142**	**0.009-1.1**	**0.1-1**	**55**	**299**	**NR**	**0.1-5**
Duration of Use								
Leave-On	*165*	*128*	*0.05-1.1*	*0.1-1*	*52*	*254*	*NR*	*0.1-5*
Rinse-Off	*3*	*11*	*0.009-0.15*	*0.1*	*3*	*31*	*NR*	*0.1-1*
Diluted for (Bath) Use	*NR*	*3*	*NR*	*0.1*	*NR*	*14*	*NR*	*0.1-1*
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	7	0.05	0.1-1	NR	NR	NR	NR
Incidental Inhalation-Spray	6	8;5[a];2[c]	NR	0.1;0.1-1[a];0.1[c]	44;3[a]	157;39[a];8[c]	NR	0.1-5;0.1[a];0.1[c]
Incidental Inhalation-Powder	NR	2[c]	NR	0.1[c]	NR	8[c]	NR	0.1[c]
Dermal Contact	9	25	0.15-0.5	0.1-1	54	274	NR	0.1-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair – Non-Coloring	NR	14	NR	0.1-1	1	25	NR	0.1-1
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	159	96	0.009-1.1	0.1-1	NR	NR	NR	NR
Mucous Membrane	NR	10	0.05	0.1-1	NR	15	NR	0.1-1
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Benzophenone-3				**Benzophenone-4**			
	2021[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2021[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**376**	**47**	**0.001-0.5**	**0.1-1**	**1226**	**240**	**0.000035-1.6**	**0.1-10**
Duration of Use								
Leave-On	*322*	*43*	*0.014-0.5*	*0.1-1*	*250*	*102*	*0.0001-1.6*	*0.1-10*
Rinse-Off	*47*	*3*	*0.001-0.5*	*0.1*	*948*	*121*	*0.000035-0.5*	*0.1-5*
Diluted for (Bath) Use	*7*	*1*	*NR*	*0.1*	*28*	*17*	*0.15*	*0.1*
Exposure Type								
Eye Area	1	NR	NR	NR	7	1	0.2	0.1-1
Incidental Ingestion	17	NR	0.5	NR	10	NR	NR	NR
Incidental Inhalation-Spray	170;54[a];27[c]	2;1[a]	0.014-0.5; 0.1-0.5[b]	0.1-1;0.1[a]	40;98[a];33[c]	20;35[a];9[c]	0.001-0.1;0.0001-0.5[a]	0.1;0.1-10[a];0.1[c]
Incidental Inhalation-Powder	3;27[c]	NR	0.3-0.35[b]	NR	33[c]	9[c]	0.1-0.2[b]	0.1[c]
Dermal Contact	296	10	0.0092-0.5	0.1-1	989	104	0.005-0.5	0.1-10
Deodorant (underarm)	2[a]	NR	0.08 (spray)	NR	NR	NR	NR	NR
Hair – Non-Coloring	36	1	0.014-0.5	0.1	191	133	0.000035-1.6	0.1-5
Hair-Coloring	1	NR	0.15	NR	33	1	0.05-0.1	0.1-1
Nail	26	36	0.001-0.4	0.1-1	3	2	0.2	0.1
Mucous Membrane	26	1	0.05-0.5	0.1	641	19	0.15-0.2	0.1-1
Baby Products	NR	NR	0.05-0.25	NR	NR	2	NR	0.1
	Benzophenone-5				**Benzophenone-6**			
	2021[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2021[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**10**	**10**	**0.06**	**0.1**	**NR**	**90**	**NR**	**0.1-1**
Duration of Use								
Leave-On	*10*	*10*	*NR*	*0.1*	*NR*	*84*	*NR*	*0.1-1*
Rinse-Off	*NR*	*NR*	*0.06*	*NR*	*NR*	*4*	*NR*	*0.1*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*2*	*NR*	*0.1*
Exposure Type								
Eye Area	NR	NR	0.06	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	6[a];3[c]	NR;3[a];7[c]	NR	NR;0.1[a];0.1[c]	NR	3	NR	0.1-1
Incidental Inhalation-Powder	3[c]	7[c]	NR	0.1[c]	NR	NR	NR	NR
Dermal Contact	9	10	0.06	0.1	NR	8	NR	0.1-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair – Non-Coloring	1	NR	NR	NR	NR	4	NR	0.1
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	78	NR	0.1-1
Mucous Membrane	NR	NR	NR	NR	NR	2	NR	0.1
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 3. Current and historical frequency and concentration of use of benzophenones according to duration and exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Benzophenone-8				**Benzophenone-9**			
	2021[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2021[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**NR**	**4**	**NR**	**0.1-1**	**13**	**123**	**NR**	**0.1-1**
Duration of Use								
Leave-On	*NR*	*1*	*NR*	*0.1*	*8*	*41*	*NR*	*0.1-1*
Rinse-Off	*NR*	*2*	*NR*	*0.1-1*	*5*	*27*	*NR*	*0.1-1*
Diluted for (Bath) Use	*NR*	*1*	*NR*	*0.1-1*	*NR*	*55*	*NR*	*0.1*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	2	NR	NR	NR
Incidental Inhalation-Spray	NR	1[a]	NR	0.1[a]	4[c]	4;13[a];14[c]	NR	0.1-1;0.1-1[a];0.1-1[c]
Incidental Inhalation-Powder	NR	NR	NR	NR	4[c]	14[c]	NR	0.1-1[c]
Dermal Contact	NR	2	NR	0.1-1	11	96	NR	0.1-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair – Non-Coloring	NR	2	NR	0.1-1	NR	23	NR	0.1-1
Hair-Coloring	NR	NR	NR	NR	NR	1	NR	0.1
Nail	NR	NR	NR	NR	NR	3	NR	0.1
Mucous Membrane	NR	1	NR	0.1-1	7	55	NR	0.1
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Benzophenone-11							
	2021[11]	**1983[1]**	**2020[12]**	**1983[1]**				
Totals*	**NR**	**168**	**NR**	**0.1-5**				
Duration of Use								
Leave-On	*NR*	*140*	*NR*	*0.1-5*				
Rinse-Off	*NR*	*19*	*NR*	*0.1*				
Diluted for (Bath) Use	*NR*	*9*	*NR*	*0.1-1*				
Exposure Type								
Eye Area	NR	NR	NR	NR				
Incidental Ingestion	NR	NR	NR	NR				
Incidental Inhalation-Spray	NR	85;25[a];2[c]	NR	0.1-5;0.1-1[a];0.1[c]				
Incidental Inhalation-Powder	NR	2[c]	NR	0.1[c]				
Dermal Contact	NR	144	NR	0.1-1				
Deodorant (underarm)	NR	NR	NR	NR				
Hair – Non-Coloring	NR	21	NR	0.1-5				
Hair-Coloring	NR	NR	NR	NR				
Nail	NR	3	NR	0.1				
Mucous Membrane	NR	12	NR	0.1-1				
Baby Products	NR	NR	NR	NR				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.

[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

NR – no reported use

Table 4. Benzophenones with no reported uses[11,12]

Benzophenone-6
Benzophenone-8
Benzophenone-10
Benzophenone-11
Benzophenone-12

Table 5. Biomonitoring studies in humans

Compound	Subjects	Concentration/dosage	Procedure	Results	Reference
Benzophenone-1, Benzophenone-2, Benzophenone-3, and 4-hydroxybenzophenone	20 males	Unknown - undefined sources	Benzophenones and 4-hydroxybenzophenone (metabolite of Benzophenone-1 and Benzophenone-3) detected in urine samples, using dispersive liquid-liquid microextraction (DLLME), followed by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Regarding method validation in terms of linearity, concentration range from minimal quantified amount (limit of quantification) to 40 ng/ml selected.	Conjugated form of Benzophenone-1 detected and quantified in 100% of the samples at concentrations ranging from 0.1 to 25 ng/ml. Free form of Benzophenone-1 detected in 95% and quantified in 90% of the samples in a concentration range of 1.2 to 5.7 ng/ml. Conjugated form of Benzophenone-2 detected and quantified in 85% of the samples in concentrations ranging from 0.1 to 7.1 ng/ml. Free form of Benzophenone-2 detected in all samples and quantified in 65% of the samples, at concentrations ranging from 0.5 to 2.2 ng/ml. Conjugated form of Benzophenone-3 detected and quantified in almost all samples (n = 19/20) at concentrations ranging from 0.6 to 44 ng/ml. Free form of Benzophenone-3 detected in 100% of the samples, but was not quantified in any of them. Free form of 4-hydroxybenzophenone detected and quantified in all samples, in a range of 0.9 to 2.0 ng/ml. Conjugated form of 4-hydroxybenzophenone detected in 60% of the samples in concentrations ranging from 0.1 to 0.9 ng/ml	[53]
Benzophenone-1 and Benzophenone-3	157 subjects (59 females, 39 males, and 59 children) in Germany	Unknown - undefined sources	Spot urine samples (157 total) obtained between October of 2007 and February of 2009.	Benzophenone-1 and Benzophenone-3 had high detection rates (26%). Urinary concentrations (µg/l) of the 2 benzophenones not reported. High detection rates also reported for bisphenol A (95%) and triclosan (45%)	[54]
Benzophenone-1, Benzophenone-3, and 4-Hydroxybenzophenone	12 volunteer mothers in Spain	Unknown - undefined sources	Study performed to investigate exposure of human embryos and fetuses to UV filters. Placentas (12) from volunteer mothers collected at delivery. Presence of UV filters analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Benzophenone-1 detected in all samples, and at concentrations below method limit of quantification (MLOQ, between 0.02 and 0.07 ng/g fresh weight). Benzophenone-3 not detected in any sample. 4-Hydroxybenzophenone, metabolite of Benzophenone-1 and Benzophenone-3, detected in 3 of the 12 placental samples at a concentration (0.07 ng/g fresh weight) below the MLOQ	[55]
Benzophenone-1 and Benzophenone-3	34 women in Tunisia	Unknown - undefined sources	Urinary concentrations determined using dispersive liquid-liquid microextraction and UHPLC-MS/MS.	Benzophenone-1 and Benzophenone-3 found in 91.2% and 64.7% of the analyzed samples, respectively. Geometric mean concentrations of Benzophenone-1 and Benzophenone-3 were 1.3 and 1.1 ng/ml, respectively	[56]
Benzophenone-1 and Benzophenone-3	143 reproductive aged women	Unknown - undefined sources	Total of 143 women provided 509 spot urine samples, collected across 2 months of study (3 to 5 samples per woman). Urinary concentrations measured and biomarker variability characterized using the intraclass correlation coefficient (ICC). ICC defined as the ratio of between-subject variance to total variance, with 95% CI. ICC values close to 0 indicate little to no reproducibility, while values close to 1 indicate perfect reproducibility, where most of the variance is attributed to differences between individuals as opposed to within-person differences.	Geometric mean urinary concentrations of Benzophenone-3 and Benzophenone-1 were 4.3 µg/l and 3.3 µg/l, respectively. ICCs for Benzophenone-3 and Benzophenone-1 were 0.66 and 0.55, respectively.	[57]

Table 5. Biomonitoring studies in humans

Compound	Subjects	Concentration/dosage	Procedure	Results	Reference
Benzophenone-1, Benzophenone-3, and 4-Hydroxybenzophenone	200 pregnant women	Unknown - undefined sources	Prospective study involved simultaneously-collected, paired samples, of amniotic fluid and maternal serum and urine. Additionally, samples of human fetal blood (n = 4) obtained during cordogenesis; cord blood (n = 23) obtained at time of delivery. Samples collected from September of 2012 to August of 2014.	The following benzophenones were all detectable in amniotic fluid and cord blood, and, except for 4-hydroxybenzophenone, also in fetal blood: Benzophenone-1, Benzophenone-3, 4 methylbenzophenone, and 4-hydroxybenzophenone. Benzophenone-1 and Benzophenone-3 detected at ~ 10 times lower concentrations in fetal and cord blood, when compared to maternal serum, and at a 1000 times lower concentration when compared to maternal urine concentrations. Therefore, Benzophenone-1 and Benzophenone-3 were only detectable in the fetal circulation in cases of high maternal exposure, indicating some protection by placental barrier. 4-Methoxybenzophenone appeared to pass into fetal and cord blood more freely, with a median 1:3 ratio between cord blood and maternal serum levels. Women appeared to have been most exposed to Benzophenone-3, and this was the only benzophenone in which the measured concentrations in the maternal urine and serum correlated with concentrations measured in amniotic fluid. Based on these data, the authors determined that for Benzophenone-3, but not the other benzophenones, maternal urinary concentrations seem to be a valid proxy for fetal exposure.	[58]
Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-4, Benzophenone-8, and 4-hydroxybenzophenone.	1576 subjects in South Korea	Unknown - undefined sources	Urine samples were collected from July to September in 2010 and 2011. Liquid chromatography-mass spectrometry was used for analysis.	Detection rates for Benzophenone-1 and 4 hydroxybenzophenone were 56% (limit of detection = 0.59 ng/ml) and 88% (limit of detection = 0.04 ng/ml), respectively. Geometric means of urinary Benzophenone-1 and 4-hydroxybenzophenone concentrations were 1.24 ng/ml and 0.45 ng/ml, respectively. Detection rate for the following benzophenones was below 25%: Benzophenone-2, Benzophenone-3, Benzophenone-4, and Benzophenone-8.	[59]
Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-6, and Benzophenone-8	25 female subjects in Southern Spain	Unknown - undefined sources	Benzophenones detected in human menstrual blood	Benzophenone-3 detected most frequently (in 24 of 25 subjects), followed by Benzophenone-6 (in 17 of 25 subjects), and Benzophenone-1 (in 11 of 25 subjects). Neither Benzophenone-2 nor Benzophenone-8 detected in any of the samples. Maximum concentrations were very similar for Benzophenone-1, Benzophenone-3, and Benzophenone-6 (3.1 to 3.7 ng/ml).	[60]
Benzophenone-1, Benzophenone-3, and Benzophenone-8	441 adult, pre-menopausal females in South Korea	Unknown - undefined sources	Study performed from 2015 to 2016. Benzophenones detected in urine.	Detection frequencies in urine samples were: Benzophenone-1 (98.4%), Benzophenone-3 (74.6%), and Benzophenone-8 (22.9%). Authors noted that Benzophenone-1 is a major urinary metabolite of Benzophenone-3.	[61]
Benzophenone-1 and Benzophenone-3	300 men	Unknown - undefined sources	Presence of UV filters in semen, serum, and urine studied. Samples collected during February to December of 2013, Only 6 men had used sunscreen during the 48 h preceding sample collection.	Benzophenone-1 and Benzophenone-3 detected in 19% and 27% of the seminal fluid samples, respectively, albeit at levels of 1 to 2 orders of magnitude lower than were detected in urine. For Benzophenone-1 and Benzophenone-3, levels in the urine and seminal fluid were significantly correlated.	[62]

Table 5. Biomonitoring studies in humans

Compound	Subjects	Concentration/dosage	Procedure	Results	Reference
Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-8, and 4-hydroxbenzophenone	Children and adults in the United States and China	Unknown - undefined sources	Urine samples (166 total) collected from the subjects. In United States, urine samples collected from children in 2012, and from adults during May to July of 2011. In China, urine samples collected from children in March and April of 2002, and from adults during August and September of 2010. Samples analyzed for free and total forms (free + conjugated) of Benzophenone-3 as well as the following 4 of its metabolic derivatives: 4-hydroxbenzophenone; Benzophenone-1; Benzophenone-2; and Benzophenone-8.	Benzophenone-3 detected in practically all urine samples from US and China. Concentrations of Benzophenone-3 in children (geometric mean = 9.97 ng/ml) and adults (geometric mean: 15.7 ng/ml) from the US, significantly higher when compared to children (geometric mean = 0.622 ng/ml) and adults (geometric mean = 0.099 ng/ml) from China. Statistically significant positive relationship found between concentrations of urinary Benzophenone-3 and its derivatives. Profiles of Benzophenone-3 derivatives in urine suggested that demethylation was major route of Benzophenone-3 metabolism. Statistically significantly lower percentage of free form of Benzophenone-3 found in urine from US population than in the Chinese population	[63]
Benzophenone-3	352 subjects	Personal care products and unknown sources	Urinary excretion of ingredients over 6-d period studied.	Benzophenone-3 was frequently detected, i.e., in 70% of the total urine samples. Authors noted that exposure to Benzophenone-3 likely also occurred via food pathway or other unknown sources.	[64]
Benzophenone-3	20 subjects	Unknown - undefined sources	Human adipose fat samples collected during years 2003 to 2004.	High concentrations of Benzophenone-3 (maximum of 4940 ng/g wet weight) detected. Results suggest that adipose tissue is important repository for Benzophenone-3 in human body.	[65]
Benzophenone-3	24 subjects	Unknown - undefined sources	Postmortem brain material (hypothalamus and white-matter tissue) analyzed for presence of Benzophenone-3. Limit of detection was 0.18 ng/g.	In the hypothalamus, the mean amount (n = 24) of Benzophenone-3 was below the limit of detection. In the white-matter, the mean amount (n = 10) of Benzophenone was 0.32 ng/g.	[66]
Benzophenone-3 and 4,4'-dihydroxybenzophenone	79 mothers (71 primiiparous and 8 multiparous nursing) in Spain	Unknown - undefined sources	Study on human UV filters in human breast milk performed, Milk samples provided from day 1 up to 31 months after childbirth. Between April and October of 2014, individual breast milk samples obtained. Most samples collected 4-6 months after delivery.	Percentage of samples that contained UV filters was 24%; two of the major contributors were Benzophenone-3 (779.9 ng/g milk) and its metabolite, 4,4'-dihydroxybenzophenone (73.3 ng/g milk). Additionally, plastic containers for the milk had high concentrations (up to 10.6 µg/g plastic) of Benzophenone-3 and 4,4'-dihydroxybenzophenone.	[67]
Benzophenone-3	40 women undergoing mastectomy for breast cancer	Unknown - undefined sources	Concentrations of UV filters in breast tissue (3 serial locations within) measured. Tissue samples collected between 2005 and 2008. For ethical reasons, cancerous tissue unavailable, but location of cancer was known. Mann-Whitney U-tests used to investigate any link between chemical concentration and whether or not a tumor was present in that region.	Benzophenone-3 measured in 83 of 120 (69%) tissue samples, and at least 1 breast region for 33 of 40 women (range: 0 to 26 ng/g tissue). Spearman's analyses showed statistically significant positive correlations between concentrations of Benzophenone-3 in each of the 3 breast regions. In the lateral region, more Benzophenone-3 was measured when a tumor was present (P = 0.007).	[68]

Table 6. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
DERMAL						
Benzophenone-3	Wistar albino rats	12 males and 12 females	Sunscreen formulation	0.6% to 0.9%. OECD TG 402. Formulation (2000 mg/kg) applied to 2" x 2", 4-ply gauze pad, and patch placed (secured with surgical tape) on hairless, dorsal skin. Patch remained in place for 24 h. Animals observed for 14 d, after which animals killed.	No statistically significant changes in terminal bw between test and controls. Hematological and serum biochemistry parameters normal. No abnormalities at necropsy or microscopic examination. LD_{50} > 2000 mg/kg.	[69]
Benzophenone-3	New Zealand rabbits	6 males	Sunscreen formulation	0.6% to 0.9%. OECD TG 404. Formulation applied to 25 cm^2 area of dorsal skin, using 2" x 3", 4-ply gauze pad (secured with surgical tape). 72-h application period.	Systemic toxicity not observed.	[69]
Benzophenone-12	Albino rabbits	5 rabbits	Water	10,000 mg/kg. OECD TG 402. Applied, under an occlusive or semi-occlusive patch, for 24 h to skin. Patch removal followed by 7-d observation period.	No deaths, and no clinical signs or adverse findings. The LD_{50} > 10,000 mg/kg.	[5]
ORAL						
Benzophenone-1	Rats	Number and strain not stated	Not stated	Details not stated	LD_{50} = 8600 mg/kg. Practically non-toxic.	[6]
Benzophenone-3	Female Wistar albino rats.	10 rats	Sunscreen formulation in 0.5% carboxymethyl cellulose	0.6% to 0.9%. OECD TG 423. Formulation (2000 mg/kg) administered by gavage to 1 fasted rat. Thereafter, each 48 h, the same dose administered to 4 rats. 5 control rats. Dosing followed by 14-d observation period, after which animals killed. Following organs examined macroscopically: heart, lungs, liver, kidneys, and spleen.	All animals survived and gained normal; no clinical signs of toxicity observed. No evidence of gross abnormalities, adverse pharmacological effects, or abnormal behavior. LD_{50} > 2000 mg/kg.	[69]
Benzophenone-8	Female Wistar rats of the CLR:(WI) strain	6 rats	Propylene glycol	Test substance (200 mg/ml) administered via gavage at dose of 2000 mg/kg. Dosing followed by 14-d observation period. Animals killed; macroscopic examinations performed.	None of the animals died. No treatment-related adverse effects. No evidence of macroscopic changes. The LD_{50} > 2000 mg/kg.	[8]
Benzophenone-12	Male rats of the CF Nelson strain	10 rats	Water	20% suspension. Test substance administered at dose of 10,000 mg/kg. Dosing followed by a 7-d observation period.	None of the animals died. No clinical signs and no findings at necropsy. LD_{50} > 10,000 mg/kg	[5]

Table 7. Repeated dose toxicity studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
				DERMAL		
Benzophenone-3	B6C3F$_1$ mice; 5 males and 5 females	2 wk	Acetone or lotion	0.5 to 8 mg applied topically.	Minimal, variable increases in liver and kidney weights, primarily in the higher dose groups.	[70]
Benzophenone-3	F344/N rats; 5 males and 5 females	2 wk	Acetone or lotion	1.25 to 20 mg applied topically	Small and variable increases in liver and kidney weights, reaching a statistical significance primarily in the higher dose groups.	[70]
Benzophenone-3	Male Sprague-Dawley rats; groups of 4 to 6	4 wk	Ointment base	100 mg/kg applied topically twice daily	Body weight, organ-to-bw ratios, and hematological and clinical chemistry parameters not affected. Pathological examinations revealed no significant changes between control and treated animals. No gross external abnormalities observed. Non-toxic to rats.	[71]
Benzophenone-3	Female Sprague-Dawley rats and their offspring	Adults: first to last day of pregnancy (~22 to 23 d). Offspring: from 43 to 56 d age	Cream	Adults received dermal applications (10% in cream; dose = 100 mg/kg) twice daily. At 21 d after birth, offspring (male and female) divided into groups of 5 males and groups of 5 females. From 43 to 56 d age, test substance administered dermally to male offspring.	Dosing of adult pregnant females did not significantly alter bw or cause apparent adverse effects, when compared to controls. No significant differences in bw and sex-ratio observed in offspring, when compared to controls.	[35]
				ORAL		
Benzophenone-1	Male and female rats (number and strain not stated)	90 d	Unknown	Details relating to test protocol not stated	NOAEL of 236 mg/kg/d. Regarding organ toxicity endpoint, critical effects observed unspecified.	[6]
Benzophenone-3	B6C3F$_1$ mice (5 males and 5 females per group)	2 wk	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm	Dose-related increase in liver weight, associated with hepatocyte cytoplasmic vacuolization. NOAEL for microscopic lesions was 6250 ppm.	[70]
Benzophenone-3	F344/N rats (5 males and 5 females per group)	2 wk	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm	One high-dose female rat died. Liver and kidney weights increased. Enlarged livers associated with marked hepatocyte cytoplasmic vacuolization at ≥ 6250 ppm. Renal lesions, consisting of dilated tubules and regeneration of tubular epithelial cells, found primarily in high-dose rats. NOAEL for microscopic lesions was 6250 ppm.	[70]
Benzophenone-3	B6C3F$_1$ mice (10 males and 10 females per group)	13 wk	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm	Decreased, bw gains (dose-related). Mild increases in liver weights observed in dosed mice of both sexes. Kidney weights increased variably in dosed females. Microscopic lesions noted only in kidneys of males at 50,000 ppm: eosinophilic protein casts in dilated renal tubules and mild inflammation associated with dilated tubules. NOAEL for microscopic lesions of 6250 ppm.	[70]
Benzophenone-3	F344/N rats (10 males and 10 females per group)	13 wk	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm.	Body weight gains of high-dose male and female rats reduced. Liver and kidney weights increased. Kidney lesions progressed to include papillary degeneration, or necrosis, and inflammation, while liver lesion appeared to regress. Liver enzymes in serum remained elevated. NOAEL for microscopic lesions of 6250 ppm.	[70]

Table 7. Repeated dose toxicity studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Benzophenone-3	Sprague-Dawley rats (10 male and 10 females per group)	14 wk	Feed	0 or 10,000 ppm	Mean bw of 10,000 ppm males not significantly different from control males, but mean bw of 10,000 ppm females significantly decreased, and was approximately 87% of control value. In males, absolute and relative liver and right kidney weights increased in 10,000 ppm group, compared to control group. In females, absolute kidney weight significantly decreased, and relative liver weight significantly increased, relative to control group. Incidence of mixed-cell cellular infiltration in liver significantly increased in 10,000 ppm males, relative to the control group. Cellular infiltrates composed of mononuclear cells with scarce neutrophils, and had no specific predisposition to specific area of liver lobule. Unlikely that cellular infiltrates, all of minimal severity, would be responsible for changes in liver weights observed in male rats at this time point. No other histologic findings observed that would explain differences in organ weights, but in females, bw changes could have influenced absolute kidney weight decrease and relative liver weight increase. However, increase in relative liver weight in exposed females accompanied by nonsignificant absolute liver weight increase. Unlikely that bw was responsible for liver weight change. Transcriptome analysis was performed on RNA extracted from microarray study of male rat livers from 10,000 ppm and control groups. Observed effects on transcription consistent with mild induction of xenobiotic metabolism-related processes, likely related to observed liver weight increases. Analysis of subset of estrogen-responsive genes showed no change in response to Benzophenone-3.	[72]
Benzophenone-4	Groups of 26 Wistar rats (13 males, 13 females/group).	48 d of dosing (males); 66 d of dosing (females)	Corn oil	OECD TG 422. 0, 750, 1000, and 1250 mg/kg/d (by gavage). Male rats were treated 2 wk before mating and thereafter. Female rats treated 2 wk before mating, and during mating, gestation, and lactation. Recovery groups of male and female rats (5/sex/dose) treated at 0 or 1250 mg/kg bw/d for 66 d total. Animals in recovery groups allowed to recover for 2 wk after final dose given.	No morbidity observed during dosing period. No test substance-related mortalities. Clinical findings sporadic and of no biological significance. Body weight changes restricted to statistically significant decrease in % bw change in recovery group of male rats treated at 1250 mg/kg from day 1 – 22, as compared to the control group. This effect on bw was considered incidental and not test substance-related. Food consumption unaffected by treatment. Observed changes in hematology and clinical chemistry not of toxicological importance. Detailed clinical examinations and microscopic examination of eyes, with optic nerve (in 0 and 1250 mg/kg groups), did not reveal abnormalities. Hormonal data showed no significant effects on concentrations of T4 or TSH (male and females), testosterone (males), or E2 (females). No significant effects on either the absolute or relative weight of brain, adrenals, heart, liver, kidneys, spleen, thymus, thyroid with parathyroid, testes, or epididymides. All adult animals were normal externally. Visceral findings included case of mild splenic enlargement at 1000 mg/kg and one case of mild testicular shrinkage at 1250mg/kg. Microscopic examination revealed no treatment-related effects, that is, incidences and types of lesions observed at 1250 mg/kg comparable to concurrent control groups. In recovery groups, no morbidity was observed during the study, and any mortalities observed were due to gavage error. NOAEL (systemic toxicity) established at 1250 mg/kg/d for male and female rats.	[7]
Benzophenone-12	Groups of 6 male rats (Carworth Farms Elias strain)	35 d	Feed	OECD TG 417. Concentrations of 1.25% and 5% daily	No significant gross lesions observed in rats killed on day 11, 22, or 35. No lesions of liver or kidneys at histological examination.	[5]

Table 7. Repeated dose toxicity studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Benzophenone-12	Groups of Wistar rats (F_0 animals: 12 males, 12 females/group)	Premating to post-weaning.	0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80	OECD TG 416. Doses of 100, 300, and 1000 mg/kg/d, administered by gavage during 10-wk premating period (males), 2-wk premating period (females), 2-wk mating period (both sexes), ~2 d post-mating (males), entire gestation period, up to 30 d of lactation (corresponding to 21 d of lactation and up to 9 d post-weaning), and 35 d post-mating (for sperm-negative females). Pups from the F_1 litter were selected (F_1 rearing animals) for specific post-weaning examinations. The study was terminated with the terminal sacrifice of the F_1 rearing animals. All F_0 parental animals were also killed. Gross necropsy and histopathological examination performed on animals killed.	No treatment-related gross pathological or histopathological findings; no deaths. No clinical signs or changes in general behavior observed in male or female F_0 parental animals of any dose group. No treatment-related bw changes or effects on food consumption. No hematological findings or treatment-related clinical biochemical findings. NOAEL of 1000 ppm/d for general, systemic toxicity.	[5]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
				EMBRY/OVARY CULTURES		
Benzophenone-3	Zebrafish embryos (40 per test concentration)	DMSO	4.38 µM, 21.9 µM, 30.7 µM, 52.6 µM, 78.9 µM, and 110 µM	Modified OECD TG 236. Fish embryotoxicity test (4 replicates). Dosing up to 120 h post-fertilization, because this period includes time points at which different developmental states can be observed. The positive control was 3,4-dichloroaniline (24.7 µM), and water served as the negative control. DMSO served as the solvent control. Endpoints evaluated: mortality, malformations, hatching, and inflation of the swim bladder.	Cumulative mortality under 10% in negative and solvent control groups at the end of the experiment. In positive control group, cumulative mortality of 75%. %. In negative and solvent control groups, percentage of hatched embryos was 95%. No hatched embryos observed in positive control group. Except for one in solvent control group (no swim bladder was observed), no malformations in negative and solvent control groups. LC_{50} values reported: 76.6 µM (at 72 h post-fertilization), 69.8 µM (at 96 h), and 57.3 µM (at 120 h). At 0.438 µM, all embryos able to inflate swim bladder. At higher concentrations, absence of swim bladder inflation in concentration-dependent manner. EC_{50} value of 29.5 µM after 120 h post-fertilization. At 72 h post-fertilization, deformation of tail observed (EC_{50} = 41.9 µM). Malformation of somites at 52.6 and 78.9 µM. Decreased number of hatched embryos after 96 h post-fertilization (EC_{50} = 54.3 µM). Other malformations observed, but frequency not concentration-dependent: pericardial and yolk sac edema, deformed jaw and ventricle or dilated gut, and jaw deformity. Benzophenone-3 caused mortality, unsuccessful hatching, and different malformations to zebrafish.	[73]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Benzophenone-3	Whole ovary cultures collected from Wistar rats. Ovaries (n = 120) collected from rats at birth (postnatal day 0).	DMSO	0.0058 µM, 0.276 µM, 0.576 µM, and 0.876 µM.	Effect on follicular assembly studied. Pups from the same litters were randomly assigned to different treatment groups so that each group contained ovaries of different pups from different litters. ovary cultures were treated for 7 d. Vehicle control cultures were treated with 0.01% DMSO. Positive control cultures were treated with the estrogen receptor β (ESR2) antagonist, 4-(2-phenyl-5,7-bis(trifluoromethyl) pyrazolo-1,5-α-pyrimidin-3-yl) phenol (PHTPP) in DMSO.	Exposure to 0.0058 µM decreased the population of total oocytes, number of nests per ovary, and number of early primary follicles. 0.0058 µM stimulated process of germ cell nest breakdown and caused decrease in reserve of total oocytes. 0.276 µM increased population of total oocytes and number of nests per ovary, but decreased number of primary follicles. At 0.576 µM and 0.876 µM, no changes observed in number of oocytes, germ cell nests per ovary, and assembled follicles in ovaries.	[74]
DERMAL						
Benzophenone-3	$B6C3F_1$ mice (10 males and 10 females per group)	Acetone	22.75 to 364 mg/kg	13-wk dermal dosing study	Epididymal sperm density decreased (whether or not statistically significant not stated) at all 3 dose levels evaluated (22.75, 91.0, and 364.0 mg/kg). Not possible to establish NOAEL for decreased epididymal sperm density. In female mice, no significant difference in estrous cycle length between control group and each dose group.	[75]
Benzophenone-3	Pregnant mice (number not stated)	Olive oil	50 mg/kg/d	Exposed dermally from GD 0 to 6. High-frequency ultrasound imaging was used to follow fetal and placental growth in vivo. Blood flow parameters in uterine and umbilical arteries were analyzed by Doppler measurements. Mice killed on GD 5, 10, and 14 (during first pregnancy), and on GD 10 and 14 (during second pregnancy). Benzophenone-3 levels analyzed in serum and amniotic fluid.	Dosing resulted in reduced fetal weight at GD 14 and feto-placental index (first pregnancy), with 16.13% of fetuses under 5th percentile; uterine artery parameters showed altered pattern at GD 10. Benzophenone-3 detected in serum 4 h after exposure on GD 6, and in amniotic fluid, on GD 14. Weight of offspring of first progeny lower in test group. Placental weights in test group decreased in second pregnancy. First and second progenies of exposed mothers showed higher percentage of females (female sex ratio). Dermal exposure during early pregnancy resulted in intrauterine growth restriction. (IUGR) phenotype, disturbed sex ratio, and alterations in the growth curve of the offspring in the mouse model.	[76]
ORAL						
Benzophenone-1	Female rats (number and strain not stated)	Unknown	100 mg/kg/d. Other administered doses not stated	Oral dosing for 3 d.	NOAEL of 100 mg/kg/d. Any reproductive effects observed not specified.	[6]
Benzophenone-2	Groups of 5 timed pregnant C57BL/6NCr mice.	10% ethanol/90% corn oil vehicle	6.25 mg/d	Administered via gavage on GD 12 through 17. Control pregnant mice dosed with vehicle only. Animals killed on GD 18. Anogenital distance in male fetuses measured and genital tubercles examined histologically. Quantitative reverse transcriptase-polymerase chain reaction analysis of genes purportedly involved in genital tubercle development also performed. Also co-administration of Benzophenone-2 with estrogen receptor antagonist (10 µg in vehicle (s.c.) during gestation,	In the test group, 8 of 57 male fetuses had hypospadias ($p = 0.0064$, when compared to controls). No changes in body mass-adjusted anogenital distance. Co-administration of Benzophenone-2 with estrogen receptor antagonist yielded normal genital tubercles; i.e., no hypospadias in 26 of 26 mice. Hypospadias was not observed after dosing with the estrogen receptor antagonist only or after dosing with vehicle only. Reverse transcriptase-polymerase chain reaction analysis showed that genital tubercles of treated male mice had higher levels of estrogen receptor-β, when compared to male controls ($p = 0.04$). Results indicated that Benzophenone-2 may cause hypospadias via signaling through estrogen receptor.	[77]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Benzophenone-3	Swiss CD-1 mice	Feed	1.25%, 2.5%, and 5.0% (w/w)	Continuous breeding protocol. Male and female mice continuously exposed for a 7-d precohabitation and a 98-d cohabitation period. F_1 generation from control, 2.5%, and 5.0% groups weaned for second generation studies.	Feed consumption in the 2.5% and 5.0% groups consistently higher, but F_0 bw consistently lower. These findings suggest that Benzophenone-3 may be adversely affecting metabolism or digestive process. In 2.5% and 5.0% dose groups, number of live pups per litter significantly reduced. During lactation and nursing of F_1 pups, pup survival significantly below control value in 2.5% and 5.0% groups. Minimal effects on fertility in F_1 generation, but pup weights significantly reduced. Epididymal sperm motility, sperm count, and percentage of abnormal sperm not affected by treatment. No apparent effects on estrual cyclicity or the average estrous cycle length in treated females. Results indicated that Benzophenone-3 caused systemic toxicity, but had minimal effects on fertility and reproduction.	[78]
Benzophenone-3	B6C3F_1 mice (10 males and 10 females per group)	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm	13-wk oral dosing study	Mice in the highest dose group (50,000 mg/kg in feed) exhibited a decrease in epididymal sperm density and an increase in length of the estrous cycle.	[70]
Benzophenone-3	3 groups of mated BALB/c female mice	Tocopherol-stripped corn oil	0.03 mg/kg/d, 0.212 mg/kg/d, and 3 mg/kg/d	Oral dosing from pregnancy day 0 until the day before weaning (lactational day 21). Sample sizes for treatment groups were: 0.03 mg/kg/d (10 litters), 0.212 mg/kg/d (11 litters), and 3 mg/kg/d (9 litters). Sample size for controls was 11 litters. Pups were weaned on postnatal day 21 and co-housed with same-sex animals of the same treatment group for the remainder of the experiment.	Developmental exposures reduced size and growth of mammary gland in males prior to (at postnatal day 21, statistically significant reduction) and during puberty (reduction not statistically significant). In females, reduced mammary cell proliferation (statistically significant at 0.03 mg/kg/d), decreased number of cells expressing estrogen receptor α (statistically significant at 0.03 or 0.212 mg/kg/d), and altered mammary gland morphology (dose response) in adulthood. In males, anogenital index reduced after exposure to 0.03 and 0.212 mg/kg/d at postnatal day 21 and in puberty. In adult males, no differences in anogenital distance observed. No effect on male bw observed. In females, anogenital index unaffected at postnatal day 21, but decreased (at 0.212 mg/kg/d) when measured at puberty. No effects on female anogenital index observed in adulthood.	[79]
Benzophenone-3	F344/N rats (10 males and 10 females per group)	Feed	0, 3125, 6250, 12,500, 25,000, or 50,000 ppm	13-wk oral dosing study.	Rats receiving diet with 50,000 mg/kg showed markedly lower epididymal sperm density and an increase in the length of the estrous cycle at the end of the study.	[70]
Benzophenone-3	Groups of 25 mated Wistar rats of the Crl:WI (Han) strain.	Corn oil	40, 200, and 1000 mg/kg/d	OECD TG 414. Dosing once daily (by gavage) on days 6 through 19 post-coitum. Dose volume of 5 ml/kg. Animals killed on day 20.	All fetal pathological findings were indicative of a minor disturbance and delay in ossification at the highest dose tested (1000 mg/kg/d). No test substance-induced effects on fetal morphology were observed at doses of 40 or 200 mg/kg/d. In all dose groups, was scattered occurrence of few external, soft tissue, and skeletal malformations without a consistent pattern. Findings also occurred without clear dose-response relationship and/or incidence, and not test substance-related. External variations not observed in any fetuses. Authors concluded that Benzophenone-3 did not possess any selective teratogenic properties. NOAEL of 200 mg/kg/d.	[9]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Benzophenone-3	Groups of 25 pregnant Sprague-Dawley rats	Chow (for pregnant females) and chow and milk (for male offspring)	3000 or 30,000 ppm	Dosing from GD 6 until postnatal day 21. Male offspring weaned on postnatal day 28 and then dosed with same concentrations. Animals killed on postnatal day 30. Controls received diet without test substance	Daily observation of male offspring did not reveal any clinical observations related to perinatal exposure. At necropsy on postnatal day 30, bw 22% lower in 30,000 ppm group when compared to control group. Rats exposed perinatally to 30,000 ppm also had statistically significantly lower weights of paired-testis, paired-epididymis, and prostate. These weights lower in males exposed to 30,000 ppm when compared to controls (26%, 17.6%, and 18.5%, respectively). Paired-testis weight to bw ratio also statistically significantly lower in 30,000 ppm group; however, no changes in relative weights of paired epididymis and prostate in 30,000 ppm group. Rats exposed did not have differences in seminal vesicle weight. Serum testosterone concentrations in rats exposed perinatally to 3000 and 30,000 ppm Benzophenone-3 were 13.5% and 28.3% lower when compared to controls, with statistical significance obtained in the 30,000 ppm Benzophenone-3 exposure group. Also, liver and paired-kidney weights lower in dose-dependent manner in 30,000 ppm group, attaining statistical significance. However, relative liver and paired-kidney weights similar to controls.	[80]
Benzophenone-3	Groups (7 to 8 animals per group) of mated female Sprague-Dawley rats	low-phytoestrogen chow	1000; 3000; 10,000; 25,000; or 50,000 ppm.	Feeding from GD 6 until weaning on postnatal day 23. Control group fed low-phytoestrogen chow only.	No exposure-related clinical signs were observed. On GD 10, 15, and 20, the bw of dams decreased in a dose-dependent manner. Absolute and relative kidney weights in dams statistically significantly higher in 50,000 ppm exposure group, when compared to control group. Exposure associated with reduced body and organ weights (kidney) in male and female offspring. No statistically significant differences in mean number of implantation sites/litter, mean resorptions per litter, % litters with resorptions, number and weights of live fetuses, or sex ratios between control and dose groups. One fetus in 50,000 ppm group had hydrocephaly, but no other malformations. Normalized anogenital distance in male pups at postnatal day 23 decreased in 50,000 ppm exposure group. Exposure to 50,000 ppm also caused impairment of spermatocyte development in testes of male offspring. In females, follicular development delayed in 50,000 ppmexposure group. Authors concluded that few adverse effects in dams and offspring dosed maternally and lactationally at 10,000 ppm or less. At higher concentrations, possible that dosing produced delay in postnatal growth, which could have adversely affected reproductive organ development; however, this is not clear. Authors noted that further work needed to clarify possible decreases in spermatogenesis and folliculogenesis observed.	[40]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Benzophenone-3	Groups of 42, 35, 35, and 43 F_0 time-mated female Sprague-Dawley rats	Feed	Dietary concentrations of 1000, 3000, and 10,000 ppm Benzophenone-3 resulted in average daily doses of approximately 70, 206, and 660 mg Benzophenone-3/kg bw/d during gestation, and 157, 478, and 1609 mg/kg/d over lactation days 1 - 14.	39-d feeding period, beginning on GD 6. Groups of 50 (1000 and 3000 ppm) or 60 (0 and 10,000 ppm) F_1 rats per sex continued on study after weaning, and were fed diets containing same concentrations for 105 wk; 10 F_1 rats per sex from 0 and 10,000 ppm groups were evaluated at 14 wk.	Gestation bw of dams receiving 10,000 ppm slightly lower (~3%) than those of control group and showed statistically significant differences. Dams receiving 3000 or 10,000 ppm displayed slight decreases in GD 6 - 21 bw gain (~10%) relative to control group, which attained statistical significance. Lower bw gain over GD 6 - 9 (10,000 ppm) and 18 - 21 (3000 and 10,000 ppm) intervals, which was associated with slightly lower feed consumption over GD 18 - 21 interval. Authors noted that these collective effects are minimal and would not be expected to affect normal development of offspring. Dosing had no effects on percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups. Authors noted that decrease in percentage of females pregnant in 10,000 ppm group can be attributed to 7 animals with no evidence of pregnancy, as shown by absence of implantation sites. Therefore, lower pregnancy rate not exposure-related, given that exposure began after implantation. Dams dosed did not display any adverse clinical findings before or after parturition. Litter size of 10,000 ppm group slightly lower on postnatal days 7 and 10.	[72]
Benzophenone-4	Groups of 26 Wistar rats (13 males, 13 females/group)	Corn oil	750, 1000, and 1250 mg/kg/d	OECD TG 422. Male rats treated 2 wk before mating and thereafter for total of 48 d of dosing (by gavage). Female rats treated 2 wk before mating, during mating, during gestation and during lactation, for total of ~ 63 d of dosing. Control rats dosed with corn oil only. Recovery groups of male and female rats (5/sex/dose) treated at 0 or 1250 mg/kg bw/d for 66 d total. Animals in recovery groups allowed to recover for 2 wk after final dose given.	No morbidity observed. Estrous cyclicity unaffected by treatment. All females showed evidence of copulation after cohabitation/mating period. Pregnancy rates of 77, 62, 77, and 77% at 0, 750, 1000, and 1250 mg/kg, respectively. No significant effects observed on gestation length or litter size. Likewise, no significant effects were observed on the number of live births, pup survival, pup weight or sex ratio. Four pups in 750 mg/kg dose group cannibalized. All other pups at 0, 750, 1000, and 1250 mg/kg normal externally. Internal examination of pups revealed no test substance-related abnormalities. Microscopic examination of pups' thyroid and parathyroid glands in 0 and 1250 mg/kg dose groups revealed no abnormalities. NOAEL (reproductive toxicity) of 1250 mg/kg/d.	[7]
Benzophenone-12	Groups of 50 Wistar rats (25 males (for mating), 25 females)	0.5% carboxymethyl-cellulose suspension in drinking water + 5 mg/100 ml Tween 80	100, 300, and 1000 mg/kg/d.	Administered (gavage) to mated females from implantation 1d prior to expected day of parturition (GD 6 to 19). Female rats killed on GD 20, and fetuses removed from uterus.	Neither clinical signs nor effects on bw (or organ/bw ratios) were observed. No test substance-related necropsy findings were observed after dosing of dams. No evidence of dead/aborted fetuses or pre- and post-implantation loss. Test substance-related external, skeletal, or visceral malformations not observed. NOAEL (for maternal and prenatal developmental toxicity) of 1000 mg/kg/d.	[5]

Table 8. Developmental and reproductive toxicity studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Benzophenone-12	Groups of Wistar rats (F_0 animals: 12 males, 12 females/group)	0.5% carboxymethylcellulose suspension in drinking water + 5 mg/100 ml Tween 80	100, 300, and 1000 mg/kg/d.	Administered (gavage) as follows: 10-wk premating period (males), 2-wk premating period (females), 2-wk mating period (both sexes), ~2 d post-mating (males), entire gestation period, up to 30 d (corresponding to 21 d of lactation and up to 9 d post-weaning), and 35 d post-mating (for sperm-negative females). Control group (12 males, 12 females) dosed with vehicle only. Pups from F_1 litter selected (F_1 rearing animals) for specific post-weaning examinations. Terminal sacrifice of F_1 rearing animals. All F_0 parental animals also killed.	Clinical examinations of F_0 parental animals did not reveal test substance-related adverse findings, and no effects on reproductive performance. No test substance-related adverse findings at clinical or gross examination of F_1 pups. For F_1 rearing animals, no test substance-related findings during clinical examinations and sexual maturation, and no gross findings. NOAEL (for reproductive performance and fertility of F_0 parental rats and develop-mental toxicity in offspring) of 1000 mg/kg/d.	[5]

Table 9. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
				In Vitro		
Benzophenone-1	1-25 µg/ml	Culture medium	Human keratinocytes (HaCaT cells).	Photo-genotoxicity of Benzophenone-1 and apoptotic parameters assessed by western blot, immunocytochemistry, flow cytometry, the comet assay (for DNA damage), and transmission electron microscopy (TEM) imaging. Apoptotic cells detected by annexin V/pro-propidium iodide (PI) staining and sub G1 population of cell cycle. Annexin V is a protein that is commonly used to detect apoptotic cells. PI is a fluorescent agent that is used to stain cells.	Benzophenone-1 photosensitized and generated intracellular reactive oxygen species (2.02 folds) under sunlight/UV radiation. Decrease in cell viability was recorded as 80.06%, 60.98%, and 56.24% under sunlight, UVA, and UVB, respectively. Benzophenone-1 enhanced lipid peroxidation, and leakage of lactate dehydrogenase (LDH) enzyme (61.7%). Benzophenone-1 induced upregulation of apoptotic proteins Bax.Bcl2 ratio, Apaf-1, cytochrome c, Smac/DIABLO, and cleaved caspase3 observed.	[81]
Benzophenone-1	5-25 µg/ml	Culture medium	HaCaT cells	HaCaT cells treated with Benzophenone-1 in presence of UVB (1.08 J/cm^2) or UVA (2.7 J/cm^2). Genotoxicity potential of Benzophenone-1 confirmed through photo-micronuclei and cyclobutene pyrimidine dimers (CPDs) formation (detected using immunostaining and fluorescence microscopy).	Immunostaining results showed maximum CPD formation by Benzophenone-1 at a concentration of 25 µg/ml (in presence of UVB). CPD formation not observed in control cells (exposed in dark or in light). Micronuclei formation detected in HaCaT cells treated with 10 µg/ml in presence of UVB . Simultaneously, micronuclei not detected in control cells exposed in dark or in light. Maximum tail DNA (29.1%) recorded at 25 µg/ml, compared to control value of 4.8%. Cells exposed to different concentrations in presence of UVA (2.7 J/cm^2) exhibited statistically significant ($p > 0.01$) DNA damage when compared to control cells. Similarly, highest olive tail moment (OTM) of 3.57 units recorded at concentration of 25 µg/ml (with UVA irradiation), when compared to control cells (0.54 units). Results indicated that Benzophenone-1 induced photogenotoxicity and apoptosis via the release of cytochrome c and Smac/DIABLO.	[81]

Table 9. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
Benzophenones -1, -3, -6, and -8	Benzophenone-1 (doses up to 600 µg/plate), Benzophenone-3 (up to 200 µg/plate), Benzophenone-6 (up to 2000 µg/plate), and Benzophenone-8 (up to 300 µg/plate)	DMSO	*S. typhimurium* strains TA98 and TA100 (with and without metabolic activation).	Ames test. Benzo[a]pyrene (BaP) was positive control (with activation), and 2-(20-furyl)-3-(5-nitro-2-furyl) acrylamide (AF2) was positive control (without activation).	None of test substances produced clear positive results with or without metabolic activation. Results classified as negative.	[82]
Benzophenones -1, -3, -6, and -8	Doses up to 10 µg/well	DMSO or methanol	*S. typhimurium* strain TL210	Luminescent *umu*-test	Positive results for Benzophenone-3 and pseudo-positive results for Benzophenone-1 and Benzophenone-8.	[82]
Sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%)	5000 µg/plate	Sunscreen	*S. typhimurium* strains: TA 98, TA100, TA1535 and TA1538 (with and without metabolic activation)	Ames test. Positive controls: sodium azide, 2-nitrofluorene, and 2-aminofluorene	No observable increase in number of revertant colonies with or without metabolic activation. Benzophenone-3 was non-genotoxic. Positive controls were genotoxic.	[69]
Benzophenone-3	Doses up to 6000 µg/plate		*S. typhimurium* strains TA98 and TA100, and *Escherichia coli* strain *uvr*A pKM101	Ames test (with and without metabolic activation)	Non-genotoxic with and without metabolic activation.	[72]
Benzophenone-3	0.20 µg/ml, 0.10 µg/ml, 0.05 µg/ml, 0.025 µg/ml, and 0.0125 µg/ml	DMSO	Human peripheral lymphocytes	Chromosomal aberrations (24-h exposure) assay. Positive control (mitomycin C)	Benzophenone-3 induced following 7 types of structural chromosomal aberrations in the chromosomal aberrations assay: gaps, chromatid and chromosome breaks, dicentric chromosomes, rings, tri- or tetra-radials, acentric fragments, and rearrangements. Most frequent aberrations were acentric fragments and chromatid aberrations; numerical aberrations not found. Statistically significant increase in chromosomal aberrations and aberrant cell frequencies at all test concentrations, when compared to solvent control. No statistically significant differences between the solvent and untreated control cultures were observed. Positive control caused statistically significant increase in chromosomal aberrations and aberrant cell frequencies (when dose-response also observed), considering that regression analysis revealed statistically significant ($p < 0.001$) correlation between Benzophenone-3 concentrations and level of genomic damage, compared to all test concentrations of Benzophenone-3 and negative and untreated controls.	[84]
Benzophenone-3	0.20 µg/ml, 0.10 µg/ml, 0.05 µg/ml, 0.025 µg/ml, and 0.0125 µg/ml	DMSO	Human peripheral lymphocytes	Micronuclei (48-h exposure) assay. Positive control (mitomycin C)	Benzophenone-3 caused statistically significant increase in micronuclei formation at all test concentrations. A dose-response was also observed, considering that a regression analysis revealed a statistically significant correlation ($p < 0.001$, compared to negative control) between Benzophenone-3 concentrations and frequencies of micronuclei and cells with micronuclei. Results for vehicle, untreated, and positive controls were same as those reported in chromosome aberrations assay above.	[84]

Table 9. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
Benzophenone-3	1 µM and 5 µM		Human breast epithelial cells.	DNA damage assay. Immunostaining with antibodies against markers of DNA damage, γ-H2AX (phosphorylated histone H2AX) and p53-binding protein 1 (53BP1).	Benzophenone-3 increased DNA damage in manner similar to E2, and in an estrogen-receptor alpha (ERα)-dependent manner. However, Benzophenone-3 had limited transactivation of target genes at same 2 concentrations. Exposure caused R-loop formation in normal human breast epithelial cell line when ERα introduced. Authors concluded that Benzophenone-3 induces DNA damage, mediated by formation of ERα-dependent R-loops at concentrations 10-fold lower than those required for transactivation.	[85]
Benzophenone-3 and Benzophenone-8	4 to 10 µl per plate	Seawater	*S. typhimurium* strain TA98 (without metabolic activation).	Ames test. Positive control was 2,4,7-trinitro-fluorene	Neither ingredient was genotoxic. Positive control was genotoxic.	[83]
Benzophenone-3 and Benzophenone-8	Each ingredient (chlorinated, doses up to 10 µl per plate) tested in seawater at ratios of 1:10 and 1:1000.	Chlorinated bromide-rich water (artificial seawater)	*S. typhimurium* strain TA98 without metabolic activation.	Ames test. Positive control was 2,4,7-trinitro-fluorene	Only Benzophenone-8 (1:10) had clear genotoxic activity that was dose-related (doses of 4, 6, 8, and 10 µl). No genotoxic activity observed for either ingredient at a ratio of 1:1000. Positive control was genotoxic.	[83]
Benzophenone-8	0.008 to 700 µg/plate	Ethanol	*S. typhimurium* tester strains: TA98, TA100, TA1535, TA1537, and TA1538.	*Salmonella*/mammalian microsome mutagenicity assay (with and without metabolic activation)	Benzophenone-8 caused weak, but reproducibly significant, increase in number of TA1537 revertants per plate. Increase was dependent upon increasing concentrations of the test substance, and was totally dependent on the presence of metabolic activation.	[86]
Benzophenone-8	Doses up to 1500 µg/plate (strain TA100) and up to 5000 µg/plate (strain WP2vurA)	DMSO	*S. typhimurium* strain TA100 and *E. coli* (*E. coli*) strain WP2vurA	OECD TG 471. Bacterial reverse mutation assay (with and without metabolic activation). Benzophenone-8 caused visible reduction in growth of the bacterial background lawns of both strains (with and without metabolic activation), initially from 500 µg/plate. Therefore, test substance evaluated up to either maximum recommended dose of 5000 µg/plate or the toxic limit (depending on the bacterial strain type).	No significant increases in frequency of revertant colonies were noted for either bacterial strain, at any dose level either with or without metabolic activation. Authors concluded that Benzophenone-8 was negative for genotoxicity.	[8]
Benzophenone-8	13 to 56 µg/ml	Ethanol	L5178Y TK+/- mouse lymphoma cells	L5178Y TK+/- mouse lymphoma mutagenesis assay (with and without metabolic activation)	Cultures treated without metabolic activation exhibited mutant frequencies not significantly different from those of solvent controls. Cultures treated with metabolic activation exhibited significant increase in mutant frequencies, and dose response evident. Two highest concentrations, 24 and 32 µg/ml, exhibited mutant frequencies that were 3.8 and 2.0 times greater, respectively, than average mutant frequency of solvent controls. Benzophenone-8 was genotoxic.	[87]
Benzophenone-12	Doses up to 50 µg/ml (with metabolic activation) and up to 52 µg/ml (without metabolic activation).	DMSO	Mouse lymphoma L5178Y cells	OECD TG 476. Mammalian cell gene mutation assay (with and without metabolic activation). Positive effect defined as doubling of mutant frequency over concurrent solvent-treated control value, together with evidence of dose-related increase.	Benzophenone-12 was non-genotoxic without metabolic activation. Results ambiguous with metabolic activation. Relative to these results (with metabolic activation), authors noted that a less than 3-fold increase in mutant frequency occurred at highly toxic concentrations.	[5]

Table 9. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
				In Vivo		
Benzophenone-3	0, 3000, or 3500 ppm		Larva from a mating of "multiple wing hair" (mwh) females with heterozygous "flare" (flr) males (*Drosophila melanogaster*)	*Drosophila* somatic mutation and recombination test (SMART). Test substance exposure for 72 h. Positive control: 25 ppm dimethylnitrosamine. A recombination between the mwh and flr genes produces twin wing spots, while events such as deletions produce single spots.	None of the Benzophenone-3-treated larva produced flies with significantly more single or multiple wing spots than controls. In contrast, DMN-treated larva produced flies with significantly more single or multiple wing spots than controls.	[88]
Benzophenone-3	0.6% to 0.9% (Doses of 500, 1000, and 2000 mg/kg)	Sunscreen formulation	Groups of 10 Wistar albino rats dosed prior to assay	OECD TG 474. Mammalian erythrocyte micronucleus test. Doses administered dermally (method not stated) for 2 consecutive days (at intervals of 24 h). Positive control group dosed i.p. with cyclophosphamide (40 mg/kg); negative control group dosed dermally with placebo formulation (2000 mg/kg). At 48 h after first dose, all rats killed and bone marrow extracted from the femur. 200 erythrocytes in bone marrow cells of each animal used to score total number of mature and immature erythrocytes. Number of micro-nuclei per 2000 immature erythrocytes recorded.	Neither sunscreen formulation (all doses) nor placebo statistically significantly increased ratio of micronucleus polychromatic erythrocyte (MNPCE)/ polychromatic erythrocyte (PCE) and PCE/(PCE + normochromatic erythrocyte (NCE)). Positive control statistically significantly increased these ratios. Authors concluded that sunscreen (2 g/kg) did not statistically significantly increase number of micronucleated immature erythrocytes or systemic toxicity at 48 h, classifying sunscreen formulation as non-genotoxic.	[69]
Benzophenone-3	0.6% to 0.9%	Sunscreen formulation	Groups of 10 Wistar albino rats dosed prior to assay	Mammalian bone marrow chromosome aberration test (modification of OECD TG 475). Doses of the sunscreen, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg, administered according to procedure stated immediately above. Same is true for positive and negative controls (cyclophosphamide and placebo formulation; same doses). Animals killed after dosing, bone marrow extracted from femur, and slides prepared. Light microscopy used to evaluate any evidence of chromosomal abnormalities.	No increment in the total number of aberrant cells or in the chromosome aberration percentage for the sunscreen formulation or placebo formulation observed. Positive control facilitated increase in number of aberrant cells. Authors concluded that sunscreen formulation was non-genotoxic.	[69]
Benzophenone-3	0.0, 500, 1670, or 5000 mg/kg		Sprague-Dawley rat bone marrow cells	In vivo cytogenetics assay to evaluate clastogenicity. Rats treated by oral gavage with single administration of each dose for 5 consecutive days. Cyclophosphamide (CP) was positive control, administered at dose of 20 mg/kg. Colchicine growth-arrested bone marrow cells collected 8 and 12 h after single treatment, and 12 h after last daily treatment.	Under either treatment protocol, none of the Benzophenone-3 concentrations caused significant increase in chromosomal aberrations.	[88]
Benzophenone-3	3 mg/kg/d	Tocopherol-stripped Corn oil	12 ovariectomized mice (Balb/c female mice) dose prior to assay	DNA damage assay. Mice exposed to Benzophenone-3 at 10 d after surgical procedure. 8 mice dosed orally with E2, and 12 mice dosed orally with Benzophenone-3 daily for 4 d. Each mouse administered 1 µl of tocopherol-stripped corn oil per gram of bw to deliver E2 (0.25 mg/kg/d) or Benzophenone-3 (3 mg/kg/d). Immunostaining of mouse mammary epithelium was performed to quantify R-loops and DNA damage in vivo.	Results indicated that R-loops and DNA damage detected in mammary epithelial cells of mice treated with Benzophenone-3. Authors concluded that acute exposure to Benzophenone-3 in mice induces DNA damage, mediated by formation of ERα-dependent R-loops at concentrations 10-fold lower than those required for transactivation.	[85]

Table 10. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN CHEMICO / IN VITRO STUDIES		
Sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3.	(0.005 wt %)	Hen's egg (embryonated membrane)	The hen's egg-chorioallantoic membrane test (HET-CAM). Nanocapsules contained poly(Ɛ-caprolactone), carrot oil, a non-ionic surfactant, and Benzophenone-3 (0.005 wt%). Eggs incubated for 10 d, after which membrane removed and CAM was exposed. Formulation then added on embryonated hen's egg membrane; effects studied for 300 s. As positive control (for vascular hemorrhage and lysis), 300 µl of sodium hydroxide solution (0.1 M) applied. Sodium chloride solution (0.9 wt%) applied as a negative control. Diluted (distilled water) formulation (300 µl) applied to eggs also. Assay monitored for any event (hemorrhage, lysis, and coagulation) for 300 s.	Formulation classified as non-irritant.	[112]
Benzophenone-4	25 mg	Three-dimensional human epidermis model	Dermal corrosion potential studied according to OECD TG 431. Before dosing, tissues moistened with sterile water (25 µl). Solid test article evenly applied to apical surface of each tissue. Each treatment (test article or control) conducted in duplicate. Exposure period for test article and control was 3 and 60 min. For 60-min exposure, dosed tissues placed in incubator for remainder of 60-min exposure. MTT assay performed using tissues transferred to 24-well plates. Mean optical density for test chemical determined to be 2.098 and 0.315 for 3-min endpoint and 1-h endpoint, respectively. Mean % tissue viability, compared to the negative control (n = 3), determined to be 85.7 % and 13.4 % for 3-min endpoint and 1-h endpoint, respectively.	Benzophenone-4 considered corrosive to skin.	[7]
Benzophenone-8	up to 200 mM (in DMSO)	KeratinoSens cell line (immortalized adherent human keratinocyte cell line (HaCaT cell line), transfected with a selectable plasmid to quantify luciferase gene induction)	OECD TG 442D. In vitro antioxidant response element (ARE)-nuclear erythroid 2-related factor 2 (Nrf2) Luciferase test method. Experiment involved 2 independent runs. Maximal average fold induction of luciferase activity (I_{max}) response for luciferase gene expression as well as sensitization potential determined. In both repetitions, induction of luciferase above threshold of 1.5 noted. I_{max} was > 1.5-fold and statistically significantly different, as compared to negative control (DMSO).	Benzophenone-8 classified as positive. Authors stated that further testing is required, having noted that this test is part of tiered strategy for evaluation of skin sensitization potential.	[8]
			ANIMAL		
Sunscreen formulation containing Benzophenone-3	0.6% to 0.9% in formulation (formulation dose = 2000 mg/kg)	Wistar albino rats (12 males, 12 females)	OECD TG 402. Dose applied to 2" x 2", 4-ply gauze pad, and patch placed (secured with surgical tape) on hairless, dorsal skin. Patch remained in place for 24 h.	No signs of erythema or edema.	[69]
Sunscreen formulation containing Benzophenone-3	0.6% to 0.9% in formulation	18 male New Zealand rabbits (3 groups of 6)	OECD TG 404. 3 groups: test, positive control (0.8% aqueous formaldehyde), and negative control (placebo sunscreen formulation), respectively. Each material applied to 25 cm^2 area of dorsal skin, using 2" x 3", 4-ply gauze pad (secured with surgical tape). Application period of 72 h, after which patches removed. Reactions scored for erythema and edema at 24 h, 48h, and 72 h; primary irritation index (PII) calculated.	No evidence of erythema or edema in test or placebo groups (PII = 0). Positive control was severely irritating (PII = 10.43). No signs of systemic toxicity.	[69]

Table 10. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Sunscreen formulation containing Benzophenone-3	0.6% to 0.9% in formulation	30 adult male guinea pigs (3 groups of 10)	OECD TG 406. One group treated with the sunscreen formulation. The other 2 groups ere treated with 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB) in 10% propylene glycol (positive control group) and a placebo formulation (cream base only, negative control group). Induction applications (sunscreen formulation, positive control, or placebo) made to the groups of animals. Inducing agents loaded on 2 cm x 4 cm filter paper secured with occlusive dressing. Observations relating to challenge reactions assessed after 24 h of induction, and reactions scored.	None of the animals treated with sunscreen formulation or placebo had sensitization reactions. Positive control induced skin sensitization. Authors classified sunscreen formulation as non-sensitizer.	[69]
Benzophenone-3	12.5%, 25%, and 50%	Groups of 4 female mice of the CBA strain	Applications (volume not stated) made to dorsum of each ear lobe on 3 consecutive days. No local findings or clinical signs of toxicity, and no mortalities. At 5 d after topical application, animals killed. Lymph nodes excised and single cell suspensions prepared. Incorporation of [^{3}H]methyl thymidine measured.	At each concentration, stimulation index less than limit criterion of 3. Benzophenone-3 classified as non-sensitizer.	[9]
Benzophenone-8	0.5 g in water (0.5 ml)	3 New Zealand white rabbits	The test substance applied to skin for 4 h using semi-occlusive patch. Reactions scored for up to 72 h post-application.	Skin irritation not observed in animals tested, and Benzophenone-8 classified as a non-irritant.	[8]
Benzophenone-12	Fine powder (0.5 g)	3 male New Zealand white rabbits	OECD TG 404. Test substance applied (abraded and intact skin of back) for 4 h under occlusive patch. Reactions scored at 24 h, 48 h, and 72 h after patch removal using Draize system. Modified PII calculated using 24-h and 72-h scores.	No evidence of erythema or edema during study (modified PII = 0); no clinical signs observed. Benzophenone-12 classified as non-irritating to skin of rabbits.	[5]
Benzophenone-12	Intradermal injection at induction: 5% in oleum arachidis (w/v), and 5% in the adjuvant/saline mixture (w/v). Induction patch application: 30% in petrolatum (w/w). Challenge patch application: 20% in petrolatum (w/w).	20 test (10 males, 10 females) and 10 controls (5 males, 5 females) guinea pigs of the Pirbright white (Tif:DHP) strain.	Maximization test. During first week of induction, intradermal injections (neck, 0.1 ml per injection; 3 pairs): adjuvant /saline mixture 1:1 (v/v), Benzophenone-12 (5%) in oleum arachidis (w/v), and Benzophenone-12 (5%) in the adjuvant/saline mixture (w/v). During the second week of induction (filter paper patch application), Benzophenone-12 (30%) in petrolatum (w/w) applied to the neck for 48 h (2 cm x 4 cm occlusive patch contained 0.4 g of paste). Control group was also treated during induction. Challenge phase (wk 5; i.e., 2 wk after induction) consisted of single, 24-h application of Benzophenone-12 (20% in petrolatum (w/w)). Test substance (0.2 g paste) applied to flank using 2 cm x 2 cm occlusive challenge patch. Reactions scored at 24 h and 48 h using Draize scale. During challenge, control group treated with vehicle and test substance to check for maximum sub-irritant concentration of test substance in adjuvant-treated animals.	Results indicated that 65% and 60% of animals sensitized to Benzophenone-12 under experimental conditions employed at 24 h and 48 h after challenge, respectively. Authors classified Benzophenone-12 as sensitizer.	[5]

Table 10. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-12	Intradermal induction at 15% (in PEG 300 and in emulsion of Freund's Complete Adjuvant (FCA)/physiological saline); epidermal induction and challenge with 40% in PEG 300	10 test and 5 control female albino guinea pigs	Maximization test. Intradermal induction of sensitization in test group performed in nuchal region. Epidermal induction of sensitization conducted for 48 h under occlusion 1 wk after intradermal induction, and following pretreatment of test areas with 10% sodium lauryl sulfate (SLS) 23 h prior to application of test substance. Control animals intradermally induced with PEG 300 and FCA/physiological saline, and epidermally induced with PEG 300 under occlusion following pretreatment with 10% SLS. Two wk after epidermal injection, control and test animals challenged by epidermal application of test substance and PEG 300 alone under occlusive dressing. Cutaneous reactions evaluated at 24 h and 48 h after removal of dressing.	No toxic symptoms evident in test or control group. Seven of 9 surviving test animals had discrete/patchy to moderate/confluent erythema at the 24- and 48-h reading after challenge treatment with Benzophenone-12. No skin effect observed in control group. Benzophenone-12 classified as skin sensitizer.	[116]
HUMAN					
Benzophenone-4	2%, 5%, and 10% in petrolatum (20 µl dose of each applied)	80 subjects	Three concentrations tested on each subject. Dose applied to 8-mm diameter Finn chamber, secured with adhesive tape. Patches applied for 2 d to upper back. Reactions scored according to International Contact Dermatitis Research Group (ICDRG) grading scale.	Benzophenone-4 (5% in petrolatum) induced skin irritation in 4 subjects. Benzophenone-4 (10% in petrolatum) induced skin irritation in 6 subjects.	[115]

Table 11. Ocular irritation studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN VITRO		
Benzophenone-4	50 mg (solid)	MatTek EpiOcular™ model (normal human-derived keratinocytes in the 3-dimensional human tissue model; progressively stratified, but not cornified, cells)	OECD TG 492. MTT cytotoxicity assay. Exposure to Benzophenone-4 for ~ 6 h.	Tissue viability of Benzophenone-4 determined to be 3.6%. Benzophenone-4 classified as irritating to human eye	[7]
Benzophenone-8	20% w/v in paraffin oil (volume = 750 µl)	Corneas from 3 cattle	OECD TG 437. Bovine corneal opacity and permeability test. Corneas exposed to test substance for 4 h. Test substance then removed from front opening of the anterior chamber and epithelium was rinsed. For evaluation of corneal permeability, passage of sodium fluorescein dye measured using ultraviolet-visible (UV/Vis) spectrophotometry.	Benzophenone-8 did not cause corneal opacity or permeability, resulting in mean in vitro irritancy score of 1 after 4 h of exposure. Authors concluded that Benzophenone-8 not a severe irritant or corrosive agent.	[8]
			ANIMAL		
Benzophenone-3	Sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) (100 mg)	3 adult New Zealand albino rabbits	Formulation instilled into conjunctival sac of right eye of each animal. After instillation, eyes examined macroscopically (in accordance with the Draize scale) at intervals of 24 h, 48 h, and 72 h, and daily from 4 to 10 d.	No signs of gross toxicity or adverse effects. Corneal opacity and iritis not observed during study. At 1 h post-instillation, conjunctival discharge observed in 1 of 3 eyes, but subsided within 96 h. Highest maximum mean total score (MMTS) value (0.67) for ocular irritation observed within 1 h after instillation, classifying sunscreen formulation as practically non-irritating to the eye.	[69]
Benzophenone-12	0.1 g	6 New Zealand white rabbits (3 males, 3 females)	OECD TG 405. Undiluted test substance instilled once, and ocular irritation evaluated on days 1, 2, 3, 4, and 7.	No evidence of ocular irritation (PII = 0).	[5]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenones-2 and-3	1% and 10% (in petrolatum)	11 patients (10 women, 1 man) with reactions due to sunscreen allergy (itchy bumps and burning)	Photopatch testing. Finn chambers (8 mm, secured with tape) containing filter paper wetted with test substance and applied to back. Chambers applied in duplicate for patch and photopatch testing. After 24 h, photopatches removed and one set irradiated with UVA (10 J/cm^2). Immediately after UVA exposure, photopatch tests read to determine immediate-type sensitivity reactions. Patch areas then covered with opaque tape material. Another reading made 24 h later (day 3), and final reading made at 5 to 7 d.	At reading immediately after UVA exposure, all reactions were negative, indicating absence of contact urticaria. One patient had delayed-type hypersensitivity photopatch test reaction to Benzophenone-2 (1% in petrolatum), and another patient had a photopatch test reaction to Benzophenone-3 (10% in petrolatum).	[128]
Benzophenone-3	2% (in petrolatum)	187 patients (76 males, 111 females) with history of photosensitivity	Over 6-year period, patients photopatch tested using standard techniques. Test substance applied in duplicate to patient's midback, on either side of the midline, using aluminum disks (Finn Chambers) and paper (Scanpor) tape. For first 2 testing periods (January 1985 through February 1987 and March 1987 through August 1989), test substance remained in place for 48 h. In 3rd period (September 1989 through December 1990), test substance removed after 24 h. Test site then irradiated with UVA, 8 J/cm^2 (January 1985 through August 1989) or 10 J/cm^2 (September 1989 through December 1990). Site then covered with light opaque material (gauze pads and aluminum foil held in place with paper (Scanpor) tape). All sites evaluated for reactions at 48 hours post-irradiation. Second readings at day 7 post-irradiation done in third test period (September 1989 through December 1990). Second readings not done during first 2 test periods. Reactions graded on scale of ± to 3+.	Nine clinically relevant photoallergic contact dermatitis responses to Benzophenone-3 (2% in petrolatum).	[123]
Benzophenone-3	2% (in petrolatum)	355 consecutive patients with suspected photosensitivity	Study based on 7 years of testing (standard photopatch protocol) at 2 Swedish dermatology clinics	Results indicated 15 photocontact allergic reactions and 1 contact allergic reaction to test substance.	[126]
Benzophenone-3	3%	4094 patients with suspected allergic contact dermatitis	Patch tested by 12 North American Contact Dermatitis Group (NACDG) dermatologists, with screening series of 50 allergens. Patients patch tested (July 1, 1996 to June 30, 1998) using Finn chambers on Scanpor tape. Patches remained in place for 48 h. Sites evaluated initially at 48 to 72 h, and, again, between 72 and 168 h after initial placement. Positive allergic patch test result was generally interpreted as a 1+, 2+, or 3+ reaction manifested by erythematous papules, vesicles, or spreading reaction with crust and ulceration. Relevance of patch test reactions determined in combination with patient's history and skin examination findings	Of the patients patch-tested, 0.5% had allergic reactions (73.7% relevant reactions, i.e., definite, probable, or possible relevance to patient's present dermatitis).	[117]
Benzophenone-3	3% (in petrolatum)	5800 patients	NACDG study. Patch testing performed from July of 1998 to December of 2000. Patches remained in place for 48 h. Test sites evaluated twice, initially at 48 h to 70 h and, again, at between 72 h and 178 h after initial placement. Positive allergic patch test result was interpreted to be a +, ++, or +++ reaction. Reactions of these types manifested by erythematous papules, vesicles, or a spreading reaction with crust and ulceration.	Incidence of positive reactions was 0.6%. Relevance of this incidence of positive reactions classified as follows: 20.6% (definite relevance), 50% (possible relevance) and 2.9% (past relevance).	[118]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-3	3% (in petrolatum)	5085 patients. 589 patients (11.8%) had an occupationally-related skin condition and 3319 (65.3%) had at least 1 allergic patch test reaction.	Standardized patch testing at 13 centers in North America. NACDG patch test results from January of 2007 to December of 2008. Patches (Finn chambers, secured with tape) remained in place for 48 h. Reactions scored at 48 h and 72 h to 168 h. At end of testing, clinical relevance of positive patch test reactions determined by consideration of patient's history and clinical findings. Relevance of a positive allergen categorized	Allergic reactions in 0.9% of the patients. Other values relating to clinical relevance were: definite relevance (22.7%), probable relevance (36.4%), possible relevance (22.7%), and past relevance (6.8%).	[119]
Benzophenone-3	3% aqueous solution	4 patients	Applied, in duplicate, to the midback using Finn chambers. At 48 h post-application, test substance removed, and sites evaluated for reactions. Reactions graded on scale of +1 to +3. Other site containing test substance irradiated with UVA (8 J/cm^2), and then covered with light-opaque material. All sites evaluated for reactions at 48 h post-irradiation. Contact allergy diagnosed as equally positive reaction at nonirradiated and irradiated sites. Photoallergy diagnosed as positive irradiated site with negative unirradiated site. Allergy and photoallergy diagnosed when both sites were positive, but with irradiated site having greater reaction than unirradiated site	Results were as follows: +2 reaction (without UVA) and +3 reaction (with UVA) - Patient 1; +2 reaction (without UVA) and +3 reaction (with UVA) - Patient 2; +2 reaction (with UVA) - Patient 3; and +2 reaction (without UVA) and +3 reaction (with UVA). All four patients were photoallergic to Benzophenone-3.	[122]
Benzophenone-3 (in sunscreens)	Concentrations between 1% and 6% (in sunscreen products)	19,570 patients	Data from 64 allergenicity studies (between 1992 and 2006) aggregated and analyzed. Done in order to evaluate the irritation and sensitization potential of sunscreen products.	Forty-eight of 19,570 possible dermal responses considered suggestive of irritation or sensitization. Mean rate of responses across all formulations was 0.26%. Sensitization rates did not correlate with Benzophenone-3 concentration. Available re-challenge data indicated that only 8 of these responses were contact allergies due to Benzophenone-3. Mean rate of contact allergy to Benzophenone-3 was 0.07%. Authors concluded that sunscreen products formulated with 1 to 6% Benzophenone-3 do not possess a significant sensitization or irritation potential for general public.	[119]
Benzophenone-3	3% and 10% (in petrolatum)	23,908 patients. Of these patients patch tested, 219 (0.9%) had sunscreen coded as allergen source	Cross-sectional analysis of patients patch tested by the NACDG between 2001 and 2010 performed	Frequent allergen in sunscreens was Benzophenone-3, whereby 70.2% of the patients (26 of 37 patients patch tested) had an allergic reaction to 10% Benzophenone-3 (in petrolatum) and 64.4% of the patients (56 of 87 patients patch tested) had an allergic reaction to 3% Benzophenone-3 (in petrolatum). Values for clinical relevance of allergic reactions to 10% Benzophenone-3 (in petrolatum) in 26 of 37 patients were: definite relevance (5 of 26 patients (19.2%)), probable relevance (11 of 26 patients (42.3%)), possible relevance (9 of 26 patients (34.6%)), and past relevance (1 of 26 patients (3.8%). Clinical relevance values reported for 3% Benzophenone-3 (in petrolatum) positive reactions in 56 of 87 patients were: definite relevance (13 of 56 patients (23.2%)), probable relevance (27 of 56 patients (48.2%)), possible relevance (15 of 56 patients (26.8%)), and past relevance (1 of 56 patients (1.8%).	[120]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-3	10% (in white petrolatum)	21 patients	Patients with positive photopatch tests to sunscreen agents retrospectively selected from the database of the contact dermatitis clinic at the Skin and Cancer Foundation in Australia. Test substance applied, in duplicate, using Finn chambers on Scanpor tape. Sites covered with opaque material. After 24 h, the test sites examined and results recorded. One site irradiated with 5 J/cm^2. Reactions scored on day 5 according to ICDRG standards, and final reading performed on day 7.	Nine patients had positive photopatch test reaction to Benzophenone-3. Two patients had positive reactions at non-irradiated sites.	[124]
Benzophenone-3	10% (in petroleum jelly)	35 patients (11 men, 24 women) with confirmed photosensitivity	Descriptive cross-sectional study (in Argentina) performed to determine proportion of photosensitive patients with photoallergic contact dermatitis to Benzophenone-3. Two sets of patches containing test substance applied to back, 1 on the right and 1 on left. At 48 h after patch application, 1 patch irradiated with cumulative UVA dose (5 J/cm^2; peak wavelengths of 350 nm, 365 nm, and 370 nm) over an 18-min period. Reactions scored at 30 min post-irradiation and at 96 h after patch application. Late reading also taken after 1 wk.	Photoallergic contact dermatitis identified in 6 patients (17.14%). Five of these patients (14.28%) had at least 1 positive reaction to Benzophenone-3 in photocontact test. Four patients had reaction at irradiated sites only, and 1 patient had reaction at both irradiated and nonirradiated sites.	[125]
Benzophenones-3 and -4	Benzophenone-3 (10% in petrolatum); Benzophenone-4 (2% in petrolatum)	347 patients (from centers across 12 European countries)	Investigation of photoallergic contact dermatitis frequency performed using 347 patients from centers across 12 European countries. Test substance applied to skin of back, and removed at 48 h. One site irradiated with UVA (5 J/cm^2), and other site covered with UV-impermeable material. Reactions scored at 48 h	Benzophenone-3 elicited photoallergic contact dermatitis in 37 patients: + reaction (14 patients), ++ reaction (18 patients), and +++ reaction (5 patients). Benzophenone-4 elicited photoallergic contact dermatitis in 3 patients: + reaction (1 patient) and ++ reaction (2 patients). Allergic contact dermatitis reactions (+ reactions) to Benzophenone-3 observed in 6 patients.	[137]
Benzophenones-3 and -4	Benzophenone-3 (10% (in petrolatum); Benzophenone-4 (2% in petrolatum)	1000 patients (consecutive dermatology outpatients in Poland)	Prospective study to evaluate frequency and causes of photoallergic contact dermatitis among dermatology outpatients. In study group, 36 (3.6%; 95% CI: 2.4 - 4.8%) individuals required photopatch testing based on their clinical symptoms. Because total number of patients requiring patch tests of any kind amounted to 205, percentage of photopatch tested patients among all patch-tested patients was 17.5% (95% CI: 12.2 - 22.8%). Patch tests (2 identical sets) mounted on back and remained under occlusion for 48 h. Some sites irradiated with UVA (5 J/cm^2) and some non-irradiated. Skin reactions scored 24 h and 48 h after irradiation. Presence of inflammatory reaction at irradiated sites and no reaction to same hapten at non-irradiated sites interpreted as confirmation of photoallergy. In case of positive reactions to a hapten, at both irradiated and non-irradiated sites, the classical contact allergy was recognized.	Photoallergic contact dermatitis ultimately confirmed in 15 (1.5%; 95% CI: 0.7 to 2.3%) patients: 7 females and 8 males. Of these, 2 patients had a positive reaction to Benzophenone-3 (10% in petrolatum). One patient had a positive reaction to Benzophenone-4 (2% in petrolatum).	[139]
Benzophenones-3 and -4	10% (in petrolatum)	402 patients with suspected clinical photosensitivity	Patch and photopatch tests performed according to ICDRG guidelines. UVA dose of 5 or 10 J/cm^2 used for photopatch testing.	3 allergic and 9 photoallergic reactions to Benzophenone-3. No photoallergic or allergic reactions to Benzophenone-4.	[133]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenones-3 and -4	Benzophenone-3 (10% in white paraffin); Benzophenone-4 (5% and 10% in white paraffin)	1155 patients (from 17 centers across the United Kingdom, Ireland, and the Netherlands)	Photopatch testing (over 2-year period) of Benzophenone-3 and Benzophenone-4 performed. Photopatch testing involved application of test substance (on aluminum Finn chamber) to skin of mid-upper back (paravertebral area avoided) for 24 h or 48 h (depending on the center). Contact dermatitis units traditionally applied allergens for 48 h, and photobiology units traditionally applied allergens for 24 h. Following patch removal, one set (dark control) covered with light-impermeable occlusive dressing, and the other set irradiated with fluorescent UVA (5 J/cm^2). Reactions scored at 48 h post-irradiation, and, if possible, at 24 h and 72 h. ICDRG visual scoring system used.	Benzophenone-3 (10% in white paraffin) caused photoallergic contact reactions in 27 patients. Benzophenone-4 (5% in white paraffin) and Benzophenone-4 (10% in white paraffin) caused photoallergic contact reactions in 2 and 5 patients, respectively. The following allergic reactions also reported: 5% Benzophenone-4 (2 patients), 10% Benzophenone-3 (9 patients), and 10% Benzophenone-4 (9 patients). Photoaugmentation and photoinhibition of contact allergy observed in 1 patient tested with 10% Benzophenone-3 and in 1 patient tested with 10% Benzophenone-4. Irritation reactions observed included: 5% Benzophenone-4 (2 patients), 10% Benzophenone-3 (2 patients), and 10% Benzophenone-4 (4 patients). with 1	[133]
Benzophenones-3 and-4	10% (in petrolatum)	1527 patients	A retrospective analysis involved the reviewing of 1527 charts in the University of British Columbia Contact Dermatitis Clinic patch test database from January of 2009 to July of 2012. 23 of the patients tested with the sunscreen series at the clinic. All 1527 patients patch tested with 70 allergens on NACDG screening series. Patch test chambers containing test substance applied to upper back and secured with tape for 48 h. Reactions scored (using the ICDRG grading scale) at time of patch removal and at 96 h to 120 h.	Of the 23 patients tested, 2 had positive reactions (allergic contact dermatitis) to Benzophenone-3 and 1 had positive reaction to Benzophenone-4. Of the 1527 patients screened 8 reacted to Benzophenone-3 in the NACDG series. This number does not include the 2 patients who tested positive to Benzophenone-3.	[129]
Benzophenones-3 and -4	10% (in petrolatum)	5595 patients tested with Benzophenone-3; 5592 patients tested with Benzophenone-4	NADCG study. Patch testing with Finn chambers.	Of the 5592 patients patch tested with Benzophenone-4, 93 had a positive (allergic) reaction. Values for clinical relevance of allergic reactions were: definite relevance (3 of 93 patients (3.2%)), probable relevance (12 of 93 patients (12.9%)), possible relevance (45 of 93 patients (48.4%)), and past relevance (8 of 93 patients (8.6%)). Of the 5595 patients patch tested with Benzophenone-3, 24 had an allergic reaction. Values for clinical relevance of allergic reactions were: definite relevance (4 of 24 patients (16.7%)), probable relevance (5 of 24 patients (20.8%)), possible relevance (11 of 24 patients (45.8%)), and past relevance (1 of 24 patients (4.2%)).	[130]
Benzophenones-3 (10% in petrolatum) and -4 (2% in petrolatum)		1390 patients tested with Benzophenone-4; 4224 patients tested with Benzophenone-3	British Society for Cutaneous Allergy (BSCA) retrospectively reviewed results from their facial patch test series. Review involved 12 centers in United Kingdom and Ireland for 2-yr period (January of 2016 to December of 2017).	Of the 1390 patients patch tested with Benzophenone-4 (2% in petrolatum), 0.79% (confidence interval (CI): 0.44% to 1.41%) had allergic reactions. Of the 4224 patients patch tested with Benzophenone-3 (10% in petrolatum), 0.17% (CI: 0.08% to 0.35%) had allergic reactions.	[131]
Benzophenones-3 and -4	Not stated	15 patients (4 males, 11 females)	Fifteen patients (4 males, 11 females) had reacted to sunscreens. Of these, 8 had used sunscreens before occasional sun exposure, and 6 had used them regularly for chronic lupus erythematosus, melasma, vitiligo, rosacea, drug photosensitivity, and atopic dermatitis. One patient had reacted to her daily cream containing Benzophenone-3. Patch testing (procedure not stated) performed	Positive patch test results were as follows: 4 allergic contact dermatitis reactions to Benzophenone-4, and 2 allergic contact dermatitis and 5 photoallergic contact dermatitis reactions to Benzophenone-3.	[132]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenones-3 and -4	Not stated	12 patients with history of acute eruption on photoexposed areas (induced by ketoprofen or tiaprofenic acid)	At least 1 mo after acute episode of contact dermatitis, patients patch tested using Finn Chamber technique. Finn chambers mounted on Scanpor tape, and patches removed after 2 d. For UV irradiation, 2 sources of light used (UVA alone and UVA + UVB).	Photopatch test results positive for Benzophenone-3 (reactions in 3 of 12 patients) and negative for Benzophenone-4.	[134]
Benzophenones-3 and -4	Not stated	82 patients (with clinical diagnosis of photoallergic contact dermatitis)	Study performed to identify photoallergens that caused photoallergic contact dermatitis in population attending outpatient clinic in Columbia. Test substances applied, in duplicate, to skin on back. Test sites covered with opaque tape for 24 h. Panel on right irradiated with UVA (dose = 5 J/cm^2; irradiance = 10.4 mW/cm^2). Reactions scored 24 h after application and at 24 h and 72 h post-irradiation.	Both Benzophenone-3 and Benzophenone-4 induced a positive photopatch reaction. Benzophenone-3 photoallergenic in 22 of 82 patients (26.8%), and Benzophenone-4 photoallergenic in 2 of 82 patients (2.4%).	[136]
Benzophenones-3 and -4	Not stated	160 patients (37 male, 123 female)	Retrospective chart review on patients who underwent photopatch testing in Canada between January of 2001 and December of 2010. Duplicate sets of allergens applied to back. At 24 h, 1 set of allergens uncovered and exposed to UVA at a dose of 5 J/cm^2. Other set of allergens shielded from UVA exposure. 24-h reactions to non-irradiated compounds assessed at 15 to 20 min later. On following day, irradiated patches read at 24 h post-irradiation. Reactions at non-irradiated patch test sites read 48 h after application.	Benzophenone-3 induced photoallergic reactions in 12 patients, allergic reactions in 17 patients, and both allergic and photoallergic reactions in 6 patients. Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions.	[136]
Benzophenones-3 and -4	Not stated	157 children (69 male, 88 female)	Duplicate series of UV filters and children's own sunscreen products applied to back. Reactions scored at time of sample removal and at 24 h and 48 h after exposure to UVA (5 J/cm^2).	Ten children (5 to 7%) had positive photopatch reactions to UV filters and/or their sunscreen products (4 to 5% to UV filters; 5 to 7% to their sunscreen products). Benzophenone-3 induced photoallergy (2+ reaction) in 33% of the children (n = 3). Single case of photoaugmentation reaction to Benzophenone-4 reported. Patient had + reaction in control panel, but had ++ reaction in irradiated panel.	[138]
Benzophenones-3,-4, and -10	10%	553 patients	Over a period of 3 yr, patients patch tested (Finn chambers) with each test substance. Results recorded at 48 h (day 2) and 96 h (day 4). Positive reactions (+ to +++) graded according to international recommendations (not specified).	13 patients (8 females, 5 males) and 1 patient with positive reactions to Benzophenone-3 and Benzophenone-10, respectively. 13 patients with positive reactions to Benzophenone-4. 1 patient with positive reaction to both Benzophenone-3 and Benzophenone-4.	[142]
Benzophenones-3, -4, and -10	Not stated	7 patients with ketoprofen-induced photodermatitis	Study performed to evaluate possibility of cross-reactivity between ketoprofen and benzophenones and other chemicals. Patch tests (uninvolved skin of back) performed using Finn chambers. At 24 h post-application, separate series of patch tests exposed to suberythemal doses of UVB and UVA. Irradiated and non-irradiated sites evaluated at 72 h post-application.	All non-irradiated patch test results for each test substance were negative. Four and 2 patients had positive UVA photopatch tests to Benzophenone-3 and Benzophenone-10, respectively. Photopatch test results for Benzophenone-4 were negative.	[145]
Benzophenones-3 and -10	Not stated	214 patients (45 with photosensitivity dermatitis/actinic reticuloid syndrome; 54 with polymorphic light eruption)	From 1989 to 1991, patients patch tested with sunscreen series. Standard closed patch testing using Finn Chambers applied to upper back. Patches removed at 2 d, and readings made at time of patch removal and at 3 or 4 d.	16 patients reacted to 1 or more sunscreens. Benzophenone-3 and Benzophenone-10 accounted for 27 and 8 positive patch tests, respectively.	[141]

Table 12. Retrospective and multicenter studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenones-3 and -10	Not stated	62 patients (32 men and 30 women)	Retrospective analysis of positive photopatch test episodes undertaken using results retrieved from environmental dermatology database, and further verified with original archived patch test documentation for each individual patient. On day 0, standard photoallergens were applied to patient's back in duplicate. On day 2, patches removed, and one series irradiated with 5 J/cm^2 of broadband UVA (2.5 J/cm^2 used if history indicated clear episodes of severe photosensitivity or patient suspected of having chronic actinic dermatitis).	80 photoallergic reactions observed in 62 (2.3%) patients, with UV filters accounting for 52 positive reactions. 34 of the 62 patients (55%) had preceding underlying photodermatosis. Most common UV filter photoallergen was Benzophenone-3 (14 positive results), followed by Benzophenone-10 (9 positive results). Benzophenone-10 accounted for 13 allergic contact reactions, and Benzophenone-3 accounted for eight allergic contact reactions.	[143]
Benzophenones-3 and -10	Not stated	23 patients (with variety of photosensitive disorders)	Study conducted to determine threshold UVA elicitation dose in photopatch testing.	Benzophenone-3 and Benzophenone-10 produced positive responses at 0.7 and 1.07 J/cm^2, respectively. Isopropyl dibenzoyl dibenzoylmethane produced positive response at 1.0 J/cm^2. Results demonstrate that high doses of UVA (e.g., 10 to 15 J/cm^2) unnecessary, and that 5 J/cm^2 should become current standard.	[144]
Benzophenone-4	2%, 5%, and 10% in petrolatum (20 µl per concentration)	80 subjects	Phototoxicity test. Each test concentration applied to 8-mm diameter Finn chamber, secured with adhesive tape. Patches applied (in duplicate) for 2 d to upper back, i.e., on non-paravertebral skin to the left and right of the upper back. At time of patch removal, one side of back covered with UV-opaque material, while other side irradiated with UV light (5 J/cm^2; 99.2% UVA and 0.8% UVB). Reactions scored according to ICDRG grading scale.	One subject had weak positive reaction (+ reaction), with no concomitant erythema score, at irradiated site.	[115]
Benzophenone-4	10% (in petrolatum)	4857 patients	NACDG study. Patch testing performed using Finn chambers	Positive reaction rate of 2.1% (100 allergic reactions). Values for clinical relevance of allergic reactions: definite relevance (0), probable relevance (20 of 100 patients (20%)), possible relevance (53 of 100 patients (53%)), and past relevance (9 of 100 patients (9%)).	[127]
Benzophenone-10	2% (in petrolatum)	280 patients with photosensitivity (and other patients suspected of sunscreen dermatitis)	From February 1985 to March 1987, patients patch and photopatch tested with series of contact allergens and photoallergens. All tests read at 2 d, and, at this time, duplicate light series exposed to UVA (1 J/cm^2). Second and final reading of all tests carried out at 4 d.	During first 16 mo of the study period (February 1985 to May 1986), there were 2 patients who were allergic to Benzophenone-10. In remaining 10 mo, 4 patients were allergic to Benzophenone-10. Photopatch results for Benzophenone-10 were negative.	[146]

Table 13. Case reports

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-2	1% (in petrolatum)	1 female patient	Severe dermatitis observed in a female patient. Dermatitis worsened after sun exposure, and was accompanied by severe itching. Cosmetic contact dermatitis suspected and patch tests (protocol not stated) were performed.	Patch test results for Benzophenone-2 were positive. Reaction classified as +++ (strong reaction: erythema, papules, and vesicles) observed on days 2, 4, and 7.	[149]
Benzophenone-2	2% (in petrolatum)	1 male patient; 15 control subjects	Male patient presented with subacute chest and arm eczema after use of toilet water product. Repeated open application test (ROAT) performed.	ROAT of product elicited positive reaction after 2 applications. Patch testing with ingredient of product, Benzophenone-2, yielded positive reaction (++). No reactions observed in 15 control subjects.	[148]

Table 13. Case reports

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-2	Concentration not stated	3 patients	Epicutaneous tests performed on two patients (both with itching erythema) who had been using nail varnish and nail varnish remover, and one patient who had artificial nails (itching erythema at perionychium of several fingers; also marked erythema and edema).	The 3 patients had sensitization reactions to important allergens in nail varnish (toluenesulfonamide-formaldehyde resin), nail varnish remover (Benzophenone-2), and artificial nails (ethyl acrylate), respectively. Symptoms and skin changes disappeared when use of 3 items discontinued.	[146]
Benzophenone-3	2% (in petrolatum)	1 female patient	After sunscreen applied, patient experienced itching and a burning sensation of the nose, cheeks, and dorsa of the hands after 3 h of direct sun exposure. Open photopatch testing (2 cm^2 area on forearm) performed.	Open photopatch testing of sunscreen produced erythematous, papular response 24 h after single exposure to UVA (25 J/cm^2), suggesting photoallergy. Patch testing with ingredient of sunscreen, Benzophenone-3, yielded a +++ reaction. Histology of biopsy from Benzophenone-3 photopatch-test reaction showed striking epidermal spongiotic response and vesicle formation, with absence of vacuolation and sunburn cells. Prominent mononuclear inflammatory cell filtrate observed in dermis.	[151]
Benzophenone-3	3% (in petrolatum)	1 male patient	Patient history: intensely pruritic bilateral lip; perioral, cheek, ear, hand, and forearm dermatitis; and painful ulcerations of the oral mucosa. On examination, 1 to 4 mm papules and papulovesicles (coalescing into edematous plaques) present on dorsal hands, fingers, volar wrists, dorsal forearms, and upper arms. Patch testing performed according to NACDG methods, using Finn chambers secured with tape.	Strong patch test at 48 h (+++ reaction) and 96 h (+++ reaction).	[154]
Benzophenone-3	10% (in petrolatum)	1 female patient	Patient experienced anaphylactic reaction (generalized wheals) 15 min after applying sunscreen all over her body. Patient previously had pruritus and erythema within 30 min of contact with garment exposed to sunscreen. Patch testing performed. Assay for the detection of IgE to Benzophenone-3 performed by incubating Benzophenone-3 with human serum albumin	Patch testing resulted in urticarial reaction at test site within 20 min, but no anaphylaxis. No specific IgE to Benzophenone-3 detected.	[155]
Benzophenone-3	Not stated	1 female patient	Erythema and blistering (at application site) observed after a application of ketoprofen gel topically to right popliteal fossa and right shoulder. After intermittent exposure to sunlight (over 24-h period), eruption extended to involve the legs, neck, hands, and other parts of body. Authors noted that, when irradiated with sunlight, ketoprofen is broken down into various benzophenones that are structurally related to Benzophenone-3. Patient patch tested using Finn Chambers on Scanpor tape. Photopatch testing (irradiation with 6 J/cm^2 UVA) also performed	Positive patch and photopatch test reactions to ketoprofen (up to 2% in petrolatum) reported. Negative patch test results for Benzophenone-3; however, positive photopatch test reaction to Benzophenone-3 (+++) reported on day 4.	[150]
Benzophenone-3	Not stated	1 female patient; 2 control subjects	Patient had history of atopic dermatitis and allergic rhinoconjunctivitis. Anaphylaxis (with generalized cutaneous wheal and flare reaction) observed after widespread application of sunscreen. A few days before testing, patient experienced contact urticaria. Patch testing (blinded and non-blinded, on normal skin) and prick tests performed.	Patch testing with the sunscreen yielded 2-cm diameter wheal and flare reaction within 5 min. Blinded patch testing with Benzophenone-3 (sunscreen ingredient) induced wheal (16 mm) and flare reaction after 15 min. Non-blinded patch tests for Benzophenone-3 in 2 control subjects yielded negative results. In prick tests, results for sunscreen and Benzophenone-3 were positive (wheal, 6 x 7 mm).	[152]
Benzophenone-3	Not stated	1 female patient; 5 control subjects	Acute, itchy rash observed on face, trunk, and limbs after application of sunscreen to daughter's skin. Patient also subsequently applied 'false tan' product to and developed severe cutaneous and systemic anaphylactic reaction. Patient patch tested with Benzophenone-3 and reactions scored 20 min after patch application.	Acute urticarial wheal and flare (50 mm) reaction observed at 20 min, and reaction settled within 1 h after oral drug treatment. Patch testing of 5 control subjects with Benzophenone-3 did not reveal reactivity at 20 min, 48 h, or 96 h.	[153]

Table 13. Case reports

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-3	Not stated	1 female patient	In same report, patient with 1-year history of perioral itching and erythema, and 3-d history of erythematous swelling over face and front of her neck. Patient had been sitting in sun for a few hours, several days before swelling began. Had also used lip balm and shampoo, both of which contained Benzophenone-3. Perioral itching resolved within several days after discontinuing lip balm. Facial erythema improved after shampoo replaced with another that did not contain benzophenone. Patch and photopatch testing performed	1+ reaction to Benzophenone-3 at both patch and photopatch test sites.	[158]
Benzophenones-3 and -4	10% (in petrolatum)	1 female hairdresser	Hand dermatitis observed over 2-year period. When use of hair care products with sun protection ceased, dermatitis began to improve. Patch testing performed	Patch testing with Benzophenone-4 yielded positive (++) reaction. Negative patch test results for Benzophenone-3.	[157]
Benzophenones-3 and -4	Not stated	1 male patient	History of persistent erythema on light-exposed skin after application of sunscreen on several occasions. Photopatch testing with sunscreen ingredients performed. Finn chambers applied to back, followed by UVA irradiation (dose = 10 J/cm^2) at 24 h. Reactions scored at 20 min, and 24 h, 48 h, and 72 h post-irradiation	Photopatch test results negative Benzophenone-4. Photopatch test results for Benzophenone-3 were: + (at 24 h), ++ (at 48 h), and +++ (at 72 h).	[155]
Benzophenones-3 and -4	Not stated	1 female patient	Patient with 2- to 3-yr history of intermittent burning and pruritic facial eczema. Erythema of the cheeks bilaterally and on the neck, and minimal scale (but no vesicles) observed. Patient had used facial moisturizer and shampoo, both of which contained Benzophenone-3, for 2 yr. Burning, itching, and erythema resolved when avoided contact with benzophenones in personal care products avoided. Patch testing and photopatch (10 J of UVA exposure) testing performed using Finn chamber technique. Results scored on days 3 and 7	Results significant for 2+ photocontact reaction to Benzophenone-3. No reaction to Benzophenone-3 at non-irradiated site. Immediately after irradiation, urticaria at Benzophenone-3 photopatch test site observed. Reaction consistent with photoallergic contact urticaria. Questionable photocontact reaction to Benzophenone-4.	[158]
Benzophenones-3, -4, and -10	Not stated	1 female patient	Patient presented with eyelid dermatitis for 1 yr and facial dermatitis for 2 mo. Patch tests performed	Patch test results: Benzophenone-3 (++), Benzophenone-4 (+), and Benzophenone-10 (negative results).	[159]
Benzophenones-3, -8, and -10	Not stated	1 female patient	Patient referred for phototesting and patch testing after recurrent episodes of dermatitis and systemic symptoms. First episode (at 24 h after application of sunscreen) described as follows: edematous, painful pruritic eruption on the arms and neck; voice changes; and tachycardia. NACDG patch and photopatch test panels applied.	At 2 h after patch application and 1 h later, the patient experienced the following: raspy voice, dry mouth, difficulty with swallowing, and tachycardia. Severe urticarial reactions (and systemic symptoms) to Benzophenones-3, -8, and -10 at test sites observed. Because of the severe reactions, UVA irradiation not completed. Authors stated that immediate reactions to and systemic symptoms caused by these benzophenones are rare.	[162]
Benzophenones-3 and -10	Not stated	1 female patient	Face eczema developed after use of cosmetic cream. Patch tests performed using polyethylene chamber secured with tape. Reactions scored on days 2 and 4 according to ICDRG methodology. Photopatch testing also performed, test substances applied in duplicate. Test substances removed after 24 h, and sites irradiated with UVA (5 J/cm^2). Reactions scored at day 1 and day 3 post-irradiation	For Benzophenone-3, positive patch test (++) and photopatch test (+++) reactions reported. For Benzophenone-10, patch test results negative, but photopatch test results positive (+++ reaction).	[161]
Benzophenone-4	Not stated	1 patient (diver)	Case of acute facial swelling after ascending to surface of water. Standard patch test for contact dermatitis performed. yielded a positive reaction to Benzophenone-4.	Positive reaction to Benzophenone-4.	[159]

Table 14. Other clinical reports

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-3	Not stated	1598 participants	A study was performed to identify association between exposure to potentially endocrine-activating chemicals and age of menarche in adolescent girls. Participants had completed the reproductive health questionnaire and laboratory examination for the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) for years 2003 to 2008. Exposures were assessed based on creatinine-corrected natural log urine concentrations of selected environmental chemicals and metabolites found in at least 75% of samples in this study sample. The weighted mean age of menarche was 12 yr of age.	Results for Benzophenone-3 indicated that exposure to this chemical was not significantly associated with the age of menarche.	[163]
Benzophenone-3	Not stated	588 participants	Association of Benzophenone-3 with serum total testosterone levels examined using child and adolescent participants in NHANES (2011–2012). Multivariable linear regression performed to estimate associations between natural log–transformed serum testosterone and quartiles of urinary Benzophenone-3 in male and female children and adolescents. Serum testosterone analyzed by isotope dilution LC-MS/MS, and was natural log–transformed for analyses because distribution of this variable was skewed left. Spot urine samples collected from study participants, and Benzophenone-3 measured by solid phase extraction, coupled on-line to HPLC/MS/MS. Statistical tests for linear trends were conducted by modeling quartiles as an ordinal variable using integer values.	Male adolescents in 3rd and 4th quartiles of Benzophenone-3 had statistically significantly lower testosterone than males in lowest quartile. Although the association was strongest for 3rd quartile, overall trend was statistically significant (p-trend = 0.01). In female adolescents, testosterone was statistically significantly higher for girls in second versus first quartile of Benzophenone-3 exposure, but positive associations were closer to the null and nonsignificant for the 3rd and 4th quartiles of exposure (p-trend = 0.14). No significant associations between testosterone and Benzophenone-3 in male or female children, and no evidence of consistent trends with increasing quartiles of exposure. Thus, Benzophenone-3 was associated with statistically significantly lower testosterone in adolescent boys only. Authors concluded that urinary levels of Benzophenone-3 were associated with lower levels of serum testosterone in male adolescents.	[164]
Benzophenone-3	Not stated	200 girls	The influence of Benzophenone-3 and other chemicals on age of menarche was studied.	Log w/v increase in childhood (pre-pubertal) urinary levels of Benzophenone-3 associated with decreased time to menarche. Benzophenone-3 urinary concentrations not reported.	[165]
Benzophenone-3	Not stated	476 mothers (had participated in birth cohort between 2006 and 2008)	Association between maternal urinary phenol concentrations during pregnancy and fetal growth studied	Association between urinary Benzophenone-3 and lower abdominal circumference in males was made. However, authors noted that this association should be verified in larger study populations with planned repeated ultrasound measures during pregnancy.	[166]
Benzophenone-3	Not stated	Cohort of 922 pregnant women	Longitudinal cohort study involving 338 children performed to evaluate association between prenatal exposure to Benzophenone-3 and gestation age and birth weight. Relationships between birth outcomes and urinary concentrations of Benzophenone-3 evaluated. Urinary Benzophenone-3 measured at 3 time points in pregnancy (visit 1: 16 - 20 wk; visit 2: 20 - 24 wk; visit 3: 24 - 28 wk). Multiple linear regression (MLR) models performed to regress gestational age and birthweight z-scores against each woman's log average concentrations of exposure biomarkers. Logistic regression models performed to calculate odds of preterm birth, small or large for gestational age (SGA and LGA), in association with each of the exposure biomarkers. Results transformed into change in the birth outcome for inter-quartile-range difference in biomarker concentration (Δ)	Average Benzophenone-3 urinary concentrations associated with an increase in gestational age.	[167]

Table 14. Other clinical reports

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-3	Not stated	338 children	A longitudinal cohort study for determining an association between urinary phthalates, parabens, and phenols found in personal care products with pubertal timing in girls and boys was performed.	No such association relating to urinary Benzophenone-3 found.	[168]
Benzophenone-3	Not stated	473 mother-son pairs (in cohort)	Placental weights and birth weights were available for cohort whereby Benzophenone-3 was measured in spot urine samples. Urine collected between wk 23 and 29 of gestation.	Positive association between Benzophenone-3 and both placental weight and child birth weight observed.	[168]
Benzophenone-3	Not stated	417 females and 229 males (participants in EARTH study, who gave birth to 418 singleton infants between 2005 and 2018)	Study performed to examine whether maternal and paternal pre-conception urinary concentrations of Benzophenone-3 (e.g., from dietary and personal care product exposure) and other chemicals associated with risk of preterm birth among couples attending fertility care. Mothers and fathers provided average of 4 and 3 urine samples during the preconception period, respectively. Geometric mean of Benzophenone-3 calculated to estimate preconception exposure of each participant. Risk ratios (RRs) of preterm birth (live birth before 37 completed weeks of gestation) estimated using modified Poisson regression models adjusted for covariates. Mean gestational age among singletons was 39.3 (1.7) wk and 8% born preterm.	No consistent pattern of association observed for Benzophenone-3 in either parent.	[170]
Benzophenones-4, and -10	10% (in petrolatum)	15 eczematous dermatitis patients	Photoallergenicity testing performed at least 3 mo after complete disappearance of the dermatitis. In photopatch tests, test substance applied to back, under occlusion, over 2-d period. At 24 h, occlusive patch removed and site exposed to UVA (5 J/cm^2). Reactions scored at 48 h and 96 h (day 2 and day 4).	No positive reactions to Benzophenone-4. 3 subjects with positive reactions to Benzophenone-10.	[171]

Table 15. Epidemiological studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenone-1, -2, -3, and -8	Not stated	413 men	Urine and semen samples (years 2005 to 2009) provided, and relationship between benzophenone urinary concentrations and semen quality studied. Linear mixed models with fixed and random effects used to assess changes in semen endpoints associated with benzophenones quantified in the urine. Investigators estimated change (β-coefficients and accompanying 95% CI) in semen endpoints (e.g., sperm concentration, total sperm count, and sperm motility) for men above the 75th percentile for each benzophenone concentration relative to men below this percentile. Initially, regression models run, including only the benzophenone and creatinine concentrations. Rationale for modeling creatinine continuously was to account for the interindividual variation in concentration, to more closely reflect men's urinary dilution while preserving statistical power	Benzophenone-2 associated with findings such as diminished sperm concentration, more immature sperm, and decreased percentage of other tail abnormalities. Benzophenone-8 associated with decreased hypoosmotic swelling and higher acrosome area. No associations observed for Benzophenone-1 or Benzophenone-3. Overall, authors noted that Benzophenone-2 and Benzophenone-8 associated with changes in semen endpoints, including sperm concentration, sperm viability, motility, sperm head, and morphology. They also noted that whether such changes are sufficient to affect couple fecundity, as measured by the time needed to achieve pregnancy, or other couple-dependent fertility outcomes, remains to be established.	[174]

Table 15. Epidemiological studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Benzophenones-1, -2, -3, and -8, and 4-hydroxybenzo-phenone (not a cosmetic ingredient)	Not stated	215 male students	Cross-sectional study performed to examine associations between urinary concentrations of benzophenone-type UV filters and semen quality and reproductive hormone levels. Urine, blood, and semen samples provided on single day. Semen quality evaluated by measuring volume, sperm counts, motility, and morphology. Serum samples analyzed for the following reproductive hormones: FSH, LH, testosterone (T), inhibin B, and E2. Associations between urinary benzophenone concentrations, semen quality parameters, and reproductive hormone levels examined using linear regression, adjusting for potential cofounders	97% of men tested had detectable urinary concentrations of at least 1 of the 5 benzophenone filters quantified. After adjusting for important covariates (i.e., body mass index, smoking status, and time of blood sample collection), the following results were reported: statistically significant positive association between urinary Benzophenone-1 and Benzophenone-3 concentrations and serum FSH levels; urinary Benzophenone-1 concentration statistically significantly positively associated with T/E2; and urinary Benzophenone-1 concentration negatively associated with inhibin B/FSH ratio. Authors concluded that, in young men, urinary benzophenone-type UV filters may be associated with modest alteration of some reproductive hormones, but reported effects on reproductive function are likely to be small, and of unclear clinical significance.	[175]
Benzophenone-1 and Benzophenone -3	Not stated	300 men	Presence of UV filters in semen, serum, and the urine studied. Samples collected during February to December of 2013, and only 6 of the men had used sunscreen during the 48 h preceding sample collection.	Benzophenone-1 and Benzophenone-3 detected in 19% and 27% of the seminal fluid samples, respectively, albeit at levels of 1 to 2 orders of magnitude lower than were detected in urine. For Benzophenone-1 and Benzophenone-3, levels in the urine and seminal fluid were significantly correlated. Authors concluded that chemical UV filters are present in men's seminal fluid, some of which can activate human sperm-specific CatSper Ca^{2+} channel (calcium cation channel of sperm) and thereby potentially interfere with the fertilization process.	[62]
Benzophenone-3	Not stated	877 idiopathic infertile men and 713 fertile controls	Case-control study on idiopathic male infertility and exposure to phenols in the environment. Urinary concentrations and semen parameters (semen volume, sperm concentration, and sperm number per ejaculate) measured	No evidence of association between exposure to Benzophenone-3 and idiopathic male infertility.	[170]
Benzophenone-3	Not stated	423 patients	Urinary levels of Benzophenone-3 and the incidence of Hirsch-sprung's disease investigated in patients in China. Hirschsprung's disease is a neonatal intestinal abnormality that is derived from a failure of enteric neural crest cells migration during embryogenesis from 5 to 12 wk.[52] Patients tested for Benzophenone-3 in urine via spot test, and then divided into groups based on presence of Hirschsprung's disease. Group 1 comprised 101 neonates with Hirschsprung's disease who presented with intestinal obstruction and chronic constipation, and were treated with surgery. Group 2 comprised 103 surgical control infants without Hirschsprung's disease. Third group (Group 3, non-surgical control) consisted of 219 neonates without Hirschsprung's disease.	Results indicated positive association between women identified with medium to high levels of Benzophenone-3 (maximum detection level = 22,800 ppb) in the urine and the incidence of Hirschsprung's disease.	[173]

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