Amended Safety Assessment of Benzophenones as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: August 21, 2020
Panel Date: September 14-15, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, CIR and Jinqiu Zhu, Ph.D., Toxicologist.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst, CIR
Date: August 21, 2020
Subject: Amended Safety Assessment of Benzophenones as Used in Cosmetics

The Expert Panel for Cosmetic Ingredient Safety (Panel) first reviewed the safety of benzophenones in 1981. The Panel subsequently published a final report (in 1983) with a conclusion stating 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. In the same year, the Panel published an addendum to the final report, 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 Cosmetic Ingredient Review (CIR) Procedures, because it had been at least 15 years since the safety assessment was published, the Panel considered whether the safety assessment of benzophenones should be reopened at the September 2001 Panel meeting. At the conclusion of their discussion, the Panel determined to not reopen the 1983 published safety assessment until results from National Toxicology Program (NTP) carcinogenicity studies on benzophenones were available. A re-review summary with this determination was published in 1985.

Because an NTP oral carcinogenicity study on Benzophenone-3 was published earlier this year, the Panel is asked to determine whether the 1983 published safety assessment should be reopened to include these and other current safety test data, and to add Benzophenones-7, -10, and -12, along with any available safety test data on these 3 ingredients. These data are included in the attached Draft Amended Report (*benzop092020rep*) for the Panel's review. This report also contains summaries of data from the published final report and addendum (indicated by *italicized text*), as well as studies dated 1983 forward. The published final report, addendum, and re-review summary (*benzop092020orig1*, *benzop092020orig2, and benzop092020orig3*, respectively) are included for your reference. Of the more recent data in the Draft Amended Report are 2020 ingredient use frequency data from FDA (*benzop092020FDA*).

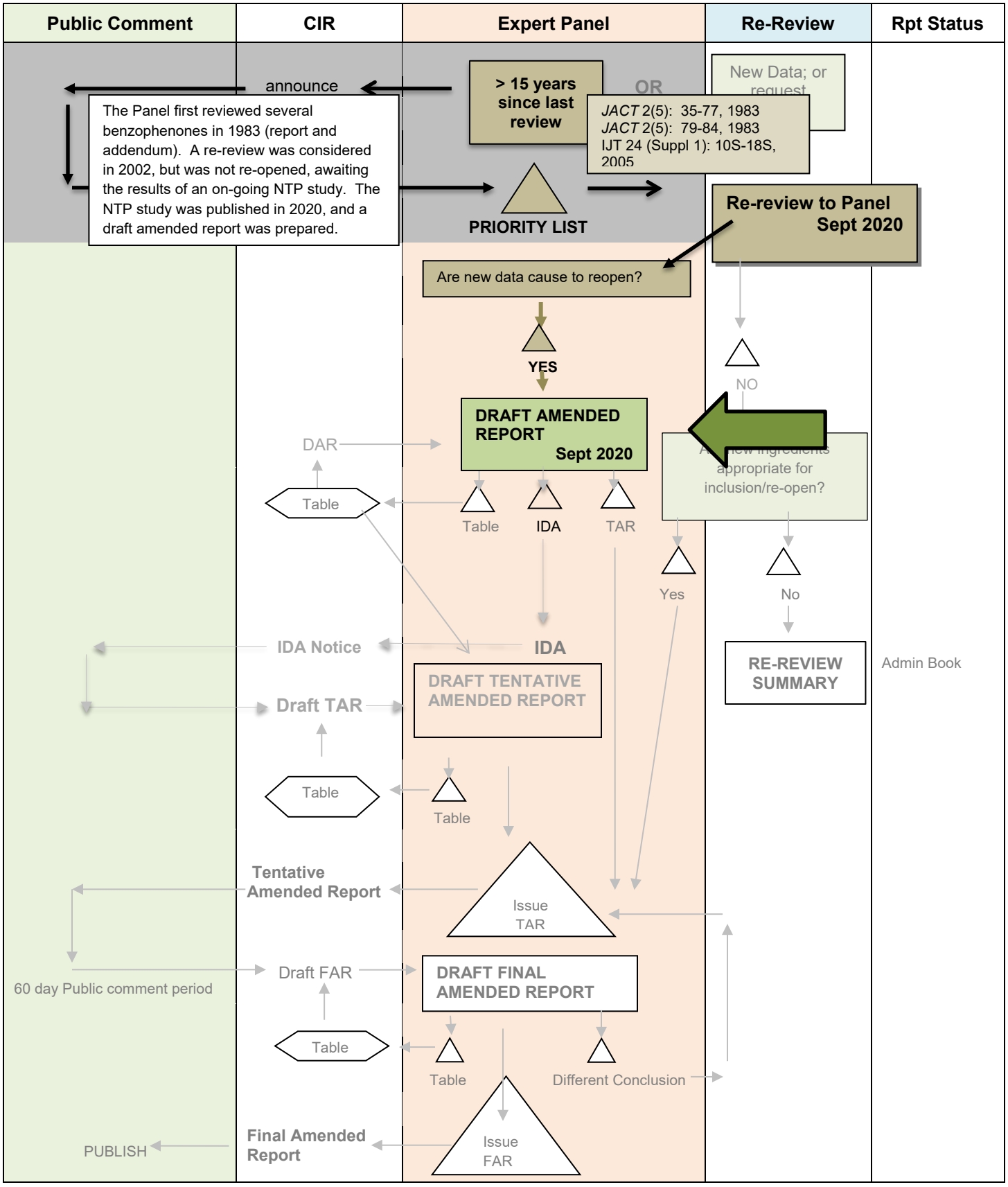
Also included in this package for your review are the report history (*benzop092020hist*), flow chart (*benzop092020flow*), literature search strategy (*benzop092020strat),* 2020 FDA VCRP data (*benzop092020FDA*), meeting minutes relating to the original reviews as well as the Panel's decision on re-opening the safety assessment (*benzop092020min*), data from the Council's 2020 use concentration survey on benzophenones (*benzop092020data1*), and the ingredient data profile (*benzop092020prof*). This profile identifies information from the original report as well as any new information that was identified since that original report was issued.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Amended Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Benzophenones-1 through -12

MEETING September 2020



*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

CIR History of:

Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12

Panel: June of 1981

The Panel agreed there were sufficient data to make a safety determination on Benzophenones-1, -3, -4, -5, -7, -9, -10, -11, and -12. However, there was discussion of the need for additional mutagenicity testing on Benzophenones-2, -6, and -8.

It was suggested the report be reconsidered by the Panel after the additional testing is done by industry; however, if testing is not done, the report would have to be separated, and Benzophenones-2, -6, and -8 deleted from the recommendation. An insufficient data report would be issued for Benzophenones -2, -6, and -8.

Tentative Report, Panel: November of 1981

The following conclusion was unanimously accepted: "On the basis of the available animal data and clinical human experience presented in this report, the Panel concludes that Benzophenone-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics."

Benzophenones-7, -10, and -12 were deleted from the approved safety recommendation and from the title of the report since they are not used in cosmetics. However, data on Benzophenones-7, -10, and -12 were left in the report as useful information because they are chemically similar to the other Benzophenones.

The Panel agreed that, subject to minor revisions, the document will be issued as a Tentative Report for a 90-day comment period.

With regard to Benzophenones-2, -6, and -8, the Panel recommended an Insufficient Data Report. The Panel reviewed the available mutagenesis data on Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12, and found that the available data on Benzophenones-2, -6, and -8 were equivocal. Additional mutagenic testing is required for these three ingredients in a mammalian system, with Benzophenone-1 to be included in the study as a control.

It should be noted that the Panel published a final report on 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.

Final Report Addendum, Panel: December of 1982

The Expert Panel evaluated the mutagenicity data submitted in response to the Insufficient Data Report of November 25, 1981 on Benzophenones-2, -6, and -8, and found them to be adequate for an assessment of safety. The following conclusion of the report was unanimously approved: "The Panel concludes that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic, and that the conclusion for the Final Report on the Safety Assessment of 1, -3, -4, -5, -9, and -11 which states 'on the basis of the available animal data and clinical human experience presented in this report, the Panel concludes that Benzophenone-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics' is also applicable to these ingredients."

It should be noted that the Panel published an addendum to the final report (in 1983), 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.

Re-review, Teams/Panel: September of 2001

The Panel determined to not reopen the 1983 published safety assessment until results from National Toxicology Program (NTP) carcinogenicity studies on Benzophenones are available.

Draft Amended Report, Teams/Panel: September 14-15, 2020

The Draft Amended Report also contains use concentration data that were received from the Council and results from the 2020 NTP oral carcinogenicity study on Benzopenone-3.

Benzophenones Data Profile* –September 14-15, 2020 Panel – Wilbur Johnson, Jr.																																
	Use				Toxico-kinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	New Rpt	Old Rpt	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Benzophenone-1	**595**	**142**		O			X		O X			O X			X	O X		X			O	O			O					X
Benzophenone-2	**103**	**299**		O		X	X		O			X			X	O X	O				O	O			O	O X		O		X
Benzophenone-3	**989**	**47**		O		X	O X	O X	O X		X	O X		X	X	O X	X		X	X	O X	O		O X	O X	O X		O X	X	X
Benzophenone-4	**2259**	**240**		O		X	X	O	O X			X			X	O				X	O	O X			O X	O X		O X		X
Benzophenone-5	**14**	**10**																														
Benzophenone-6	**0**	**90**					X		O							O X	O				O	O			O			O		
Benzophenone-7	**0**	**0**																														
Benzophenone-8	**0**	**4**		O			X	O	O X			O X				O X	O				X	O	X		O	O	X			X
Benzophenone-9	**71**	**123**		O					O							O					O	O						O		
Benzophenone-10	**0**	**0**																							X	X				X
Benzophenone-11	**0**	**168**		O					O							O					O	O			O			O		
Benzophenone-12	**0**	**0**					O	O	O X			O X			X	X					X	O		X	O			O X		

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

[

BENZOPHENONES – 03/25/2020;6/17-22/2020]

Ingredient	CAS #	InfoBase	SciFinder	PubMed**	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECE-TOC	Web
Benzophenone-1	131-56-6	Yes		92/19			No	Yes	Yes (REACH dossier)	No	No	No	No	No	No	No	No	
Benzophenone-2	131-55-5	Yes		96/32		Yes	No	No	No	No	No	No	No	No	No	No	No	
Benzophenone-3	131-57-7	Yes		547/120			Yes	Yes	Yes (REACH dossier)	No	No	No	No	Yes	Yes	No	No	
Benzophenone-4	4065-45-6	Yes		124/17			Yes	Yes	Yes (REACH dossier)	No	No	No	No	No	Yes	No	No	
Benzophenone-5	6628-37-1	Yes		35/2			Yes	No	No	No	No	No	No	No	No	No	No	
Benzophenone-6	131-54-4	Yes		6/1			No	Yes	Yes (REACH dossier)	No	No	No	No	No	No	No	No	
Benzophenone-7*	85-19-8	Yes		0			No	No	Yes (REACH dossier)	No	No	No	No	No	No	No	No	
Benzophenone-8	131-53-3	Yes		25/1			No	Yes	Yes (REACH dossier)	No	No	No	No	No	No	No	No	
Benzophenone-9	76656-36-5	Yes		2/1			No	No	No	No	No	No	No	No	No	No	No	
Benzophenone-10*	1641-17-4	Yes		9/6			No	No	No	No	No	No	No	No	No	No	No	
Benzophenone-11	1341-54-4	Yes		0			No	No	No	No	No	No	No	No	No	No	No	
Benzophenone-12*	1843-05-6	Yes		4/1		Yes	No	Yes	Yes (REACH dossier)	No	No	No	No	No	No	No	No	

*Search all years; remaining ingredient searches (2002 forward)
**PubMed + toxline archive searched

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable
Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/
RIFM (the Research Institute for Fragrance Materials) should be contacted

Qualifiers

Absorption	Genotoxic	Sensitization
Acute	Irritation	Skin
Allergy	Metabolism	Subchronic
Allergic	Mutagen	Teratogen
Allergenic	Mutagenic	Teratogenic
Cancer	Penetration	Toxic
Carcinogen	Percutaneous	Toxicity
Chronic	Pharmacokinetic	Toxicokinetic
Development	Repeated dose	Toxicology
Developmental	Reproduction	Tumor
Excretion	Reproductive	

JUNE 1981 PANEL MEETING

Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12

The Panel agreed there were insufficient data to make a safety determination on Benzophenones-1, -3, -4, -5, -7, -9, -10, -11, and -12. However, there was discussion of the need for additional mutagenicity testing on Benzophenones-2, -6, and -8.

Mr. McNerney agreed to respond to the Team members request for such data as soon as possible.

Dr. Elder suggested the report be reconsidered by the Panel after the additional testing is done by industry; however, if testing is not done, the report would have to be separated, and Benzophenones-2, -6, and -8 deleted from the recommendation. An insufficient data report would be issued for Benzophenones -2, -6, and -8.

NOVEMBER 1981 PANEL MEETING

Benzophenones-1, -3, -4, -5, -9, and -11

On motion by Dr. Bergfeld, seconded by Dr. Montagna, the following conclusion was unanimously accepted: "On the basis of the available animal data and clinical human experience presented in this report, the Panel concludes that Benzophenone-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics."

Benzophenones-7, -10, and -12 were deleted from the approved safety recommendation and from the title of the report since they are not used in cosmetics. However, data on Benzophenones-7, -10, and -12 were left in the report as useful information because they are chemically similar to the other Benzophenones.

Subject to minor revisions, the document will be issued as a Tentative Report for a 90-day comment period.

With regard to Benzophenones-2, -6, and -8, the Panel recommended an Insufficient Data Report. On motion by Dr. Bergfeld, seconded by Dr. Roudabush, the following statement was unanimously approved: "The Panel reviewed the available mutagenesis data on Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12, and found that the available data on Benzophenones-2, -6, and -8 were equivocal. Additional mutagenic testing is required for these three ingredients in a mammalian system, with Benzophenone-1 to be included in the study as a control."

DECEMBER 1982 PANEL MEETING

Benzophenones-2, -6, and -8

The Expert Panel evaluated the mutagenicity data submitted in response to the Insufficient Data Report of November 25, 1981 on Benzophenones-2, -6, and -8, and found them to be adequate for an assessment of safety. The following conclusion of the report was unanimously approved: "The Panel concludes that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic, and that the conclusion for the Final Report on the Safety Assessment of 1, -3, -4, -5, -9, and -11 which states 'on the basis of the available animal data and clinical human experience presented in this report, the Panel concludes that Benzophenone-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics' is also applicable to these ingredients."

Subject to minor revisions, the document will be issued as an Addendum to the Final Report on Benzophenone-1, -3, -4, -5, -9, and -11, for a 90-day comment period.

SEPTEMBER 2002 PANEL MEETING

Benzophenone and Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12

The CIR Expert Panel has issued a Final Report on the safety of Benzophenones -1, -3, -4, -5, -9, and -11 in cosmetics, and an addendum to this Final Report to include Benzophenones -2, -6, and -8. The original Final Report Conclusion is stated as follows: 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. Additionally, the following conclusion is stated in the Final Report Addendum: The Panel concludes that Benzophenones-2, -6, and -8 are not mutagenic or genotoxic, and that the conclusion for the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11, which states "On the basis of the available animal and clinical human experience presented in this report, the Panel concludes that Benzophenones-1, -3, -4, -5, -

9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics" is also applicable to these three ingredients.

Dr. Marks said that his Team determined that the Panel should postpone any decision as to whether or not the Final Safety Assessment on Benzophenones should be reopened until after the NTP carcinogenicity studies have been completed. An NTP 2-year carcinogenicity study on Benzophenone was initiated in 1999, and the pathology quality assessment for this study is ongoing. Benzophenone-3 is listed among the chemicals that NTP has assigned to a laboratory for toxicology/carcinogenesis testing.

Dr. Marks added that during the waiting period for these studies, new photoallergic data should be captured and photosensitivity frequency should be monitored.

Dr. Belsito said that, in light of the ongoing NTP studies, a decision as to whether or not the safety assessment should be reopened cannot be made at this meeting.

Regarding the new data included in the re-review document on Benzophenones, Dr. Belsito said that his Team did not express concern over any of the findings, including the reported incidence of allergic contact dermatitis (low incidence). He then asked whether Dr. Marks' request that CIR monitor photosensitivity frequency mean that CIR should track every publication that discusses the photosensitization potential and phototoxicity of Benzophenones.

Dr. Marks said that after the NTP carcinogenicity studies have been received and the safety assessment has been formally reopened, the Panel could then revisit the photoallergy data.

Dr. Slaga added that, according to the re-review document, Benzophenone and Benzophenone-3 have weak estrogenic activity, and this is one of the reasons why NTP wanted to test these chemicals.

Dr. McEwen suggested that the Panel not reopen the safety assessment at this meeting, with the proviso that the re-review document will be updated and reviewed again by the Panel after the NTP studies have been made available.

The Panel unanimously agreed that the Panel's Final Safety Assessment on the Benzophenones should not be reopened until results from the NTP carcinogenicity studies are available.

Amended Safety Assessment of Benzophenones as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: August 21, 2020
Panel Date: September 14-15, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, CIR and Jinqiu Zhu, Ph.D., Toxicologist.

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INTRODUCTION

The Expert Panel for Cosmetic Ingredient Safety (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.[1] During the same year, the Panel also published an addendum to this published 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 Cosmetic Ingredient Review (CIR) Procedures & Support to the Expert Panel for Cosmetic Ingredient Safety, 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 on Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12 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] It should be noted that the published re-review summary includes 3 other benzophenones (Benzophenones-7, -10, and -12) that were found in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*) after publication of the original safety assessments on benzophenones.

The NTP carcinogenicity study on Benzophenone-3 was published in May 2020, and results from this study are included in the current safety assessment for the Panel's review. Other safety test data on Benzophenone-3, as well as data on Benzophenones-1, -2, -4, -5, -6, -7, -8, -9, -10, -11, and -12 that have been identified in the published literature since the original safety assessment was published in 1983, are also included. Data from the published CIR safety assessments on benzophenones appear, in *italics*, at the beginning of sections in the report text. (This information is not included in 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 *Dictionary*, the benzophenones reviewed in this safety assessment function mainly as light stabilizers in cosmetic products, but also as sunscreens (see Table 1).[4] In the United States, sunscreens are active ingredients in over-the-counter (OTC) drug products, and are not cosmetic ingredients.

The published data in this document were identified by conducting an exhaustive search of the world's literature from year 1983 forward. A list of the typical search engines and websites used, sources explored, and endpoints that Panel evaluates, is available 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 several of the benzophenones 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, methyl, and chloride 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.

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 United States (US) Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient 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.[11] 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.[12] The concentration of use survey on benzophenones was conducted for ingredient use as a light stabilizer, but not as a sunscreen.

In the 1983 original report, Benzophenone-2 was the benzophenone with the highest reported use frequency (229 uses total).[1] In 2020, Benzophenone-4 was the benzophenone with the highest reported use frequency (2259 uses total).[10] The use frequency of Benzophenone-2 (299 uses total) in the 1983 original report decreased to a value of 103 in 2020. The use frequency of Benzophenone-4 (240 uses) in the 1983 original report increased substantially to a value of 2259 in 2020. Of the ingredients reviewed in the1983 report, Benzophenone-4 had the highest use concentration (≤ 10% in suntan gels, creams and liquids (leave-on products)).[1] In 2020, Benzophenone-4 is the benzophenone with the highest reported use concentration, and is used at substantially lower concentrations of up to 1.6% in other non-coloring hair preparations (leave-on products).[12] Frequency and concentration of use data are presented in Table 3.

According to VCRP and Council survey data, the following 6 ingredients are not currently in use in cosmetic products: Benzophenone-6, Benzophenone-7, Benzophenone-8, Benzophenone-10, Benzophenone-11, and Benzophenone-12.

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 ingredient use concentrations of 0.05 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%), pump hair spray (maximum concentration of 0.05%), and in pump deodorant spray (at maximum concentration of 0.08%). A higher concentration (0.48%) is stated for perfumes, but not all perfumes are spray products. Thus, there is uncertainty that the 0.48% concentration is for a spray product. 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%). 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 (Bremmer et al 2006).[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 10% Benzophenone-3 (as UV filter) is allowed in ready for use preparations. Benzophenone-4 and Benzophenone-5 are allowed in these products at concentrations up to 5% (as acid).

Non-Cosmetic

According to the US FDA, the following benzophenone are allowed in sunscreens as active ingredients within the concentration specified for each ingredient: Benzophenone-3 (up to 6%), Benzophenone-4 (up to 10%), and Benzophenone-8 (up to 3%) (21 CFR 352.10). on February 26, 2019, the FDA published a proposed rule to establish final monograph regulations for over-the-counter (OTC) sunscreen drug products (84 FR (38) 6204).[21] The rule 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 (c).

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Benzophenone-3

Sunscreen products were applied to excised human epidermis in Franz diffusion cells, with the amount penetrating into and across the epidermis assessed by high performance liquid chromatography (HPLC) for 8 h following application.[22] All sunscreen agents investigated penetrated into the skin (0.25 g/m^2 or 14% of applied dose). Only 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 (LP), coconut oil (CO), 50:50 ethanol:coconut oil (50:50 EC), aqueous cream (AC), and oily cream (OC). 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 EC 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: LP > OC > 50:50 EC > CO. For Benzophenone-3 penetration across epidermal membrane, LP was greater than 50:50 EC; however, the difference between the 2 vehicles was not statistically significant. For the remaining vehicles, the order of flux (high to low) was OC > AC > CO. 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 CO and 9.97% from LP. A comparison of the maximum amount of Benzophenone-3 that penetrated indicated that LP and CO statistically significantly differed from each other and the remaining formulations. The highest Benzophenone-3 skin retention was observed for 50:50 EC. 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.

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.[24] 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 skin absorption parameters of intrinsically aged skin and young skin were comparable.

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.[25] 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 oil-in-water emulsion than from the water-in-oil emulsion.

Benzophenone-3 and Benzophenone-4

Static diffusion cells were used to evaluate the skin penetration of Benzophenone-3 and Benzophenone-4 in vitro.[26] 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.[27] 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. These results indicate that Benzophenone-3 penetrated very quickly and saturated the stratum corneum in less than 30 min. 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).

Animal

Benzophenone-2

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 investigate whether UVA (maximum wavelength from lamp = 365 nm) and UVB (maximum wavelength from lamp = 312 nm) affect the absorption of Benzophenone-3 through the skin.[24] The dorsal skin of female nude mice (ICR-Foxn/^{nu} strain, groups of 4) was subjected to UVA (24 and 39 J/cm^2) or UVB (150, 200, and 250 mJ/cm^2). 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. A glass cylinder (available diffusion area = 0.785 cm^2) was glued onto the mouse dorsal region. A 0.2 ml aliquot of Benzophenone-3 (3.5 mg/ml in 30% ethanol/double

distilled water) was pipetted into the cylinder. The application time was 6 h, after which the skin was excised. Senescent skin (24 wk old) was treated similarly for comparative purposes. When compared to non-irradiated skin, a statistically significant ($p < 0.05$) reduction in the in vivo skin deposition of Benzophenone-3 was observed following UVB irradiation. There were no statistically significant differences in Benzophenone-3 uptake between non-irradiated skin and skin irradiated with UVA. Benzophenone-3 uptake levels between young and old 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 performed (5 males, 7 females) was 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 obtained before and after application. A substantial amount of all sunscreen chemicals in the stratum corneum of the back was noted after 8h. 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 hours, 40% of the Benzophenone-4 remained at the level of the stratum corneum (compared to 20% for PEG-25 PABA).

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.[25] 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 potential for systemic absorption of Benzophenone-3 (10% in silicone-based water-in-oil emulsion (sunscreen)) was studied in vitro (3 experiments) using a full-thickness porcine-ear skin mimicking in-use conditions.[30] 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).[31]

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 body weight:	60 kg
No observed effect level NOAEL (oral teratogenicity-rat):	200 mg/kg body weight/d

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

MoS = NOAEL/(SED) = 112

Benzophenone-3 as a UV-filter in cosmetics at 0.5%

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 body weight:	60 kg
No observed effect level NOAEL (teratogenicity-rat):	200 mg/kg body weight/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 body weight/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.

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

Benzophenone-2

The fate of Benzophenone-2 was deciphered in 8 human and zebrafish in vitro cell models, encompassing hepatic and mammary cellular contexts.[32] 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 and T47D-KBLuc 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).[33] 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.[34] 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.[35] 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. Blood and tissue Benzophenone-2 concentrations in the frontal cortex and hippocampus were determined. After dermal application, 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.

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).[36] 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. It was measurable 24 h after skin application. 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.[37] 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 age, 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. 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.[38] 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).[39] 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 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 (ovx) rats (groups of 5).[40] Doses were administered (by gavage) once per day for 5 d. 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 20 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.[41] 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 6 until weaning on postnatal day 23: 1000; 3000; 10,000; 25,000, or 50,000 ppm.[42] Serum concentrations of Benzophenone-3 and its metabolites were measured. 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).[39] 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. 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

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

Benzophenones were detected in urine samples that were obtained in a study involving 20 male subjects.[43] The detection methodology was 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, a concentration range from the minimal quantified amount (limit of quantification) to 40 ng/ml was selected for the benzophenones. Urinary concentrations were described as follows: The free form of 4-hydroxybenzophenone was detected and quantified in all samples in a range of 0.9 to 2.0 ng/ml. The conjugated form of 4-hydroxybenzophenone was detected in 60% of the samples in concentrations ranging from 0.1 to 0.9 ng/ml. The conjugated form of Benzophenone-2 was detected and quantified in 85% of the samples in concentrations ranging from 0.1 to 7.1 ng/ml. The free form of Benzophenone-2 was detected in all samples and quantified in 65% of the samples, at concentrations ranging from 0.5 to 2.2 ng/ml. The conjugated form of Benzophenone-3 was detected and quantified in almost all samples (n = 19/20) at concentrations ranging from 0.6 to 44 ng/ml. The free form of Benzophenone-3 was detected in 100% of the samples, but it was not quantified in any of them. For Benzophenone-1, the conjugated form was detected and quantified in 100% of the samples at concentrations ranging from 0.1 to 25 ng/ml. The free form of Benzophenone-1 was detected in 95% and quantified in 90% of the samples in a concentration range of 1.2 to 5.7ng/ml. 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.[44] Benzophenone-1 and Benzophenone-3 had high detection rates (26%). Urinary concentrations (µg/l) of the two benzophenones were not reported. High detection rates were also reported for bisphenol A (95%) and triclosan (45%).

A study was performed to investigate the exposure of human embryos and fetuses to UV filters.[45] Placentas (12) from volunteer mothers in Spain were collected at delivery. The presence of UV filters was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Benzophenone-1 was detected in all samples, and at concentrations below the method limit of quantification (MLOQ, between 0.02 and 0.07 ng/g fresh weight). Benzophenone-3 was not detected in any sample. 4-Hydroxybenzophenone, metabolite of Benzophenone-1 and Benzophenone-3, was detected in 3 of the 12 placental samples at a concentration (0.07 ng/g fresh weight) that was below the MLOQ.

Urinary concentrations of benzophenones were measured in 34 Tunisian women.[46] Chemical analyses were performed using dispersive liquid-liquid microextraction and UHPLC-MS/MS. Benzophenone-1 and Benzophenone-3 were 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.

Benzophenone-1 and Benzophenone-3 were detected in the urine of reproductive-aged women.[47] A total of 143 women provided 509 spot urine samples collected across 2 months of study (3 to 5 samples per woman). Urinary concentrations were measured and biomarker variability was characterized using the intraclass correlation coefficient (ICC). The ICC is 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.

A prospective study involving 200 pregnant women was performed.[48] The study involved simultaneously-collected, paired samples, of amniotic fluid and maternal serum and urine. Additionally, samples of human fetal blood (n = 4) were obtained during cordogenesis; cord blood (n = 23) was obtained at the time of delivery. 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 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 the 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. The 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.

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.[49] The urine samples were collected from July to September in 2010 and 2011. Liquid chromatography-mass spectrometry was used for analysis. The 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. The geometric means of urinary Benzophenone-1 and 4-hydroxybenzophenone concentrations were 1.24 ng/ml and 0.45 ng/ml, 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.[50] Of the 6 benzophenones, 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). Neither Benzophenone-2 nor Benzophenone-8 was 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).

An exposure assessment on Benzophenones-1, -2, -3, - 4, and 8, conducted from 2010 to 2011, involved a population of 1576 subjects in South Korea. Urine samples were collected.[49] The detection frequency for Benzophenone-1 was above 55.8%, and the detection frequency for Benzophenone-3 was 24.8%. The detection rate for the remaining benzophenones (Benzophenones-2, -4, and -8) was lower than 14%. The geometric mean values of urinary Benzophenone-1 and Benzophenone-3 were 1.24 ng/ml and 2.66 ng/ml, respectively. Concentrations of Benzophenone-2, Benzophenone-4 and Benzophenone-8 in the urine were virtually undetectable.

A study with data on benzophenones in the urine was performed from 2015 to 2016, using 441 adult pre-menopausal females in South Korea.[51] The detection frequencies of benzophenones in urine samples were: Benzophenone-1 (98.4%), Benzophenone-3 (74.6%), and Benzophenononone-8 (22.9%). The authors noted that Benzophenone-1 is a major urinary metabolite of Benzophenone-3.

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.[52] 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).[53] 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).[54] 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 and 300 ng/ml in men. 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 estradiol 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.[55] 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 times 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. 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.[56] 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.[57] 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).

Urine samples (166 total) were collected from children and adults in the US and China.[58] The samples were 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 was detected in practically all urine samples from the US and China. 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. A statistically significant positive relationship was found between the concentrations of urinary Benzophenone-3 and its derivatives. The profiles of Benzophenone-3 derivatives in the urine suggested that demethylation was a major route of Benzophenone-3 metabolism. A statistically significantly lower percentage of the free form of Benzophenone-3 was found in urine from the US population than in the Chinese population.

The urinary excretion of ingredients in personal care products over a 6-d period was studied using 8 subjects.[59] The participants identified their usual personal care products. A total of 352 individual urine samples was collected over a 6-day period. Benzophenone-3 was frequently detected, i.e., in 70% of the total urine samples. The authors noted that exposure to Benzophenone-3 likely also occurred via the food pathway or other unknown sources.

A dermal absorption study on a sunscreen containing 5% Benzophenone-3 was performed using 9 adult subjects.[60] 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-

trihydroxybenzophenone; 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.

Human adipose fat samples were collected from 20 subjects.[61] High concentrations of Benzophenone-3 (maximum of 4940 ng/g wet weight) were detected. These results suggest that adipose tissue is an important repository for Benzophenone-3 in the human body.

Postmortem brain material (hypothalamus and white-matter tissue) obtained from up to 24 individuals was analyzed for the presence of Benzophenone-3.[62] The 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. A study on human UV filters in human breast milk was performed, and involved 76 breast milk samples from mothers in Spain.[63] 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). Additionally, the plastic containers for the milk had high concentrations (up to 10.6 µg/g plastic) of Benzophenone-3 and 4,4'-dihydroxybenzophenone.Concentrations of UV filters in breast tissue (3 serial locations within) from 40 women undergoing mastectomy for breast cancer were measured.[64] 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). Spearman's analyses showed statistically significant positive correlations between concentrations of Benzophenone-3 in each of the 3 breast regions. For ethical reasons, cancerous tissue was not available, but the location of the cancer was known. Mann-Whitney U-tests were used to investigate any link between chemical concentration and whether or not a tumor was present in that region. In the lateral region, more Benzophenone-3 was measured when a tumor was present ($P = 0.007$).

A study was performed to determine whether active sunscreen ingredients are absorbed into the systemic circulation.[65] The study involved groups of 6 subjects (1 group per product). 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.[66] 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.[67] 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.[68] Cotton shirts 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.[69] 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). The authors noted that because 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 also concluded that there is a direct association of Benzophenone-3 exposure and Hirschsprung's disease in neonates under normal conditions of use of sunscreen products.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Benzophenones-3, -4, -8, and -12

Benzophenones-3, -4, -8, and -12 were nontoxic when applied to the skin of rabbits at doses of > 5 g/kg.[1]

Benzophenone-3

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).[70] The study was performed in accordance with OECD TG 402. The formulation (2000 mg/kg) was applied to a 2" x 2", 4-ply gauze pad, and the patch was placed (secured with surgical tape) on hairless, dorsal skin. The patch remained in place for 24 h. Animals were observed for 14 d, after which the animals were killed. An untreated control group was also used. No overt signs of toxicity were observed during the 14-d observation period, and locomotor activity was normal. There were no statistically significant changes in terminal body weight between test and control animals. Hematological and serum biochemistry parameters were also normal. There were no abnormalities at necropsy or microscopic examination. 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, according to OECD TG 404.[70] The formulation was applied to a 25 cm^2 area of dorsal skin, using a 2" x 3", 4-ply gauze pad (secured with surgical tape). The application period was 72 h. Systemic toxicity was not observed.

Benzophenone-12

The acute dermal toxicity of Benzophenone-12 was evaluated using 5 albino rabbits, in accordance with OECD TG 402.[5] The test substance (aqueous paste; dose = 10,000 mg/kg) was applied, under an occlusive or semi-occlusive patch, for 24 h to the skin. Patch removal was followed by a 7-d observation period. None of the animal died, and no clinical signs or adverse findings were observed. The LD_{50} was > 10,000 mg/kg.

Oral

Benzophenones-1, -2, -3, -4, -6, -8, -9, -11, and -12

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-1

The acute oral toxicity of Benzophenone-1 was evaluated using rats (number and strain not stated).[6] Details relating to the test protocol were not included. The LD_{50} was 8600 mg/kg, and Benzophenone-1 was classified as practically non-toxic.

Benzophenone-3

The acute oral toxicity of a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was evaluated using 10 female Wistar albino rats.[70] The study was performed in accordance with OECD TG 423. A dose of the formulation (2000 mg/kg, in 0.5% carboxymethyl cellulose) was administered by gavage to 1 fasted rat. Thereafter, each 48 h, the same dose was administered to 4 rats. The negative control group (5 rats) was dosed with 0.5% carboxymethyl cellulose. Dosing was followed by a 14-d observation period, after which the animals were killed. The following organs were examined macroscopically: heart, lungs, liver, kidneys, and spleen. All of the animals survived and gained normal body weight, and no clinical signs of toxicity were observed. There also was no evidence of gross abnormalities, adverse pharmacological effects, or abnormal behavior during the study. The LD_{50} for the sunscreen formulation was > 2000 mg/kg.

Benzophenone-4

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.*

Benzophenone-8

The acute oral toxicity of Benzophenone-8 was evaluated in accordance with OECD TG 423, using 6 female Wistar rats of the CLR:(WI) strain.[8] The test substance (200 mg/ml, in propylene glycol) was administered via gavage at a dose of 2000 mg/kg. Dosing was followed by a 14-d observation period. The animals were then killed, and gross and microscopic examinations were performed. None of the animals died, and there were no treatment-related adverse effects during the observation period. There also was no evidence of macroscopic changes. The LD_{50} was > 2000 mg/kg.

Benzophenone-12

Benzophenone-12 was evaluated for acute oral toxicity using 10 male rats of the CF Nelson strain.[5] The animals were dosed orally with Benzophenone-12 (20% suspension in water; dose = 10,000 mg/kg). Dosing was followed by a 7-d observation period. None of the animals died and no clinical signs were observed. There also were no findings at necropsy. The LD_{50} was > 10,000 mg/kg.

Short-Term Toxicity Studies

Dermal

Benzophenone-3

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.[71] The only effects noted were minimal, variable increases in liver and kidney weights, primarily in the higher dose groups.

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.[71] The only effects noted were small and variable increases in liver and kidney weights, reaching a statistical significance primarily in the higher dose groups.

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.[72] Body weight, organ-to-body weight ratios, and hematological and clinical chemistry parameters were not affected. Pathological examinations revealed no significant changes between control and treated animals. No gross external abnormalities were observed. 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 2 wk.

The short-term dermal toxicity of Benzophenone-3 was studied using female Sprague-Dawley rats and their offspring.[37] 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 age, the test substance was administered dermally to the male offspring. Cream without Benzophenone-3 was applied to control offspring. The dosing of adult pregnant females did not significantly alter their body weight or cause any apparent adverse effects, when compared to control rats. No significant differences in body weight and sex-ratio were observed in the offspring.

Oral

Benzophenone-3 and Benzophenone-8

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.

Benzophenone-3

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.[71] A dose-related increase in liver weight, associated with hepatocyte cytoplasmic vacuolization, was the only finding in mice in this 2-wk study. The no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for mice.

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.[71] One high-dose female rat died. Liver and kidney weights were increased. Enlarged livers were associated with a marked hepatocyte cytoplasmic vacuolization in rats receiving diets containing concentrations of 6250 ppm Benzophenone-3 or higher. Renal lesions, consisting of dilated tubules and

regeneration of tubular epithelial cells, were found primarily in high-dose rats. The NOAEL for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for rats.

Benzophenone-4

A short-term oral toxicity study on Benzophenone-4 was performed using groups of 26 Wistar rats (13 males, 13 females/group).[7] The study was performed in accordance with OECD TG 422. The test substance was administered orally (in corn oil, by gavage) at doses of 750, 1000, and 1250 mg/kg/d. Control rats were dosed with corn oil only. 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. Recovery groups of male and female rats (5/sex/dose) were treated at 0 or 1250 mg/kg body weight/d for 66 d total. Animals in the recovery groups were allowed to recover for 2 wk after the final dose was given. No morbidity was observed during the period. There also were no test substance-related mortalities. Clinical findings were sporadic and of no biological significance. Body weight changes were restricted to a statistically significant decrease in % body weight change in the recovery group of male rats treated at 1250 mg/kg from day 1 – 22, as compared to the control group. This effect on body weight was considered incidental and not test substance-related. Food consumption was unaffected by treatment. The observed changes in hematology and clinical chemistry were not considered to be of toxicological importance. Detailed clinical examinations and microscopic examination of the eyes, with optic nerve (in 0 and 1250 mg/kg groups), did not reveal any abnormalities. Developmental and reproductive toxicity data are included in that section of this safety assessment.

Hormonal data showed no significant effects on the concentrations of tetraiodothyronine (T4) or thyroid stimulating hormone (TSH) (male and females), testosterone (males), or estradiol (females). There were no significant effects on either the absolute or the relative weight of the brain, adrenals, heart, liver, kidneys, spleen, thymus, thyroid with parathyroid, testes, or epididymides. All adult animals were normal externally. Visceral findings included a case of mild splenic enlargement at 1000 mg/kg and one case of mild testicular shrinkage at 1250 mg/kg. Microscopic examination revealed no treatment-related effects, that is, the incidences and types of lesions observed at 1250 mg/kg were comparable to that of the concurrent control groups. The NOAEL (systemic toxicity) for Benzophenone-4 in this study was established at 1250 mg/kg/d for male and female rats.

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 OECD TG 417.[5] No significant gross lesions were observed in rats that were killed on day 11, 22, or 35 of the study. Furthermore, there were no lesions of the liver or kidneys at histological examination.

The repeated dose toxicity of Benzophenone-12 was evaluated in Wistar rats, in accordance with OECD TG 416.[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 control group (12 males, 12 females) was dosed with vehicle only. 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. 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 were performed on animals that were killed. There were no treatment-related gross pathological or histopathological findings, and none of the animals died. No clinical signs or changes in general behavior were observed in male or female F_0 parental animals of any dose group. There were no treatment-related body weight changes or effects on food consumption. Additionally, there were no hematological findings or treatment-related clinical biochemical findings. A 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 StudiesOral

Benzophenones-1, -3, and -12

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 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. In a 120-d feeding study, Benzophenone-12 was nontoxic to dogs at a concentration of 0.6% in the diet.

Benzophenone-1

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] Details relating to the test protocol were not stated. The NOAEL was 236 mg/kg body weight/d. Regarding the organ toxicity endpoint, the authors stated that critical effects observed were unspecified.

Benzophenone-3

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.[71] Decreased, body weight gains (dose-related) were reported. Mild increases in liver weights were observed in dosed mice of both sexes. Kidney weights were increased variably in dosed females. Microscopic lesions were noted only in the kidneys of males receiving 50,000 ppm Benzophenone. These included eosinophilic protein casts in dilated renal tubules and a mild inflammation associated with the dilated tubules. The NOAEL for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for mice.

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.[71] Body weight gains of high-dose male and female rats were reduced. Liver and kidney weights were increased. Kidney lesions progressed to include papillary degeneration, or necrosis, and inflammation, while the liver lesion appeared to regress. Liver enzymes in serum remained elevated. The NOAEL for microscopic lesions was 6250 ppm Benzophenone-3 in the diet for rats.

Results relating to subchronic oral toxicity are included in an NTP oral carcinogenicity study on Benzophenone-3 involving male and female Sprague-Dawley rats.[73] Groups of 10 male and 10 female rats were exposed to 0 or 10,000 ppm Benzophenone-3 in the diet for 14 wk. At the 14-wk interim evaluation, the mean body weight of the 10,000 ppm males was not significantly different from that of the control males, but the mean body weight of the 10,000 ppm females was significantly decreased, and was approximately 87% of the control group value. 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.

The incidence of mixed-cell cellular infiltration in the liver was significantly increased in 10,000 ppm males, relative to the control group. These cellular infiltrates were composed of mononuclear cells with scarce neutrophils, and had no specific predisposition to a specific area of the liver lobule. It is unlikely that the cellular infiltrates, which were all of minimal severity, would be responsible for the changes in liver weights observed in male rats at this time point. No other histologic findings were observed that would explain the differences in organ weights, but in the females, body weight changes could have influenced the absolute kidney weight decrease and the relative liver weight increase. However, the increase in relative liver weight in exposed females was accompanied by a nonsignificant absolute liver weight increase. So, it is unlikely that body weight was responsible for the liver weight change.

As a part of the 14-wk interim evaluation, transcriptome analysis was performed on RNA extracted from microarray study of male rat livers from the 10,000 ppm and control groups. The observed effects on transcription were consistent with a mild induction of xenobiotic metabolism-related processes that is likely related to the observed liver weight increases. Analysis of a subset of estrogen-responsive genes showed no change in response to Benzophenone-3.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Benzophenone-3

The embryotoxicity of Benzophenone-3 was evaluated in the fish embryotoxicity test using zebrafish embryos.[74] The test was performed in accordance with a modification of OECD TG 236. The applied number of zebrafish embryos was 40 at each concentration in 4 replicates. The experiment was prolonged until 120 h post-fertilization, because this period includes time points at which different developmental states can be observed. The following 6 concentrations of Benzophenone-3 (in dimethylsulfoxide (DMSO)) were prepared: 0.116 mM, 0.0789 mM, 0.0523 mM, 0.0307 mM, 0.0219 mM, and 0.00535 mM. Each solution was supplemented by a certain volume of DMSO in order to achieve the same DMSO concentration (3.52 mM; 250 µl/l). The positive control was 3,4-dichloroaniline (0.0247 mM), and water served as the negative control. DMSO served as the solvent control. The following endpoints were evaluated: mortality, malformations, hatching, and inflation of the swim bladder. Cumulative mortality was under 10% in the negative and solvent control groups at the end of the experiment. In the positive control group, cumulative mortality was 75%. In the negative and solvent control groups, the percentage of hatched embryos was 95%. No hatched embryos were observed in the positive control group. Except for one in the solvent control group (no swim bladder was observed), there were no malformations in the negative and solvent control groups.

The following LC_{50} values were reported: 0.0766 mM (at 72 h post-fertilization), 0.0698 mM (at 96 h), and 0.0573 mM (at 120 h). At a concentration of 0.00438 mM, all embryos were able to inflate their swim bladder. However, at higher concentrations, Benzophenone-3 caused the absence of swim bladder inflation in a concentration-dependent manner. The calculated EC_{50} value was 0.0295 mM after 120 h post-fertilization. At 72 h post-fertilization, deformation of the tail was observed (EC_{50} = 0.0419 mM). Additionally, malformation of the somites was observed at concentrations of 0.0526 and 0.0789 mM. Benzophenone-3 decreased the number of hatched embryos after 96 h post-fertilization. The EC_{50} value was 0.0543 mM. Other malformations (not named) were observed, but the incidence was not concentration-dependent. These included pericardial and yolk sac edema, deformed jaw and ventricle or dilated gut, and jaw deformity. The authors concluded that Benzophenone-3 caused mortality, unsuccessful hatching, and different malformations to zebrafish.

The effect of Benzophenone-3 on follicular assembly was studied using whole ovary cultures collected from Wistar rats.[75] 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): 5.8 nM, 276 nM, 576 nM, and 876 nM. 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 5.8 mM Benzophenone-3 decreased the population of total oocytes and decreased the number of early primary follicles. Benzophenone-3 (276 mM) increased the population of total oocytes, but decreased the number of primary follicles.

Thus, these results indicate that exposure to a low concentration of Benzophenone-3 (5.8 mM) stimulated the process of germ cell nest breakdown and caused a decrease in the reserve of total oocytes. Exposure to a higher concentration (276 nM) caused the opposite effect. In ovaries exposed to even higher concentrations of Benzophenone-3 (576 nM and 876 nM), no changes were observed in the number of oocytes, germ cell nests per ovary, and assembled follicles in ovaries.

Animal

Dermal

Benzophenone-3

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 in acetone.[71] Epididymal sperm density was decreased at all 3 dose levels evaluated (22.75, 91.0, and 364.0 mg/kg). Therefore, it was not possible to establish a NOAEL for decreased epididymal sperm density.

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) or olive oil (vehicle) 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. The mice were killed on GD 5, 10, and 14 (during first pregnancy), and on GD 10 and 14 (during second pregnancy). Benzophenone-3 levels were analyzed in serum and amniotic fluid. Benzophenone-3 dosing resulted in reduced fetal weight at GD 14 and feto-placenta index (first pregnancy), with 16.13% of fetuses under the 5th percentile; uterine artery parameters showed an altered pattern at GD 10. Benzophenone-3 was detected in serum 4 h after exposure on GD 6, and in amniotic fluid, on GD 14. The weight of offspring of the first progeny was lower in the group dosed with Benzophenone-3. Placental weights in animals dosed with Benzophenone-3 were decreased in the second pregnancy. First and second progenies of mothers exposed to Benzophenone-3 showed a higher percentage of females (female sex ratio). 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

Benzophenone-1

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. Details relating to the test protocol were not included. A NOAEL of 10 mg/kg/d was reported. Regarding observations of any reproductive effects, the authors noted that reproductive effects were not specified.

Benzophenone-2

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. Control pregnant mice were dosed with vehicle only. The animals were killed on GD 18. The anogenital distance in male fetuses was measured and genital tubercles were examined histologically. Quantitative reverse transcriptase-polymerase chain reaction analysis of genes purportedly involved in genital tubercle development was also performed. In the test group, 8 of 57 male fetuses had hypospadias ($p = 0.0064$, when compared to controls). When compared to controls, there were no changes in body mass-adjusted anogenital distance. 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. 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 Benzophenone-2-treated male mice had higher levels of estrogen receptor-β, when compared to male controls ($p = 0.04$). The authors concluded that Benzophenone-2 may cause hypospadias via signaling through the estrogen receptor.

Benzophenone-3

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. The feed consumption in the 2.5 and 5.0% groups was consistently higher, but the F_0 body weights were consistently lower. These findings suggest that Benzophenone-3 may be adversely affecting metabolism or the digestive process in Swiss CD-1 mice. In the 2.5 and 5.0% Benzophenone-3 dose groups, the number of live pups per litter was significantly reduced. The F_1 generation from control, 2.5%, and 5.0% groups were weaned for second generation studies. During lactation and nursing of F_1 pups, pup survival was significantly below the control value in the 2.5 and 5.0% groups. Benzophenone-3 had minimal effects on fertility in the F_1 generation, but pup weights were significantly reduced. Epididymal sperm motility, sperm count, and percentage of abnormal sperm were not affected by Benzophenone-3 treatment. Additionally, there were no apparent effects on estrual cyclicity or the average estrous cycle length in treated females. Based on the results of the present study, 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.[71] 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: 30 µg/kg/d, 212 µg/kg/d, and 3000 µg/kg/d. Thus, the offspring were exposed in utero, and via lactation. Sample sizes for the treatment groups were: 30 µg/kg/d (10 litters), 212 µg/kg/d (11 litters), and 3000 µg/kg/d (9 litters). The 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 to Benzophenone-3 reduced the size and growth of the mammary gland in males prior to (at postnatal day 21, statistically significant reduction) and during puberty (reduction not statistically significant). In females, Benzophenone-3 reduced mammary cell proliferation (statistically significant at 30 µg/kg/d), decreased the number of cells expressing estrogen receptor α (statistically significant at 30 or 212 µg/kg/d), and altered mammary gland morphology (dose response) in adulthood. 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. No effect on male body weight was 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 of Benzophenone-3 exposure 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.[71] 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.[42] 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. The control group was fed the low-phytoestrogen chow only. No exposure-related clinical signs were observed. On GD 10, 15, and 20, the body weights of dams decreased in a dose-dependent manner. Absolute and relative kidney weights in dams were statistically significantly higher in the 50,000 ppm exposure group, when compared to the control group. Exposure to Benzophenone-3 was associated with reduced body and organ weights in male and female offspring.

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. One fetus in the 50,000 ppm group had hydrocephaly, but no other malformations were observed. Normalized anogenital distance in male pups at postnatal day 23 was decreased in the 50,000 ppm exposure group. Exposure to this concentration also caused impairment of spermatocyte development in the testes of male offspring. In females, follicular development was delayed in the 50,000 ppm exposure group. The authors concluded that few adverse effects in rat dams and offspring dosed maternally and lactationally with Benzophenone-3 (in chow) from GD 6 to postnatal day 23 were observed at Benzophenone-3 concentrations of 10,000 ppm or less. At higher concentrations, it is possible that Benzophenone-3 produced a delay in postnatal growth, which could have adversely affected reproductive organ development; however, this is not clear. The authors noted that further work is needed to clarify the possible decreases in spermatogenesis and folliculogenesis observed in this study.

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 then weaned on postnatal day 28, and subsequently dosed with Benzophenone-3 via chow and milk. Daily observation of male offspring did not reveal any clinical observations related to perinatal Benzophenone-3 exposure. At necropsy on postnatal day 30, body weights were 22% lower in the 30,000 ppm Benzophenone-3 exposure group when compared to the control group (diet without Benzophenone-3). Rats exposed perinatally to 30,000 ppm Benzophenone-3 also had statistically significantly lower weights of the paired-testis, paired-epididymis, and prostate. These weights were lower in males exposed to 30,000 ppm

Benzophenone-3 when compared to controls (26%, 17.6%, and 18.5%, respectively). The paired-testis weight to body weight ratio was also statistically significantly lower in the 30,000 ppm exposure group; however, there were no changes in the relative weights of the paired epididymis and prostate in the 30,000 ppm Benzophenone-3 exposure group. Rats exposed to Benzophenone-3 did not have any 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, the liver and paired-kidney weights were lower in a dose-dependent manner in the 30,000 ppm Benzophenone-3 exposure group, attaining statistical significance. However, relative liver and paired-kidney weights were similar to controls.

Results relating to developmental toxicity are included in an NTP oral carcinogenicity study on Benzophenone-3 involving male and female Sprague-Dawley rats.[73] 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 Benzophenone-3 resulted in average daily doses of approximately 70, 206, and 660 mg Benzophenone-3/kg body weight/d during gestation, and 157, 478, and 1609 mg/kg/d over lactation days (LD) 1 - 14. Gestation body weights of dams receiving 10,000 ppm Benzophenone-3 in the diet were slightly lower (~3%) than those of the control group and showed statistically significant differences. Dams receiving 3000 or 10,000 ppm Benzophenone-3 in the diet displayed slight decreases in GD 6 - 21 body weight gain (~10%) relative to the control group, which attained statistical significance. Lower body weight gain over the GD 6 - 9 (10,000 ppm) and 18 - 21 (3000 and 10,000 ppm) intervals, which was associated with slightly lower feed consumption over the GD 18 - 21 interval, likely contributed to this response. The authors noted that these collective effects are minimal and would not be expected to affect normal development of the offspring.

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. The authors noted that the apparent decrease in the percentage of females pregnant in the 10,000 ppm group can be attributed to the 7 animals that had no evidence of pregnancy, as shown by the absence of implantation sites. Therefore, the lower pregnancy rate was not exposure-related, given that exposure began after implantation. Dams receiving Benzophenone-3 did not display any adverse clinical findings before or after parturition. Litter size of the 10,000 ppm Benzophenone-3 group was slightly lower on postnatal days 7 and 10.

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 dose volume was 5 ml/kg. The animals were 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, there was a scattered occurrence of a few external, soft tissue, and skeletal malformations without a consistent pattern. These findings also occurred without a clear dose-response relationship and/or incidence, and were not test substance-related. External variations were not observed in any fetuses in the study. The authors concluded that Benzophenone-3 did not possess any selective teratogenic properties. The NOAEL for Benzophenone-3 was 200 mg/kg/d.

Benzophenone-4

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] Control rats were dosed with corn oil only. The study was performed in accordance with OECD TG 422. 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. Recovery groups of male and female rats (5/sex/dose) were treated at 0 or 1250 mg/kg body weight/d for 66 d total. Animals in the recovery groups were allowed to recover for 2 wk after the final dose was given. No morbidity was observed. Estrous cyclicity was unaffected by treatment. All female rats showed evidence of copulation after the cohabitation/mating period. Pregnancy rates were 77, 62, 77, and 77% at 0, 750, 1000, and 1250 mg/kg, respectively. No significant effects were 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 the 750 mg/kg dose group were cannibalized. All other pups at 0, 750, 1000, and 1250 mg/kg were normal externally. The internal examination of the pups revealed no test substance-related abnormalities. Microscopic examination of the pups' thyroid and parathyroid glands in the 0 and 1250 mg/kg dose groups revealed no abnormalities. The NOAEL (reproductive toxicity) for Benzophenone-4 in this study was established at 1250 mg/kg/d.

Benzophenone-12

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, 25 females).[5] The test substance was administered by gavage 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 female rats were killed on GD 20, and fetuses were removed from the uterus. Neither clinical signs nor effects on body weight (or organ/body weight ratios) were observed. Furthermore, no test substance-related necropsy findings were observed after dosing of dams. There was no evidence of dead/aborted fetuses or pre- and post-implantation loss. Test substance-related external, skeletal, or visceral malformations were not observed. 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 control group (12 males, 12 females) was dosed with vehicle only. 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 (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 ended with terminal sacrifice of the F_1 rearing animals. All F_0 parental animals were also killed. Parental results are described in the Short-Term Toxicity section of this safety assessment. Clinical examinations of F_0 parental animals did not reveal any test substance-related adverse findings, and there were no effects on reproductive performance. No test substance-related adverse findings at clinical or gross examination of F_1 pups were observed. For F_1 rearing animals, there were no test substance-related findings during clinical examinations and sexual maturation, and there were no gross findings. 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

In Vitro

Benzophenones-1, -2, -3, -4, -6, -8, -9, and -11

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. All other benzophenones (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.

Benzophenone-1

The photo-genotoxicity of Benzophenone-1 and apoptotic parameters were evaluated using human keratinocytes (HaCaT cells).[81] These were assessed by western blot, immunocytochemistry, flow cytometry, the comet assay (for DNA damage), and transmission electron microscopy (TEM) imaging. 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. Benzophenone-1 enhanced lipid peroxidation, and leakage of lactate dehydrogenase (LDH) enzyme (61.7%). Apoptotic cells were 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 induced upregulation of apoptotic proteins Bax.Bcl2 ratio, Apaf-1, cytochrome c, Smac/DIABLO, and cleaved caspase3 were observed.

In the same study, the genotoxicity potential of Benzophenone-1 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. Immunostaining results showed maximum CPD formation by Benzophenone-1 at a concentration of 25 µg/ml (in presence of UVB). CPD formation was not observed in control cells exposed in the dark or exposed to light. Micronuclei formation was detected in HaCaT cells treated with Benzophenone-1 (10 µg/ml) in the presence of UVB (1.08 J/cm^2). Simultaneously, micronuclei were not detected in control cells exposed to UV or control cells exposed in the dark. Maximum tail DNA (29.1%) was recorded at a Benzophenone-1 concentration of 25 µg/ml, compared to a control value of 4.8%. 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. Similarly, the highest olive tail moment

(OTM) of 3.57 units was recorded at a Benzophenone-1 concentration of 25 µg/ml (with UVA irradiation), when compared to control cells (0.54 units). The authors noted that this study established the involvement of Benzophenone-1 in photogenotoxicity and apoptosis via the release of cytochrome c and Smac/DIABLO.

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

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] The solvent for each test substance was DMSO or methanol. Results indicated positive results for Benzophenone-3 and pseudo-positive results for Benzophenone-1 and Benzophenone-8. Pseudo-positive was defined as a good dose response and with from 1.5 to 2 times as many revertant colonies as spontaneous revertant colonies.

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] DMSO served as the solvent control, benzo[a]pyrene (BaP) served as the positive control (with activation), and 2-(20-furyl)-3-(5-nitro-2-furyl) acrylamide (AF2) served as the positive control (without activation). Results indicated that none of the test substances produced clear positive results with or without metabolic activation. The results of this test were classified as negative.

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] The positive control, 2,4,7-trinitrofluorene, was genotoxic. 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. Each ingredient (chlorinated, doses up to 10 µl per plate) was tested in seawater at ratios of 1:10 and 1:1000. Only Benzophenone-8 (1:10) had clear genotoxic activity that was dose-related (doses of 4, 6, 8, and 10 µl). No genotoxic activity was observed for either ingredient at a ratio of 1:1000. The positive control, 2,4,7-trinitrofluorene, was genotoxic.

Benzophenone-3

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.[70] The formulation was tested at a dose of 5000 µg/plate with and without metabolic activation. The following substances served as positive controls: sodium azide, 2-nitrofluorene, and 2-aminofluorene. There was no observable increase in the number of revertant colonies with or without metabolic activation. Thus, Benzophenone-3 was not genotoxic in this assay. The positive controls were 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. In the chromosomal aberrations assay, the exposure period was 24 h. The exposure period was 48 h in the micronuclei assay. Benzophenone-3 induced the 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. The most frequent aberrations observed were acentric fragments and chromatid aberrations, and no numerical aberrations were found. A statistically significant increase in chromosomal aberrations and aberrant cell frequencies was observed at all test concentrations, when compared to the solvent (DMSO) control. A dose-response was also observed, considering that a regression analysis revealed a statistically significant ($p < 0.001$) correlation between Benzophenone-3 concentrations and the level of genomic damage. No statistically significant differences between the solvent and untreated control cultures were observed. The positive control (mitomycin C) caused a statistically significant increase in chromosomal aberrations and aberrant cell frequencies, when compared to all test concentrations of Benzophenone-3 and the negative and untreated controls.

In the micronuclei test, Benzophenone-3 caused a 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 the vehicle, untreated, and positive controls were the same as those reported in the chromosome aberrations assay immediately above.

The effect of Benzophenone-3 on DNA damage was studied using human breast epithelial cells.[85] DNA damage after treatment with Benzophenone-3 and 17β-estradiol (E2)was determined by immunostaining with antibodies against markers of DNA damage, γ-H2AX (phosphorylated histone H2AX) and p53-binding protein 1 (53BP1). 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. However, Benzophenone-3 had limited transactivation of target genes at the same 2 concentrations. Benzophenone-3 exposure caused R-loop formation in a normal human breast epithelial cell line when ERα was introduced. The 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.

Benzophenone-3 was evaluated for genotoxicity using *S. typhimurium* strains TA98 and TA100, and *Escherichia coli* strain *uvr*A pKM101.[73] The test substance was evaluated at doses up to 6000 µg/plate with and without metabolic activation. Benzophenone-3 was non-genotoxic with and without metabolic activation.

Benzophenone-8

A bacterial reverse mutation assay (OECD TG 471) 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. Both strains were tested with and without metabolic activation. Benzophenone-8 caused a visible reduction in growth of the bacterial background lawns of both strains (with and without metabolic activation), initially from 500 µg/plate. Therefore, the test substance was evaluated up to either the maximum recommended dose level of 5000 µg/plate or the toxic limit (depending on the bacterial strain type). No significant increases in the frequency of revertant colonies were noted for either bacterial strain, at any dose level either with or without metabolic activation. The authors concluded that Benzophenone-8 was negative for genotoxicity in this assay.

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. The increase was dependent upon increasing concentrations of the test substance, and was totally dependent on the presence of metabolic activation.

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] The cultures treated in the absence of metabolic activation exhibited mutant frequencies that were not significantly different from those of solvent controls. However, cultures treated in the presence of metabolic activation exhibited a significant increase in the mutant frequencies, and a dose response was evident. The two highest concentrations, 24 and 32 µg/ml, exhibited mutant frequencies that were 3.8 and 2.0 times greater, respectively, than that of the average mutant frequency of solvent controls. Results for Benzophenone-8 were positive in the L5178Y TK+/- mouse lymphoma assay.

Benzophenone-12

The genotoxicity of Benzophenone-12 in the mammalian cell gene mutation assay (mouse lymphoma L5178Y cells) was evaluated in accordance with OECD TG 476.[5] The assay was performed with and without metabolic activation. Benzophenone-12 (in DMSO) was evaluated at doses up to 50 µg/ml (with metabolic activation) and up to 52 µg/ml (without metabolic activation). A positive effect was defined as doubling of the mutant frequency over the concurrent solvent-treated control value, together with evidence of a dose-related increase. Benzophenone-12 was non-genotoxic without metabolic activation. Results were ambiguous with metabolic activation. Relative to these results (with metabolic activation), the authors noted that a less than 3-fold increase in the mutant frequency occurred at highly toxic concentrations.

In Vivo

Benzophenone-1

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.

Benzophenones-2, -6, and -8

Benzophenone-2 and Benzophenone-6 did not induce sister chromatid exchanges in Chinese hamster bone marrow cells from animals (species not stated) 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 the number of bone marrow micronuclei.

Benzophenone-3

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 or 25 ppm dimethylnitrosamine (DMN; positive control) for 72 hours. 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.

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 oral gavage with a single administration of 0.0, 0.5, 1.67, or 5 g/kg Benzophenone-3, or a dose of 5 g/kg/d Benzophenone-3 for 5 consecutive days. Cyclophosphamide (CP) was the positive control, and was administered at a dose of 20 mg/kg in both treatment regimens. Colchicine growth-arrested bone marrow cells were collected

8 and 12 hours after the single treatment, and 12 hours after the last daily treatment. Under either treatment protocol, none of the Benzophenone-3 concentrations 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 (OECD TG 474), using groups of 10 Wistar albino rats.[70] Doses of 0.5 g/kg, 1 g/kg, and 2 g/kg were administered dermally (method not stated in OECD TG 474) for 2 consecutive days (at intervals of 24 h). The positive control group was dosed i.p. with cyclophosphamide (0.04 g/kg), and the negative control group was dosed dermally with a placebo formulation (2 g/kg). At 48 h after the first dose, all rats were killed and bone marrow was extracted from the femur. Two-hundred erythrocytes in the bone marrow cells of each animal were used to score the total number of mature and immature erythrocytes. The number of micronuclei per 2000 immature erythrocytes was also recorded. Neither the sunscreen formulation (all doses) nor the placebo statistically significantly increased the ratio of micronucleus polychromatic erythrocyte (MNPCE)/polychromatic erythrocyte (PCE) and PCE/(PCE + normochromatic erythrocyte (NCE)). The positive control statistically significantly increased these ratios. The authors concluded that the sunscreen (2 g/kg) did not statistically significantly increase the number of micronucleated immature erythrocytes or systemic toxicity at 48 h, classifying the sunscreen formulation 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 (modification of OECD TG 475), using groups of 10 Wistar albino rats.[70] Doses of the sunscreen, 0.5 g/kg, 1 g/kg, and 2 g/kg, were administered according to the procedure stated immediately above. The same is true for the positive and negative controls (cyclophosphamide and placebo formulation; same doses). The animals were killed after dosing, bone marrow was extracted from the femur, and slides were prepared. Light microscopy was used to evaluate any evidence of chromosomal abnormalities. No increment in the total number of aberrant cells or in the chromosome aberration percentage of the sunscreen formulation or the placebo formulation was observed. The positive control facilitated an increase in the number of aberrant cells. The authors concluded that 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 body weight to deliver E2 (250 µg/kg/d) or Benzophenone-3 (3000 µg/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 were detected in mammary epithelial cells of mice treated with Benzophenone-3. The 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.

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 (10^{-7} to 10^{-5} M) 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 (10^{-6} M) 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 (10^{-9} M). 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 a National Toxicology Program (NTP) study using male and female Sprague-Dawley rats and male and female B6C3F1/N mice. [73] 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 body weight, respectively, for male mice and 109, 320, and 1278 mg/kg, respectively, for female mice) for 104 (female mice) or 105 (male mice) weeks. Survival of all exposed groups of male and female mice was not statistically significantly different from that of the control groups. Mean body weights of 1000 and 3000 ppm males and females were within 10% of those of the control groups throughout the study. Mean body weights of 10,000 ppm male and female mice were at least 10% lower than those of the control groups, generally after weeks 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.

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 Benzophenonoe-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 body weight for males and 60, 180, and 632 mg/kg 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 body weights 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 week 77, F_1 female rat mean body weights 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)).

Perinatal studies and 14-wk interim evaluations were also conducted in rats from the NTP carcinogenicity study. Results from perinatal studies are included in the section on Developmental and Reproductive Toxicity. Results from 14-wk interim evaluations are included in the Subchronic (Oral) Toxicity section.

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.

Tumor Promotion

Benzophenone-1

The xenoestrogenic effect of Benzophenone-1 on BG-1 human ovarian cancer cells expressing estrogen receptors and relevant xenografted animals models, when compared to E2, was evaluated.[92] In the in vitro cell viability assay, Benzophenone-1 (10^{-8} to 10^{-5} M) 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, liked 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 (20 µg/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 bromodeoxyuridine (BrdUrd) positive nuclei and the over-expression of cyclin D1protein. The authors noted that the results of this study suggest that Benzophenone-1 is an endocrine disrupting chemical that exerts xenoestrogenic effects by, like E2, 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.[93] 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 (10^{-8} to 10^{-5} M) 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 (10^{-6} M) and biclutamide (10^{-9} M, androgen receptor antagonist; n = 3). In the migration assay, LNCaP cells were treated with 10% charcoal/dextran-treated fetal bovine serum (FBS) containing 10^{-6} M 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 10^{-6} M and 10^{-7} M. In the MTT assay, when the cells were co-treated with Benzophenone-1 (10^{-6} M) and biclutamide (10^{-9}), 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 (10^{-6} M) 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 (10^{-9} M). 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 (10^{-4} M) 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

(10^{-6} M) 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 antitumor activity of Benzophenone-8 and Benzophenonone-12 was evaluated using a two-stage mouse skin carcinogenesis model.[94] 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 male offspring were then weaned on postnatal day 28, and subsequently dosed with Benzophenone-3 via chow and milk. 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.

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.[35] 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).[95] 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^{-5} M and 10^{-4} M in both assays. Additionally, both Benzophenone-2 and Benzophenone-3 caused an increase in caspase-3 activity at much lower concentrations (from 10^{-8} M to 10^{-7} M). 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.[36] 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.[96] 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).[97] 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.[37] 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. 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).

Effect on Melanogenesis

Benzophenone-2

The dual action of Benzophenone-2 in the biosynthetic pathway of melanin has been identified.[98] It has been observed to act as a weak competitive inhibitor of tyrosinase (inhibition constant (K_i) = 2.02 ± 0.09 mM; half maximal inhibitory concentration (IC_{50}) = 3.82 ± 0.39 mM). 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.

Behavioral Toxicity

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).[36] 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.[99] 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 (10^{-8} to 10^{-5} M). Benzophenone-2 (10^{-5} M) shifted the Th1/Th2 balance toward a Th2 response (lower IFN-γ production and higher IL-10). The authors noted that these results show that Benzophenone-2 at high doses may possess immunomodulatory effects.

Benzophenone-2 (in ethanol) was administered dermally (100 mg/kg), twice daily for 4 wk, to 10 male Wistar rats.[100] 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. The authors concluded that dosing with Benzophenone-2 increased the activity and function of the immune cells (thymocytes and splenocytes).

Benzophenone-4

The immunosuppressive activity of Benzophenone-4 (0.01%) was evaluated using human dendritic cells (e.g., CD14+ human monocytes).[101] 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 Benzophenonoe-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).[102] Also, zebrafish (*Danio rerio*) embryo exposure involved the 3 potent benzophenones (Benzophenones-1, -3, and -8) that were identified based on the transcriptional changes that were observed in the cells. In GH3 cells, Benzophenones-1, -2, -3, and -8, but not Benzophenone-4, down-regulated the *Tshβ*, *Trhr*, and *Trβ* genes. Additionally, some of the benzophenones significantly upregulated the *Nis* and *Tg* genes, while down-regulating the *Tpo* gene in the FRTL-5 cells. In the zebrafish embryo assay on Benzophenone-1, Benzophenone-3, and Benzophenone-8, significant decreases in whole-body thyroxine (T4) and triiodothyronine (T3) level were observed at day 6 post-fertilization. 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.[103] 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 estradiol 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 estradiol valerate resulted in significantly increased uterine weight. Both doses of Benzophenone-3 also had this effect on the uterus. Blood luteinizing hormone levels were statistically significantly reduced after dosing with estradiol 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 (ovx) rats (groups of 5).[40] 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.[104] Estradiol-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 estradiol benzoate (E_2).[105]

Benzophenone-2 was evaluated for its effect on the hypothalamic-pituitary-thyroid (HPT) axis.[100] 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 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.[106] 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.[106] 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).[107] 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).[108] 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 10^{-7} and 10^{-5} M. 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^{-5} M.

The effect of Benzophenone-3 on the secretory and proliferative activity of rat (adult female Wistar rats) adrenocortical cells was investigated in vitro.[109] Within 120 min of culture, Benzophenone-3 (10^{-12} to 10^{-8} M) stimulated basal corticosterone production from dispersed adrenocortical cells. The chronic, 24-h exposure to Benzophenone-3 (10^{-10} M) 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.[110] 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.[74] Concentration-response curves were fitted by nonlinear regression. In the estrogen assay, an EC_{50} (half maximal effective concentration) value of 6.44E-03 mM 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 antiestrogen assays, Benzophenone-3 showed a sigmoidal concentration-response curve. In antiandrogen assays, the EC_{50} value for Benzophenone-3 was 0.0102 mM. The results of this study indicate that Benzophenone-3 has estrogenic and antiandrogenic potential.

Effect on Hematological Parameters

Benzophenone-2

Benzophenone-2 was also evaluated for its effect on hematological parameters.[100] 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

The cytotoxicity of a sunscreen formulation composed of polymeric nanocapsules loading Benzophenone-3 was evaluated using the L929 fibroblast (murine) cell line.[111] 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

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.[112] 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 Benzophenon-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.[113] 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

Irritation

In Vitro

Benzophenone-3

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.[111] The nanocapsules contained poly(Ɛ-caprolactone), carrot oil, a non-ionic surfactant, and Benzophenone-3 (0.005 wt%). The eggs were incubated for 10 d, after which the membrane was removed and the CAM was exposed. The formulation was then added on the embryonated hen's egg membrane, and effects were studied for 300 s. As a positive control (for vascular hemorrhage and lysis), 300 µl of sodium hydroxide solution (0.1 M), was applied. Sodium chloride solution (0.9 wt%) was applied as a negative control. The diluted (distilled water) formulation (300 µl) was applied to eggs also. The assay was monitored for any event (hemorrhage, lysis, and coagulation) for 300 s. The formulation was classified as a non-irritant.

Benzophenone-4

The dermal corrosion potential of Benzophenone-4 was determined using a three-dimensional human epidermis model, according to OECD Test TG 431.[7] Before dosing, the tissues were moistened with sterile water (25 µl). Approximately 25 mg of solid test article was evenly applied to the apical surface of each tissue. Each treatment with test article or control was conducted in duplicate. The exposure period for the test articles and controls was 3 and 60 min. For the 60-min exposure, the dosed tissues were placed in an incubator for the remainder of the 60-min exposure period. The MTT assay was performed using tissues transferred to 24-well plates. The mean optical density for the test chemical was determined to be 2.098 and 0.315 for the 3-min endpoint and 1-h endpoint, respectively. The mean % tissue viability, compared to the negative control (n = 3), was determined to be 85.7 % and 13.4 % for the 3-min endpoint and 1-h endpoint, respectively. Based on these values, Benzophenone-4 was considered corrosive to the skin.

Animal

Benzophenones-1, -2, -3, -4, -6, -9, and -11

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%.

Benzophenone-3

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).[70] The study was performed in accordance with OECD TG 402. The formulation (2000 mg/kg) was applied to a 2" x 2", 4-ply gauze pad, and the patch was placed (secured with surgical tape) on hairless, dorsal skin. The patch remained in place for 24 h. There were no signs of erythema or edema.

The skin irritation potential of a sunscreen formulation (0.6% to 0.9% Benzophenone) was evaluated using 18 male New Zealand rabbits (3 groups of 6), according to OECD TG 404.[70] The 3 groups were test, positive control (0.8% aqueous formaldehyde), and negative control (placebo sunscreen formulation), respectively. Each material was applied to a 25 cm^2 area of dorsal skin, using a 2" x 3", 4-ply gauze pad (secured with surgical tape). The application period was 72 h, after which the patches were removed. Reactions were then scored for erythema and edema at 24 h, 48h, and 72 h, and a primary irritation index (PII) was calculated. There was no evidence of erythema or edema in the test or placebo groups (PII = 0). The positive control was severely irritating (PII = 10.43). Additionally, there were no signs of systemic toxicity in any of the groups.

Benzophenone-8

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. Reactions were scored for up to 72 h post-application. Skin irritation was not observed in any of the animals tested, and Benzophenone-8 was classified as a non-irritant.

Benzophenone-12

A skin irritation test on Benzophenone-12 (ground to fine powder) was performed using 3 male New Zealand white rabbits, in accordance with OECD TG 404.[5] The test substance was applied (0.5 g, abraded and intact skin of back) for 4 h under an occlusive patch. Reactions were scored at 24 h, 48 h, and 72 h after patch removal using the Draize system. A modified PII was calculated using the 24-h and 72-h scores. There was no evidence of erythema or edema during the study (modified PII = 0), and no clinical signs were observed. Benzophenone-12 was classified as non-irritating to the skin of rabbits.

Human

Benzophenones-1, -2, -3, -4, -6, -8, -9, -11, and -12

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%.

Benzophenone-4

The frequency of irritant reactions to Benzophenone-4 was studied using 80 subjects.[114] Benzophenone-4 was tested 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, secured with adhesive tape. Patches were applied for 2 d to the upper back. Reactions were scored according to the 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.

Sensitization

In Vitro

Benzophenone-8

The in vitro antioxidant response element (ARE)-nuclear erythroid 2-related factor 2 (Nrf2) Luciferase test method (OECD TG 442D) 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. This is an immortalized adherent human keratinocyte cell line (HaCaT cell line), transfected with a selectable plasmid to quantify luciferase gene induction. The experiment involved 2 independent runs. The maximal average fold induction of luciferase activity (I_{max}) response for luciferase gene expression as well as sensitization potential, was determined. In both repetitions, the induction of luciferase above the threshold of 1.5 was noted. Specifically, the I_{max} was > 1.5-fold and statistically significantly different, as compared to the negative control (DMSO). Thus, 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

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 30 adult male guinea pigs (3 groups of 10).[70] The study was performed in accordance with OECD TG 406. One group was treated with the sunscreen formulation. The other 2 groups were 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) were made to the groups of animals. Inducing agents were 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. None of the

animals treated with the sunscreen formulation or placebo had sensitization reactions. The positive control induced skin sensitization. The authors classified the sunscreen formulation 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 (left and right) on 3 consecutive days. No local findings or clinical signs of toxicity were observed, and there were no mortalities. At 5 d after topical application, the animals were killed. Lymph nodes were excised and single cell suspensions were prepared. The incorporation of [^{3}H]methyl thymidine was measured. At each concentration that was applied, the stimulation index was less than the limit criterion of 3. Benzophenone-3 was classified as a non-sensitizer.

Benzophenone-12

The maximization test was used to assess the cutaneous allergenic potential of Benzophenone-12.[115] Ten test and five control female albino guinea pigs (weights = 319 to 394 g) were used. The intradermal induction of sensitization in the test group was performed in the nuchal region with a 15% dilution of Benzophenone-12 in PEG 300 and in an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction of sensitization was conducted for 48 h under occlusion with the test substance (at 40% in PEG 300) one week after the intradermal induction, and following pretreatment of the test areas with 10% sodium lauryl sulfate (SLS) 23 h prior to application of the test substance. The animals of the control group were intradermally induced with PEG 300 and FCA/physiological saline, and epidermally induced with PEG 300 under occlusion following pretreatment with 10% SLS. Two weeks after epidermal injection, the control and test animals were challenged by epidermal application of the test substance (at 40% in PEG 300) and PEG 300 alone under an occlusive dressing. Cutaneous reactions were evaluated at 24 and 48 h after removal of the dressing. No toxic symptoms were evident in the 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 (40% w/w in PEG 300). No skin effect was observed in the control group. Benzophenone-12 was classified as a skin sensitizer.

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] Treatment during the first week of induction involved the following 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) was applied to the neck for 48 h. The 2 cm x 4 cm occlusive patch contained 0.4 g of paste. A control group of 10 guinea pigs (5 males, 5 females) was also treated during induction. 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)). The test substance (0.2 g paste) was applied to the flank using a 2 cm x 2 cm occlusive challenge patch. Reactions were scored at 24 h and 48 h using the Draize scale. It should be noted that, during challenge, the control group was treated with the vehicle as well as the test substance in order to check the maximum sub-irritant concentration of the test substance in adjuvant-treated animals. Results indicated that 65% and 60% of the animals were sensitized to Benzophenone-12 under the experimental conditions that were employed at 24 h and 48 h after challenge, respectively. The authors classified Benzophenone-12 as a sensitizer in guinea pigs.

Human

Benzophenones-1, -2, -3, -4, -6, -8, and -11

Benzophenone-1 was nonsensitizing 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 nonsensitizing at a concentration of 10%. Benzophenone-3 was also nonsensitizing 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

Benzophenones-2, -3, and -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.

Benzophenone-3

A 3% aqueous solution of Benzophenone-3 was applied, in duplicate, to the midback of 4 patients, using Finn chambers.[116] At 48 h post-application, the test substance was removed, and sites evaluated for reactions. Reactions were graded on a scale of +1 to +3. The other site containing the test substance was irradiated with UVA (8 J/cm^2), and then covered with light-opaque material. All sites were evaluated for reactions at 48 h post-irradiation. Contact allergy was diagnosed as an equally positive reaction at nonirradiated and irradiated sites. Photoallergy was diagnosed as a positive irradiated site with a negative unirradiated site. Allergy and photoallergy were diagnosed when both sites were positive, but with the irradiated site having a greater reaction than the unirradiated site. The results for a 3% aqueous solution of Benzophenone-3 are 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.

Over a 6-year period, 187 patients (76 males, 111 females) with a history of photosensitivity were photopatch tested using standard techniques. Two-thirds of the patients were between the ages of 31 and 60 years.[117] Phototest allergens (Benzophenone-3, at 2% in petrolatum) were applied in duplicate to the patient's midback, one set on either side of the midline. Test material containing antigens was applied with aluminum disks (Finn Chambers) and paper (Scanpor) tape. For the first two testing periods (January 1985 through February 1987 and March 1987 through August 1989), the antigens were left in place for 48 h. In the 3rd period (September 1989 through December 1990), the antigens were removed after 24 h. One set of antigens was then irradiated with UVA, 8 J/cm^2 (January 1985 through August 1989) or 10 J/cm^2 (September 1989 through December 1990). Both sets of antigens were then covered with light opaque material (gauze pads and aluminum foil held in place with paper (Scanpor) tape). All sites were evaluated for reactions at 48 hours post-irradiation. Second readings at day 7 post-irradiation were done in the third test period (September 1989 through December 1990). Second readings were not done during the first two test periods. Reactions were graded on a scale of $\pm$ to 3+. Testing revealed a total of 63 positive reactions, classified as follows: 14 plain contact, 41 photocontact, and eight combined contact and photocontact in 37 (20%) patients. 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.[118] Benzophenone-3 (10% in white petrolatum) was applied, in duplicate, using Finn chambers on Scanpor tape. Sites were covered with opaque material. After 24 h, the test sites were examined and results were recorded. One site was irradiated with 5 J/cm^2. Reactions were scored on day 5 according to the standards of the ICDRG, and a final reading was performed on day 7. Nine patients had a positive photopatch test reaction to Benzophenone-3. Two patients had positive reactions at non-irradiated sites.

A study was performed to determine the proportion of photosensitive patients with photoallergic contact dermatitis to Benzophenone-3.[119] The study was a descriptive cross-sectional study involving 35 patients (11 men, 24 women) in Argentina with confirmed photosensitivity reactions. These patients had experienced at least 1 episode of photosensitivity reactions. Two sets of patches containing Benzophenone-3 (10% in petroleum jelly) were applied to the back, 1 on the right and 1 on the left. At 48 h after patch application, 1 of the patches was irradiated with a cumulative UVA dose (5 J/cm^2; peak wavelengths of 350 nm, 365 nm, and 370 nm) over an 18-min period. Reactions were scored at 30 min post-irradiation and at 96 h after patch application. A late reading was also taken after 1 week. 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. Four patients had a reaction at the irradiated sites only, and 1 patient had a reaction at both irradiated and nonirradiated sites. The authors concluded that photoallergic contact dermatitis to sunscreens containing Benzophenone-3 is common and is probably underdiagnosed, due to a lack of confirmation by photopatch tests or other diagnostic tools.

Since 1990, seven sunscreen allergens have been included in the standard photopatch protocol at two Swedish dermatology clinics.[120] Three-hundred fifty-five consecutive patients with suspected photosensitivity were tested, and, in 28 of these (7.9%), a total of 42 allergic reactions was found. Eighty percent of the reactions was of photocontact origin. The most common allergen was Benzophenone-3 (2% in petrolatum), with 15 photocontact allergic reactions and 1 contact allergic reaction.

Benzophenone-2 and Benzophenone-3

Twenty-seven patients reported reactions due to sunscreen allergy (itchy bumps and burning).[121] Of these, 11 (10 women, 1 man) patients agreed to photopatch testing. Finn chambers (8 mm, secured with tape) containing filter paper wetted with the test substance (Benzophenone-2 or Benzophenone-3) were applied to the back. The chambers were applied in duplicate for patch and photopatch testing. After 24 h, photopatches were removed and one set was irradiated with UVA (10 J/cm^2). Immediately after UVA exposure, photopatch tests were read to determine immediate-type sensitivity reactions. Patch areas were then covered with opaque tape material. Another reading was made 24 h later (day 3), and the final reading was made at

5 to 7 d. At the reading immediately after UVA exposure, all reactions were negative, indicating the absence of contact urticaria. 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).

Benzophenone-3 and Benzophenone-4

Fifteen patients (4 males, 11 females; mean age = 47.7 years) reacted to sunscreens.[122] Eight patients 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 reacted to her daily cream containing Benzophenone-3. 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 UV absorbers, commercial sunscreens, facial cosmetics, fragrance materials, preservatives, and emollients.[123] Patch tests were performed according to ICDRG guidelines. A UVA dose of 5 or 10 J/cm^2 was used for photopatch testing. Benzophenone-3 and Benzophenone-4 were tested at a concentration of 10% in petrolatum. Of the 402 patients, 80 patients (20%) presented with relevant allergic or photoallergic contact dermatitis to UV absorbers. There were three allergic and nine 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.[124] At least one month after the acute episode of contact dermatitis, the patients were patch tested using the Finn Chamber technique. Finn chambers were mounted on Scanpor tape, and patches were removed after 2 d. For UV irradiation, two sources of light were used (UVA alone and UVA + UVB). Photopatch test results were positive for Benzophenone-3 (reactions 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.[125] Benzophenone-3 was tested at a concentration of 10% in white paraffin. Benzophenone-4 was tested at concentrations of 5% and 10% in white paraffin. Photopatch testing involved application of the test substance (on aluminum Finn chamber) to skin of the mid-upper back (paravertebral area avoided) for 24 h or 48 h (depending on the center). The 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) was covered with light-impermeable occlusive dressing, and the other was irradiated with fluorescent UVA (5 J/cm^2). Reactions were scored at 48 h post-irradiation, and, if possible, at 24 h and 72 h. The ICDRG visual scoring system was 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 were also reported: 5% Benzophenone-4 (2 patients), 10% Benzophenone-3 (9 patients), and 10% Benzophenone-4 (9 patients). Photoaugmentation and photoinhibition of contact allergy was observed in 1 patient tested with 10% Benzophenone-3 and in 1 patient tested with 10% Benzophenone-4. 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.[126] The study involved 82 patients with a clinical diagnosis of photoallergic contact dermatitis. Photopatch tests were performed. The test substances (allergens, concentrations not stated) were applied, in duplicate, to skin on the back. The test sites were covered with opaque tape for 24 h. The panel on the right was irradiated with UVA (dose = 5 J/cm^2; irradiance = 10.4 mW/cm^2). Reactions were scored 24 h after application of the allergen and at 24 h and 72 h post-irradiation. Both Benzophenone-3 and Benzophenone-4 (concentrations not stated) induced a positive photopatch reaction. Benzophenone-3 was photoallergenic in 22 of 82 patients (26.8%), and Benzophenone-4 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.[127] Benzophenone-3 (10% in petrolatum) or Benzophenone-4 (2% in petrolatum) was applied to skin of the back, and removed at 48 h. One site was irradiated with UVA (5 J/cm^2), and the other site was covered with a UV-impermeable material. Reactions were scored at 48 h. Benzophenone-3 (10% in petrolatum) elicited photoallergic contact dermatitis in 37 patients: + reaction (14 patients), ++ reaction (18 patients), and +++ reaction (5 patients). Benzophenone-4 (2% in petrolatum) elicited photoallergic contact dermatitis in 3 patients: + reaction (1 patient) and ++ reaction (2 patients). Allergic contact dermatitis reactions (+ 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.[128] Photoallergic, allergic, and irritant reactions were recorded for 26 common allergens. Duplicate sets of allergens (test concentration not stated) were applied to the patient's back. At 24 h, 1 set of allergens was uncovered and exposed to UVA at a dose of 5 J/cm^2. The other set of allergens was shielded from UVA exposure. Twenty-four-h reactions to the non-irradiated compounds were assessed at 15 to 20 min later. On the following day, the irradiated patches were read at 24 h post-irradiation. Reactions at non-irradiated patch test sites were 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.

A prospective study was performed to evaluate the frequency and causes of photoallergic contact dermatitis among dermatology outpatients.[129] The study involved 1000 consecutive dermatology outpatients in Poland. All patients with a history of dermatitis, induced or aggravated by exposure to light, were qualified by photopatch testing. In the study group, 36 (3.6%; 95% CI: 2.4 - 4.8%) individuals required photopatch testing based on their clinical symptoms. Because the total number of patients requiring patch tests of any kind amounted to 205, the percentage of photopatch tested patients among all patch-tested patients was 17.5% (95% CI: 12.2 - 22.8%). Patch tests (2 identical sets) were mounted on the back and remained under occlusion for 48 h. Some sites were irradiated with UVA (5 J/cm^2) and some were non-irradiated. Skin reactions were scored 24 h and 48 h after irradiation. The presence of an inflammatory reaction at the irradiated sites and no reaction to the same hapten at non-irradiated sites was interpreted as confirmation of photoallergy. In case of positive reactions to a hapten, both on irradiated and non-irradiated sites, the classical contact allergy was recognized. Photoallergic contact dermatitis was ultimately confirmed in 15 (1.5%; 95% CI: 0.7 to 2.3%) persons: 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).[130] Tests were performed in a single photo-investigation center during years 2000 to 2011. A duplicate series of UV filters and the children's own sunscreen products was applied to the back (ingredient test concentration not stated). Reactions were scored at the 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). A single case of a photoaugmentation reaction to Benzophenone-4 was reported. This patient had a + reaction in the control panel, but had a ++ reaction in the irradiated panel.

Benzophenone-4

The phototoxicity of Benzophenone-4 was studied using 80 subjects.[114] Benzophenone-4 was tested 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, secured with adhesive tape. Patches were applied (in duplicate) for 2 d to the upper back, i.e., on non-paravertebral skin to the left and right of the upper back. At the time of patch removal, one side of the back was covered with UV-opaque material, while the other side was irradiated with UV light (5 J/cm^2; 99.2% UVA and 0.8% UVB). Reactions were scored according to the ICDRG grading scale. One subject had a weak positive reaction (+ reaction), with no concomitant erythema score, to Benzophenone-4 (10% in petrolatum) at the irradiated site.

Benzophenone-3 and Benzophenone-10

A retrospective analysis of positive photopatch test episodes was undertaken using results retrieved from the environmental dermatology database, and further verified with the original archived patch test documentation for each individual patient.[131] In 111 patients with positive reactions (4.1%), there were 155 allergic contact or photoallergic reactions to allergens in the photopatch series. On day 0, the standard photoallergens were applied (test concentration not stated) to the patient's back in duplicate. On day 2, the patches were 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). Eighty photoallergic reactions were observed in 62 (2.3%) patients (32 men and 30 women), with UV filters accounting for 52 positive reactions. It should be noted that 34 of the 62 patients (55%) had a preceding underlying photodermatosis. The most common UV filter photoallergen was Benzophenone-3 (14 positive results), followed by Benzophenone-10 (n = 9). 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 eight 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.[132] Benzophenone-3 and Benzophenone-10 produced positive responses at 0.7 and 1.07 J/cm^2, respectively. Isopropyl dibenzoyl dibenzoylmethane produced a positive response at 1.0 J/cm^2. These results demonstrate that high doses of UVA (e.g., 10 to 15 J/cm^2) are unnecessary, and that 5 J/cm^2 should become the current standard.

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

Seven patients with ketoprofen-induced photodermatitis were patch tested and photopatch tested with Benzophenone-3, Benzophenone-4, and Benzophenone-10 (test concentrations not stated).[133] The aim of the study was to evaluate the possibility of cross-reactivity between ketoprofen and benzophenones and other chemicals because of their structural similarities. Patch tests (uninvolved skin of back) were performed using Finn chambers. At 24 h post-application, a separate series of patch tests was exposed to suberythemal doses of UVB and UVA. Irradiated and non-irradiated sites were evaluated at 72 h post-application. 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.

The photoallergenicity of Benzophenone-4 (10% in petrolatum) and Benzophenone-10 (10% in petrolatum) was evaluated using 15 eczematous dermatitis patients.[134] Testing was performed at least 3 months after complete disappearance of the dermatitis. In photopatch tests, the test substance was applied to the back, under occlusion, over a 2-d period. At 24 h, the occlusive patch was removed, and the site was exposed to UVA (5 J/cm^2). Reactions were scored at 48 h and 96 h (day 2 and day 4). There were no positive reactions to Benzophenone-4 (10% in petrolatum). Three subjects had positive reactions to Benzophenone-10 (10% in petrolatum).

From February 1985 to March 1987, 280 patients with photosensitivity and other patients (ages not stated) suspected of sunscreen dermatitis were patch and photopatch tested with a series of contact allergens and photoallergens (test concentration = 2% in petrolatum).[135] All tests were read at 2 d, and, at this time, the duplicate light series was exposed to UVA (1 J/cm^2). The second and final reading of all tests was carried out at 4 d. Fifteen patients had positive patch and/or photopatch tests. Three were allergic to more than one UV absorber. During the first 16 months of the study period (February 1985 to May 1986), there were two 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.

OCULAR IRRITATION STUDIES

In Vitro

Benzophenone-4

The ocular irritation potential of Benzophenone-4 was evaluated using the MatTek EpiOcular™ model, in accordance with OECD TG 492.[7] The viability of normal human-derived keratinocytes in the 3-dimensional human tissue model following exposure to the test substance was determined via the MTT cytotoxicity assay. The 3-dimensional tissue construct models the corneal epithelium, with progressively stratified, but not cornified, cells. Tissues were exposed to Benzophenone-4 (solid, 50 mg) for ~ 6 h. The mean % tissue viability of Benzophenone-4 was determined to be 3.6%. Based on the results of this test, Benzophenone-4 was classified as irritating to the human eye.

Benzophenone-8

An ocular irritation study on Benzophenone-8 was performed using the bovine corneal opacity and permeability test (OECD TG 437).[8] Corneas from 3 animals were exposed to the test substance (20% w/v in paraffin oil; volume = 750 µl) for 4 h. The test substance was then removed from the front opening of the anterior chamber and the epithelium was rinsed. For the evaluation of corneal permeability, the passage of sodium fluorescein dye was measured using ultraviolet-visible (UV/Vis) spectrophotometry. Benzophenone-8 did not cause corneal opacity or permeability, resulting in a mean in vitro irritancy score of 1 after 4 h of exposure. Based on these results, the authors concluded that Benzophenone-8 was not a severe irritant or corrosive agent in the bovine corneal opacity and permeability test.

Animal

Benzophenones-1, -2, -3, -4, -6, -8, -9, -11, and -12

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.

Benzophenone-3

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.[70] The formulation (100 mg) was instilled into the conjunctival sac of the right eye of each animal. After instillation, eyes were examined macroscopically at intervals of 24 h, 48 h, and 72 h, and daily from 4 to 10 d, in accordance with the Draize scale. There were no signs of gross toxicity or adverse effects. Corneal opacity and iritis were not observed during the study. At 1 h post-instillation, conjunctival discharge was observed in 1 out of 3 eyes, but subsided within 96 h. The highest maximum mean total score (MMTS) value (0.67) for ocular irritation was observed within 1 h after instillation, classifying the sunscreen formulation as practically non-irritating to the eye.

Benzophenone-12

Benzophenone-12 was evaluated for ocular irritation potential using 6 New Zealand white rabbits (3 males, 3 females), in accordance with OECD TG 405.[5] The undiluted test substance (0.1 g) was instilled once, and ocular irritation was evaluated on days 1, 2, 3, 4, and 7. There was no evidence of ocular irritation (PII = 0).

CLINICAL STUDIES

Retrospective and Multicenter Studies

Benzophenone-3

Over 3400 patients (age range: 3 to 96 years) with suspected allergic contact dermatitis were evaluated and then patch tested by 12 North American Contact Dermatitis Group dermatologists with a screening series of 50 allergens.[136] The patients were patch tested (July 1, 1996 to June 30, 1998) using Finn chambers on Scanpor tape. The patches remained in place for 48 hours, and sites were evaluated initially at 48 to 72 h, and, again, between 72 and 168 h after initial placement. A positive allergic patch test result was generally interpreted as a 1+, 2+, or 3+ reaction manifested by erythematous papules, vesicles, or a spreading reaction with crust and ulceration. The relevance of the patch test reactions was determined in combination with the patient's history and skin examination findings, and were integrated to determine the diagnostic group. Of the 4094 patients patch tested with 3% Benzophenone-3, 0.5% had allergic reactions (73.7% relevant reactions, i.e., definite, probable, or possible relevance to patient's present dermatitis).

A North American Contact Dermatitis Group (NACDG) study that was performed involved 5800 patients who were patch tested with Benzophenone-3 (3% in petrolatum).[137] Patch testing was performed from July of 1998 to December of 2000. The patches remained in place for 48 h. Test sites were evaluated twice, initially at 48 h to 70 h and, again, at between 72 h and 178 h after initial placement. A positive allergic patch test result was interpreted to be a +, ++, or +++ reaction. Reactions of these types were manifested by erythematous papules, vesicles, or a spreading reaction with crust and ulceration. 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.[138] This was done in order to evaluate the irritation and sensitization potential of sunscreen products containing Benzophenone-3 at concentrations between 1% and 6%. Forty-eight of 19,570 possible dermal responses were considered suggestive of irritation or sensitization. The mean rate of responses across all formulations was 0.26%. Sensitization rates did not correlate with Benzophenone-3 concentration. The available re-challenge data indicated that only 8 of these responses were contact allergies due to Benzophenone-3. The mean rate of contact allergy to Benzophenone-3 was 0.07%. The authors concluded that these data indicate that that sunscreen products formulated with 1 to 6% Benzophenone-3 do not possess a significant sensitization or irritation potential for the general public.

A cross-sectional analysis of patients patch tested by the NACDG between 2001 and 2010 was performed.[139] Of the 23,908 patients who were patch tested, 219 (0.9%) had sunscreen coded as an allergen source. 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). Values for the 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%). The 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%).

NACDG patch testing results from January of 2007 to December of 2008 were reported.[140] Standardized patch testing was used at 13 centers in North America. A total of 5085 patients was tested. Five-hundred ninety-eight patients (11.8%) had an occupationally-related skin condition and 3319 (65.3%) had at least 1 allergic patch test reaction. Patches (Finn chambers, secured with tape) remained in place for 48 h. Reactions were scored at 48 h and 72 h to 168 h. At the end of testing, clinical relevance of positive patch test reactions was determined by consideration of the patient's history and clinical findings. Relevance of a positive allergen was categorized into present, past, or unknown. Present relevance of a positive allergen was categorized as follows: definite (use test with suspected item resulted in positive reaction, or a patch test to the object or product was positive); probable (allergen could be verified as present in known skin contactants, and clinical presentation was consistent); and possible (allergic reactions to Benzophenone-3 (3% in petrolatum) were observed in 22.7% (definite relevance) of the patients. Other values relating to clinical relevance were: probable relevance (36.4%), possible relevance (22.7%), and past relevance (6.8%).

Benzophenone-4

In another study by the NACDG, 4857 patients were patch tested, and the positive reaction rate for Benzophenone-4 (10% in petrolatum) was 2.1% (100 allergic reactions).[141] Patch testing was performed using Finn chambers. The values for clinical relevance of allergic reactions were: 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%)).

Benzophenone-3 and Benzophenone-10

From 1989 to 1991, 214 patients were patch tested to a sunscreen series containing 9 constituents.[142] Standard closed patch testing was employed, using Finn Chambers applied to the upper back skin. The patches were removed at 2 d, and readings were made at the time of patch removal and at 3 or 4 d. Forty-five patients had photosensitivity dermatitis/actinic reticuloid syndrome and 54 had polymorphic light eruption. Of the 214 patients, 16 reacted to one or more sunscreens. The Benzophenones were the most frequent sensitizers. Benzophenone-3 and Benzophenone-10 accounted for 27 and 8 positive patch tests, respectively.

Benzophenone-3 and Benzophenone-4

In a retrospective review, 160 patients (37 male, 123 female) underwent photopatch testing at Toronto Western Hospital between January of 2001 and December of 2010.[128] Photoallergic, allergic, and irritant reactions were recorded for 26 common allergens. Duplicate sets of allergens were applied to the patient's back. At 24 h, 1 set of allergens was uncovered and exposed to UVA at a dose of 5 J/cm^2. The other set of allergens was shielded from UVA exposure. Twenty-four-h reactions to the non-irradiated compounds were assessed at 15 to 20 min later. On the following day, the irradiated patches were read at 24 h post-irradiation. Reactions at non-irradiated patch test sites were read 48 h after application. Benzophenone-3 induced photoallergic reactions in 12 subjects, and induced allergic reactions in 17 subjects. Benzophenone-3 caused both an allergic and photoallergic reaction in 6 patients. Additionally, Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions.

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.[143] Twenty-three of the patients were tested with the sunscreen series at the clinic. Also, as part of the regular screening at the clinic, 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. Patch test chambers containing the test substance were applied to the upper back and secured with tape for 48 h. Reactions were scored (using the ICDRG grading scale) at the 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 a positive reaction to Benzophenone-4. Additionally, of the 1527 patients screened (no specific history of sunscreen allergy), 8 patients reacted to Benzophenone-3 in the NACDG series. This number does not include the 2 patients who tested positive to Benzophenone-3, i.e., those who presented with a positive history and were additionally tested with the sunscreen series.

A total of 5592 patients was patch tested with Benzophenone-4 (10% in petrolatum, Finn chambers) in an NADCG study.[144] Of the patients patch tested, 93 had a positive (allergic) reaction. 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, Finn chambers). Of the patients patch tested, 24 had an allergic reaction. Values for the clinical relevance of allergic reactions to 10% Benzophenone-3 (in petrolatum) 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%)).

The British Society for Cutaneous Allergy (BSCA) retrospectively reviewed the results from their facial patch test series.[145] This review involved 12 centers in the United Kingdom and Ireland for a 2-year 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 4224 patients patch tested with Benzophenone-3 (10% in petrolatum), 0.17% (CI: 0.08% to 0.35%) had allergic reactions.

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

Over a period of 3 years, 553 patients were patch tested (Finn chambers) with 10% Benzophenone-3, 10% Benzophenone-4, and 10% Benzophenone-10.[146] The dose per area was not stated. Results were recorded at 48 h (day 2) and 96 h (day 4). Positive reactions (+ to +++) were graded according to International recommendations. Thirteen patients (8 females, 5 males) 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.

Case Reports

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 important allergens in nail varnish (tolunenesulfonamide-formaldehyde resin), nail varnish remover (Benzophenone-2), and artificial nails (ethyl acrylate), respectively. Symptoms and skin changes disappeared when these three items were no longer used.

A male patient presented with subacute chest and arm eczema after use of a toilet water product.[148] A repeated open application test with his product elicited a positive reaction after only two applications. Patch testing with an ingredient of the product, Benzophenone-2 (2% in petrolatum), yielded a positive reaction (++). No reactions were observed in 15 control subjects.

Severe dermatitis was observed in a female patient.[149] The dermatitis worsened after sun exposure, and was accompanied by severe itching. Cosmetic contact dermatitis was suspected and patch tests (protocol not stated) were performed. Patch test results for Benzophenone-2 (1% in petrolatum) were positive. A reaction classified as +++ (strong reaction: erythema, papules, and vesicles) was observed on days 2, 4, and 7.

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] After intermittent exposure to sunlight (over 24-h period), the eruption extended to involve the legs, neck, hands, and other parts of the body. The patient was patch tested with ketoprofen and its components using Finn Chambers on Scanpor tape. Photopatch testing (irradiation with 6 J/cm^2 UVA) was also performed. Positive patch and photopatch test reactions to ketoprofen (up to 2% in petrolatum) were reported. Patch test results for Benzophenone-3 were negative; however, a positive photopatch test reaction to Benzophenone-3 (+++) was reported on day 4. The authors noted that, when irradiated with sunlight, ketoprofen is broken down into various benzophenones that are structurally related to Benzophenone-3.

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] Open photopatch testing of the sunscreen on a 2 cm^2 area of forearm skin produced an erythematous, papular response 24 h after a single exposure to UVA (25 J/cm^2), suggesting photoallergy. Patch testing with an ingredient of the sunscreen, Benzophenone-3 (2% in petrolatum), yielded a +++ reaction. Histology of a biopsy from the Benzophenone-3 photopatch-test reaction showed a striking epidermal spongiotic response and vesicle formation, with absence of vacuolation and sunburn cells. A prominent mononuclear inflammatory cell filtrate was observed in the dermis.

Anaphylaxis (with generalized cutaneous wheal and flare reaction) was observed in a female patient after widespread application of a sunscreen to the skin.[152] The patient had a history of atopic dermatitis and allergic rhinoconjunctivitis. A few days before testing, she experienced contact urticaria after coming in contact with someone who had applied the same sunscreen to the face. Patch testing with the sunscreen (applied to normal skin) yielded a 2-cm diameter wheal and flare reaction within 5 min. Blinded patch testing with Benzophenone-3 (sunscreen ingredient, concentration not stated) induced a 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 the sunscreen and Benzophenone-3 were positive (wheal, 6 x 7 mm).

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] Additionally, the patient subsequently applied a 'false tan' product to her skin and developed a violent reaction, described as a severe cutaneous and systemic anaphylactic reaction. The patient was patch tested with Benzophenone-3 (concentration not stated), and reactions were scored 20 min after patch application. An acute urticarial wheal and flare (50 mm) reaction was observed at 20 min, and the reaction settled within 1 h after oral drug treatment. The patch testing of 5 control subjects with Benzophenone-3 did not reveal any reactivity at 20 min, 48 h, or 96 h.

In another case report, a male patient presented with the following history: intensely pruritic bilateral lip; perioral, cheek, ear, hand, and forearm dermatitis; and painful ulcerations of the oral mucosa.[154] On examination, 1 to 4 mm papules and papulovesicles (coalescing into edematous plaques) were present on the dorsal hands, fingers, volar wrists, dorsal forearms, and upper arms. Patch testing was performed according to NACDG methods, using Finn chambers secured with tape. The patient had a strong reaction to Benzophenone-3 (3% in petrolatum) at 48 h (+++ reaction) and 96 h (+++ reaction).

A female patient experienced an anaphylactic reaction 15 min after applying a sunscreen all over her body.[155] Generalized wheals were observed. The patient previously had pruritus and erythema within 30 min of putting her bathing suit on, which she had worn earlier during sunscreen application. Patch testing with Benzophenone-3 (10% in petrolatum) resulted in an urticarial reaction at the test site within 20 min. Patch testing did not elicit anaphylaxis in this patient. Photopatch tests (without Benzophenone-3) were negative. No specific immunoglobulin E (IgE) antibodies were found against inhalation allergens or latex. An assay for the detection of IgE to Benzophenone-3 was performed by incubating Benzophenone-3 with human serum albumin. No specific IgE to Benzophenone-3 was detected.

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] Patch testing with ingredients of the sunscreen was performed. The patient was patch tested using Finn chambers applied to the back, followed by UVA irradiation (dose = 10 J/cm^2) at 24 h. Reactions were scored at 20 min, and 24 h, 48 h, and 72 h post-irradiation. Photopatch test results were negative for Benzophenone-4. Photopatch test results for Benzophenone-3 were as follows: + (at 24 hours), ++ (at 48 hours), and +++ (at 72 hours).

Hand dermatitis was observed in a female hairdresser over a 2-year period.[157] Patch testing with and Benzophenone-4 yielded a positive (++) reaction. When she stopped using hair-care products with sun protection, the dermatitis began to improve. Further 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] Erythema of the cheeks bilaterally and on the neck, and minimal scale (but no vesicles) were observed. She had used a facial moisturizer and a shampoo, both of which contained Benzophenone-3, for 2 years. Patch testing and photopatch testing were performed using the Finn chamber technique. Results were scored on days 3 and 7. 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 (10 J of UVA), urticaria at the Benzophenone-3 photopatch test site was observed. This reaction was consistent with photoallergic contact urticaria. The authors noted that the patient's burning, itching, and erythema resolved when she avoided contact with benzophenones in her personal care products.

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 been sitting in the sun for a few hours, several days before the swelling began. She had also used a lip balm and shampoo, both of which contained Benzophenone-3. The patient had a 1+ reaction to Benzophenone-3 at both patch and photopatch test sites. The perioral itching resolved within several days after discontinuing the lip balm. Additionally, the facial erythema improved greatly after the shampoo was replaced with another that did not contain benzophenone.

A case of acute facial swelling in a diver has been reported.[159] The diver's face (left side) became swollen after ascending to the surface of the water. An in vitro ImmunoCap IgE assay was positive to latex. Subsequent patch testing (standard test) for contact dermatitis yielded a positive reaction to Benzophenone-4. The patch testing protocol and test concentration were not stated.

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

A female patient presented with eyelid dermatitis for 1 year and facial dermatitis for two months.[160] Patch tests (procedure not stated) results for benzophenones were as follows: Benzophenone-3 (++), Benzophenone-4 (+), and Benzophenone-10 (negative results).

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

Face eczema developed in a female patient after use of a cosmetic cream.[161] Patch tests involving Benzophenone-3 and Benzophenone-10 were performed using a polyethylene chamber secured with tape. Reactions were scored on days 2 and 4 according to ICDRG methodology. Photopatch testing was also performed, whereby the 2 benzophenones were applied in duplicate. The test substances were removed after 24 h, and the sites were irradiated with UVA (5 J/cm^2). Reactions were scored at day 1 and day 3 post-irradiation. 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] The first episode occurred 24 h after application of a sunscreen, and was described as follows: edematous, painful pruritic eruption on the arms and neck; voice changes; and tachycardia. UVA phototesting at 10, 5 and 2.5 J/cm^2 yielded normal results. Additionally, NACDG patch and photopatch test panels were 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. On the next day, 24-h patch test reactions were as follows: fragrance mix (1+), 2(2-hydroxy-5-methylphenyl)benzotriazole (++), and triclosan (+). Urticarial reactions to Benzophenone-3, Benzophenone-8, and Benzophenone-10 at test sites were also observed. Because of the severe reactions, UVA irradiation was not completed. The authors noted the occurrence of symptoms 2 h after application and severe associated urticarial and systemic symptoms. The authors also stated that immediate reactions to and systemic symptoms caused by these benzophenones are rare.

Other Clinical Reports

Benzophenone-3

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.

Benzophenone-3

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. 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 years of age. Results for Benzophenone-3 included in this study indicated that exposure to this chemical 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 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. Serum testosterone was analyzed by isotope dilution LC-MS/MS, and was natural log–transformed for analyses because the distribution of this variable was skewed left. Spot urine samples were collected from study participants, and Benzophenone-3 was 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 the 3rd and 4th quartiles of Benzophenone-3 had statistically significantly lower testosterone than males in the lowest quartile. Although the association was strongest for the 3rd quartile, the overall trend was statistically significant (p-trend = 0.01). In female adolescents, testosterone was statistically significantly higher for girls in the 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). 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. Thus, Benzophenone-3 was associated with statistically significantly lower testosterone in adolescent boys only. The authors concluded that urinary levels of Benzophenone-3 were associated with lower levels of serum testosterone in male adolescents.

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. Benzophenone-3 urinary concentrations values were not reported.

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. However, the authors noted that this association should be verified in larger study populations with planned repeated ultrasound measures during pregnancy.

A study was performed to study the association between prenatal exposure to Benzophenone-3 and gestation age and birth weight.[167] Specifically, relationships between birth outcomes and urinary concentrations of Benzophenone-3 were evaluated. The study involved a cohort of 922 pregnant women. Urinary Benzophenone-3 was 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 were performed to regress gestational age and birthweight z-scores against each woman's log average concentrations of exposure biomarkers. Logistic regression models were 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 were transformed into the change in the birth outcome for an inter-quartile-range difference in biomarker concentration (Δ). Average Benzophenone-3 urinary concentrations were associated with an increase in gestational age.

A 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.[168] The study was a longitudinal cohort study involving 338 children. 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. Urine was collected between weeks 23 and 29 of gestation.[169] A positive association between 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 of EARTH study who gave birth to 418 singleton infants between 2005 and 2018. Mothers and fathers provided an average of 4 and 3 urine samples during the preconception period, respectively. The geometric mean of Benzophenone-3 was calculated in order to estimate the preconception exposure of each participant. Risk ratios (RRs) of preterm birth (live birth before 37 completed weeks of gestation) were estimated using modified Poisson regression models adjusted for covariates. The mean gestational age among singletons was 39.3 (1.7) weeks and 8% born preterm. No consistent pattern of association was observed for Benzophenone-3 in either parent.

Benzophenone-4 and Benzophenone-10

The allergenicity of Benzophenone-4 and Benzophenone-10 was evaluated using 15 eczematous dermatitis patients (6 women, 9 men).[134] The patients were patch tested (protocol not stated) at least 3 months after complete disappearance of the dermatitis. None of the subjects had positive reactions to 10% Benzophenone-4 in petrolatum. Two subjects had positive reactions to 10% Benzophenone-10 in petrolatum.

EPIDEMIOLOGICAL STUDIES

Benzophenone-3

A case-control study on idiopathic male infertility and exposure to phenols in the environment was performed.[171] The study involved 877 idiopathic infertile men and 713 fertile controls. Urinary concentrations and semen parameters (semen volume, sperm concentration, and sperm number per ejaculate) were measured. 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.[172] The patients were tested for Benzophenone-3 in the urine via a spot test, and then divided into groups based on the 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. A third group (Group 3, non-surgical control) consisted of 219 neonates without Hirschsprung's disease. 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.

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

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.[173] Linear mixed models with fixed and random effects were used to assess changes in semen endpoints associated with the following benzophenones that were quantified in the urine: Benzophenone-1, Benzophenone-2, Benzophenone-3, and Benzophenone-8. The investigators estimated the 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 were run, including only the benzophenone and creatinine concentrations. The 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 was associated with findings such as diminished sperm concentration, more immature sperm, and a decreased percentage of other tail abnormalities. Benzophenone-8 was associated with decreased hypoosmotic swelling and higher acrosome area. No associations were observed for Benzophenone-1 or Benzophenone-3. Overall, the authors noted that Benzophenone-2 and Benzophenone-8 were associated with changes in semen endpoints, including sperm concentration, sperm viability, motility, sperm head, and morphology. The authors 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.

A study was performed to examine associations between urinary concentrations of benzophenone-type UV filters and semen quality and reproductive hormone levels.[174] The study was described as a cross-sectional study involving 215 university students. All men provided urine, blood, and semen samples on a single day. Urinary concentrations of the following benzophenones were measured: Benzophenone-1, Benzophenone-2, Benzophenone-3, Benzophenone-8, and 4-hydroxybenzophenone. In the same subjects, semen quality was evaluated by measuring volume, sperm counts, motility, and morphology. Serum samples were 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 were examined using linear regression, adjusting for potential cofounders.

Of the men tested, 97% 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: 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. No statistically significant associations between the following were found: the other benzophenones and other reproductive hormone levels or between any semen parameters and any of the urinary benzophenones. The authors concluded that, in young men, urinary benzophenone-type UV filters may be associated with a modest alteration of some reproductive hormones, but the reported effects on reproductive function are likely to be small, and of unclear clinical significance.

Benzophenone-1, Benzophenone-3

The presence of UV filters in semen, serum, and the urine was studied using 300 men.[175] Samples were 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 were 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. The authors concluded that chemical UV filters are present in men's

seminal fluid, some of which can activate the human sperm-specific CatSper Ca^{2+} channel (calcium cation channel of sperm) and thereby potentially interfere with the fertilization process.

SUMMARY

Benzophenones-1 to -12 are substituted derivatives of a 2-hydroxybenzophenone. Most benzophenones are soluble in inorganic solvents, but insoluble in water.

In the 1983 original report and in 2020, Benzophenone-2 (299 uses) and Benzophenone-4 (2259 uses) had the highest reported use frequency, respectively. The use frequency of Benzophenone-2 (299 uses) in the 1983 original report decreased to a value of 103 in 2020. The use frequency of Benzophenone-4 (240 uses) in the 1983 original report increased substantially to a value of 2259 in 2020. Of the ingredients reviewed in the1983 report, Benzophenone-4 had the highest use concentration (≤ 10% in suntan gels, creams and liquids (leave-on products)). In 2020, Benzophenone-4 is the benzophenone with the highest reported use concentration, and is being used at substantially lower concentrations of up to 1.6% in other non-coloring hair preparations (leave-on products).

According to the FDA, Benzophenones-3, -4, and -8 are active ingredients that are allowed in sunscreens. In 2019, FDA determined 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.

Results from a 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 at concentrations up to 0.5% in all types of cosmetic products does not pose a risk to the health of the consumer, apart from its contact allergenic and photoallergenic potential.

In vitro toxicokinetic studies were performed using human and in vitro (zebrafish) 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.

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 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 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 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 study on Benzophenone-12 involving rats indicated metabolism to its glucuronide conjugate, and that Benzophenone-12 had no bioaccumulation potential.

Benzophenones-1, -2, and -3 have been detected in the urine of human subjects who had not been dosed with either benzophenone. The same is true for Benzophenones-4, -6, and -8. Furthermore, Benzophenone-3 has been detected in human brain white matter and in human breast tissue.

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, Benzophenone-1 was classified as practically non-toxic (LD_{50} = 8600 mg/kg) in rats. The acute oral LD_{50} (rats) for a sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was > 2000 mg/kg. An acute oral LD_{50} of 3530 mg/kg for Benzophenone-4 was reported in a study involving rats. Acute oral 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 with Benzophenone-12.

In a short-term (2 wk) oral 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 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. A NOAEL of 1250 mg/kg/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. Repeated oral 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, a NOAEL of 1000 mg/kg/d for general systemic toxicity was determined.

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.

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 feeding study involving rats (number and strain not stated), a NOAEL of 236 mg/kg/d was reported. In a 13-wk oral toxicity study involving groups of 20 $B6C3F_1$ mice, a NOAEL of 6250 ppm was reported. 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 0.0562 and 0.0789. The number of hatched embryos at 96 h post-fertilization was also decreased.

Pregnant mice (number not stated in abstract) were exposed dermally to Benzophenone-3 (50 mg/kg/d) from GD 0 to 6. Dermal exposure resulted in an intrauterine growth restriction (IUGR) phenotype, disturbed sex ratio, and alterations in the growth curve of the offspring. In a 13-wk dermal dosing study 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 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 30, 212, and 3000 µg/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, the reproductive toxicity of Benzophenone-1 was evaluated using female rats (number and strain not stated). After 3 d of dosing, a NOAEL of 10 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 33 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 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. 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, 12500, 25000, or 50000 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, and, therefore, can affect infant neurodevelopment.

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 (maximum detection level = 22,800 ppb) in the urine and the incidence of Hirschsprung's disease. The relationship between urinary concentrations of benzophenones and semen quality was studied using 413 men. Benzophenone-2 was associated with findings such as diminished sperm concentration, more immature sperm, and a decreased percentage of other tail abnormalities. Benzophenone-8 in the urine was associated with decreased hypoosmotic swelling and higher acrosome area. No associations were observed for Benzophenone-1 or Benzophenone-3. The clinical significance of these findings was not established.

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 evaluated the gentoxicity 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 Salmonella 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 *Salmonella 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 *Salmonella 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 *Salmonella typhimurium* strains TA98 and TA100, and *Escherichia 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 *Salmonella 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 *Salmonella 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.

In the in vivo micronucleus assay using mouse erythrocytes, results for Benzophenone-1 (doses not stated) was were classified as inconclusive.

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 ppm 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 orally with doses of 0.5, 1.67, or 5 g/kg Benzophenone-3, or a single dose of 5 g/kg/d Benzophenone-3 for five consecutive days. 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 administered according to the same procedure, and results were negative. Twelve ovariectomized Balb/c female mice were dosed orally with Benzophenone-3 (3000 µg/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 (10^{-6} M) 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.

The oral carcinogenicity of Benzophenone-3 was evaluated in a National Toxicology Program (NTP) study using male and female Sprague-Dawley rats and male and female B6C3F1/N mice. On D 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 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 body weight 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 xenoestrogenic effect of Benzophenone-1 on BG-1 human ovarian cancer cells expressing estrogen receptors and relevant xenografted animals models, when compared to E2, was evaluated. In the in vitro cell viability assay, Benzophenone-1 (10^{-8} to 10^{-5} M) 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 10^{-6} M and 10^{-7} M. In the MTT assay, when the cells were co-treated with Benzophenone-1 (10^{-6} M) and biclutamide (10^{-9}), 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 10-5 M and 10-4 M. 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/cm2]) 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.

Splenocytes were cultured in the presence of different concentrations of Benzophenone-2 (10-5 to 10-8 M). Benzophenone-2 (10-5 M) 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 Benzophenonoe-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 human estrogen receptor alpha (hERα) and β (hERβ). Assays were performed at concentrations between 10^{-7} and 10^{-5} M.

Benzophenone-3 activated ERα moderately and had almost no effect on ERβ. Benzophenone-3 was not considered estrogenic at 10^{-5} M. Exposure to Benzophenone-3 (10^{-10} M) 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.

In *Saccharomyces cerevisiae* cultures, Benzophenone-2 was cytotoxic at concentrations > 2.5 x 10^{-3} M. Benzophenone-3 was also cytotoxic to the *Saccharomyces cerevisiae* (IC_{50} = 4.67E-02 mM) and *Aliivibrio fischeri* (IC_{50} = 2.40E-02). 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 1,525 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 6.44E-03 mM (estrogenic activity) was reported for Benzophenone-3. In the androgen assays, the androgenicity of Benzophenone-3 was not proven. Howevever, Benzophenone-3 was found to be antiandrogenic (EC_{50} = 1.02E-02 mM).

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.

Of the 4094 patients (with suspected allergic contact dermatitis) patch tested with 3% Benzophenone-3, 0.5% had allergic reactions. When 5,800 patients were patch tested with Benzopheonone-3 (3% in petrolatum), the incidence of positive reactions was 0.6%. Data from 64 allergenicity studies were aggregated and analyzed, in order to evaluate the irritation and

sensitization potential of sunscreen products containing Benzophenone-3 (between 1% and 6%). Forty-eight of 19,570 possible dermal responses were considered suggestive of irritation or sensitization. The mean rate of contact allergy to Benzophenone-3 was 0.07%. Of 23,908 patients patch tested, 219 (0.9%) had sunscreen coded as an allergen source. 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). In another study, 5085 patients were patch tested and allergic reactions to Benzophenone-3 (3% in petrolatum) were observed in 22.7% of the patients.

When Benzophenone-4 (10% in petrolatum) was patch tested in a study involving 4857 patients, the positive reaction rate was 2.1% (100 allergic reactions).

Of 214 patients patch tested, Benzophenone-10 and Benzophenone-3 accounted for 8 and 27 positive reactions, respectively. Twenty-three patients with a variety of photosensitive disorders were photopatch tested. Benzophenone-10 and Benzophenone-3 produced positive responses at 1.0 and 0.7 J/cm^2, respectively. In a retrospective review, 160 patients underwent photopatch testing. Benzophenone-3 caused both an allergic and photoallergic reaction in 6 patients. Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions. Another retrospective analysis involved the reviewing of 1527 charts in a patch test database. Twenty-three of the patients were tested with the sunscreen series. Of the 23, two had positive reactions (allergic contact dermatitis) to Benzophenone-3 (10% in petrolatum) and 1 had a positive reaction to Benzophenone-4 (10% in petrolatum). Of the 1527 patients screened (no specific history of sunscreen allergy), 8 patients reacted to Benzophenone-3.

A total of 5592 patients was patch tested with Benzophenone-4 and Benzopenone-3 (both at 10% in petrolatum). Values for the clinical relevance of allergic reactions were: Benzophenone-4 (definite relevance: 3 of 93 patients (3.2%)) and Benzophenone-3 (definite relevance: 4 of 24 patients (16.7%)). Results from a facial patch test series were reviewed retrospectively. Of the 1390 patients patch tested with Benzophenone-4 (2% in petrolatum), 0.79% had allergic reactions. Of the 4224 patients patch tested with Benzophenone-3 (10% in petrolatum), 0.17% had allergic reactions.

The allergenicity of Benzophenone-4 and Benzophenone-10 was evaluated using 15 eczematous dermatitis patients. Patch test reactions to 10% Benzophenone-4 in petrolatum were negative. Two subjects had positive reactions to 10% Benzophenone-10 in petrolatum. Five hundred fifty-three patients were patch tested with 10% Benzophenone-3, 10% Benzophenone-4, and 10% Benzophenone-10. Thirteen patients and 1 patient had positive reactions to 10% Benzophenone-3 and 10% Benzophenone-10, respectively. Thirteen patients also had positive reactions to 10% Benzophenone-4.

Four patients were patch tested with 3% aqueous Benzophenone-3, with and without UVA (8 J/cm^2). Each patient was photoallergic to Benzophenone-3. Positive reactions were observed with and without UVA. Patients (187 total) with a history of photosensitivity were photopatch tested with Benzophenone-3 (2% in petrolatum]. Reactions were positive in 9 patients. Nineteen patients with positive photopatch tests to sunscreen agents were retrospectively selected from the database of a contact dermatitis clinic. Of the 19 patients, 9 had a positive photopatch test reaction to 10% Benzophenone-3 in petrolatum. A study was performed, using 35 patients with confirmed photosensitivity reactions, to determine the proportion of photosensitive patients with photoallergic contact dermatitis to Benzophenone-3. Five of these patients (14.28%) had at least one positive reaction to Benzophenone-3 in the photocontact test. Four patients had a reaction at the irradiated sites only, and 1 patient had a reaction at both irradiated and nonirradiated sites.

Fifteen patients (4 males, 11 females; mean age = 47.7 years) with reactions to sunscreens were tested with sunscreen ingredients. There were 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 UV absorbers and commercial sunscreens. There were 3 allergic and 9 photoallergic reactions to Benzophenone-3 (10% in petrolatum), and no photoallergic or allergic reactions to Benzophenone-4 (10% in petrolatum). Twelve patients with a history of acute eruption on photoexposed areas were photopatch tested. Results were positive for 3 patients tested with Benzophenone-3, and negative for Benzophenone-4. In a population of 355 consecutive patients with suspected photosensitivity, the most common allergen was Benzophenone-3 (2% in petrolatum), with 15 photocontact allergic reactions and 1 contact allergic reaction.

Photopatch tests on Benzophenone-3 (10% in white paraffin) and Benzophenone-4 (5% and 10% in white paraffin) were performed using 1155 patients. Benzophenone-3 (10% in white paraffin) caused photoallergic contact reactions in 27 patients. Benzophenone-4 (10% in white paraffin) and Benzophenone-4 (5% in white paraffin) caused photoallergic contact reactions in 5 and 2 patients, respectively. The following allergic reactions were also reported: 10% Benzophenone-3 (9 patients), 10% Benzophenone-4 (9 patients), and 5% Benzophenone-4 (2 patients).

Eighty-two outpatients with photoallergic contact dermatitis were photopatch tested with Benzopheone-3 and Benzopenone-4 (concentrations not stated). Benzophenone-3 was photoallergenic in 22 of 82 patients (26.8%), and Benzophenone-4 was photoallergenic in 2 of 82 patients (2.4%). An investigation of photoallergic contact dermatitis frequency was performed using 347 patients. Benzophenone-3 (10% in petrolatum) Benzophenone-4 (2% in petrolatum) elicited photoallergic contact dermatitis in 37 patients. 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 underwent photopatch testing. 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 using 1000 consecutive dermatology outpatients. Photoallergic contact dermatitis was confirmed in 15 patients. Two of the 15 photopatch tested 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. Benzophenone-3 induced photoallergy in 3 children, and a single case of a photoaugmentation reaction to Benzophenone-4 was reported. The phototoxicity of Benzophenone-4 at concentrations of 2%, 5%, and 10% in petrolatum was studied using 80 subjects. One subject had a weak positive reaction (+ reaction), with no concomitant erythema score, to Benzophenone-4 (10% in petrolatum) at the irradiated site.

A retrospective analysis of positive photopatch test episodes was performed using results retrieved from a dermatology database (111 patients positive). The 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. Seven photodermatitis patients were patch tested and photopatch tested with Benzophenone-3, Benzophenone-4, and Benzophenone-10 (test concentrations not stated). Four and 2 patients had positive photopatch tests to Benzophenone-3 and Benzophenone-10, respectively. Photopatch test results for Benzophenone-4 were negative. The photoallergenicity of Benzophenone-4 (10% in petrolatum) and Benzophenone-10 (10% in petrolatum) was evaluated using 15 eczematous dermatitis patients. There were no positive reactions to Benzophenone-4 (10% in petrolatum). Three subjects had positive reactions to Benzophenone-10 (10% in petrolatum). Patients (280) patients with photosensitivity were patch and photopatch tested with a series of contact allergens and photoallergens (test concentration = 2% in petrolatum). Six patients were found to be allergic to Benzophenone-10, but photopatch test results were negative.

A sunscreen formulation containing Benzophenone-3 (0.6% to 0.9%) was classified as practically non-irritating to the eyes of 3 New Zealand albino rabbits. The ocular irritation potential of Benzophenone-4 (solid, 50 mg) was evaluated using the MatTek EpiOcular™ model. Test results classified Benzophenone-4 as irritating to the human eye. Benzophenone-12 (0.1 g) was evaluated for ocular irritation potential using 6 New Zealand white rabbits, and results were negative.

Benzophenone-3 had no effect on thyroid function when applied topically to 15 men (dose = 40 g) and 17 women (dose = 35 g) daily for 4 d. A study was performed to assess the relationship between exposure to endocrine-disrupting chemicals and the age of menarche in adolescent girls. It was concluded that Benzophenone-3 exposure was not significantly associated with the age of menarche. The influence of Benzophenone-3 on the age of menarche was also evaluated in a study involving 200 girls. A log ng/ml increase in childhood (pre-pubertal) urinary levels of Benzophenone-3 was associated with decreased time to menarche. The association of Benzophenone-3 with serum total testosterone levels was examined in a study involving child and adolescent participants. Benzophenone-3 was associated with statistically significantly lower testosterone in adolescent boys only.

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. An association between urinary Benzophenone and lower abdominal circumference in males was made. However, the authors noted that this association should be verified in a larger population. A study was performed to examine paternal and maternal preconception and maternal prenatal urinary phenol concentrations in relation to birth weight and head circumference. Singletons (346) born to 346 mothers and 184 fathers (184 couples) from a prospective preconception cohort of subfertile couples were evaluated. Benzophenone-3 concentration was associated with a 137 g increase in birth weight.

A study was performed to study the association between prenatal exposure to Benzophenone-3 and gestation age and birth weight. Relationships between birth outcomes and urinary concentrations of Benzophenon-3 were studied. Average Benzophenone-3 urinary concentrations were associated with an increase in gestational age. Another study was designed to determine an association between urinary phthalates, parabens, and phenols found in personal care products with pubertal timing in girls and boys (338 children total). No association relating to urinary Benzophenone-3 was found. Placental weights and birth weights were available for 473 mother-son pairs in a cohort whereby Benzophenone-3 was measured in spot urine samples. A positive association between 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 Benzopheonone-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. This study involved 417 female and 229 male participants and 418 singleton infant births. No consistent pattern of association was observed for Benzophenone-3 in either parent.

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.

TABLES

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

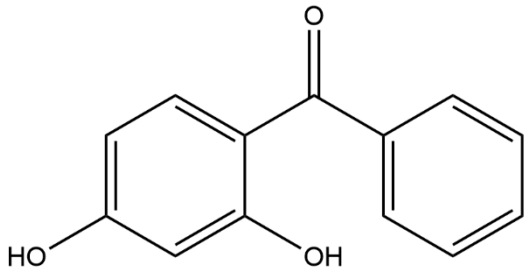
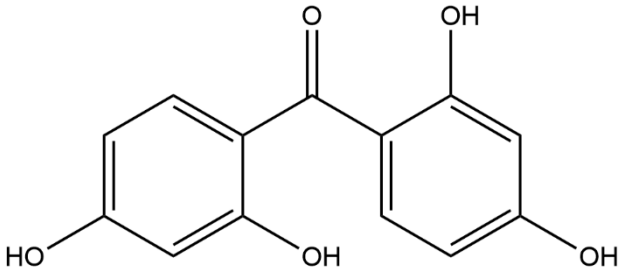
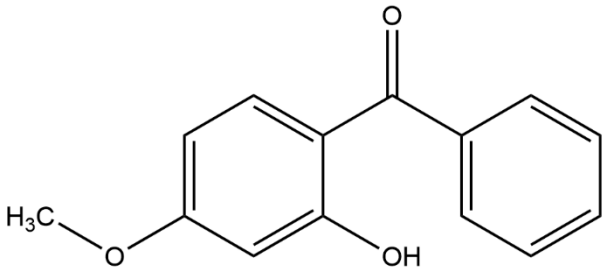
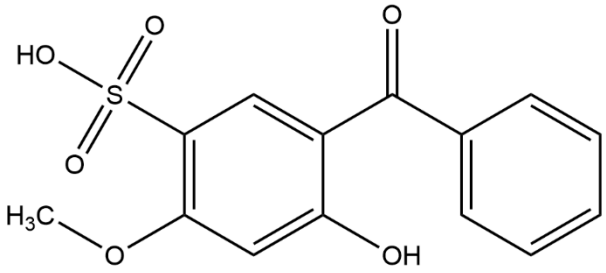
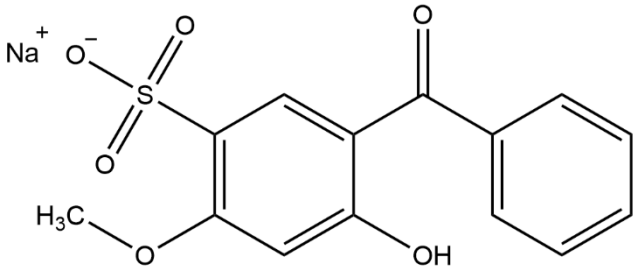
Ingredient /CAS No.	Definition & Structures	Function(s)
Benzophenone-1 131-56-6	Benzophenone-1 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-2 131-55-5	Benzophenone-2 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
Benzophenone-3 131-57-7	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

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

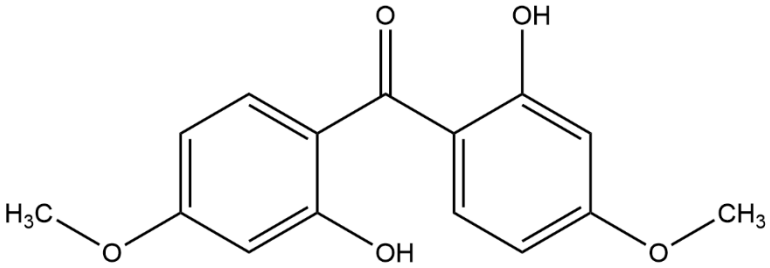
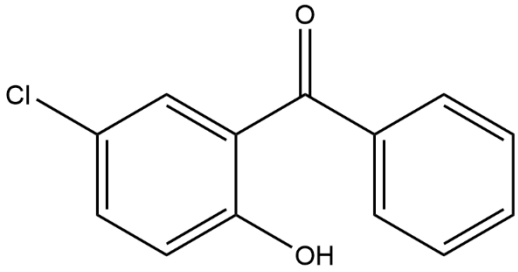
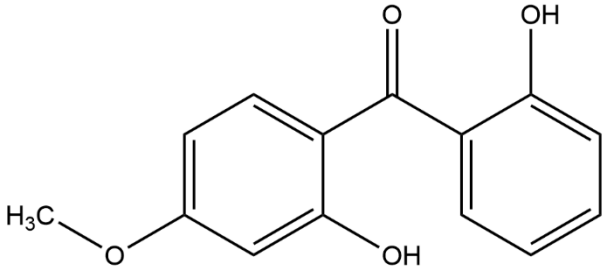
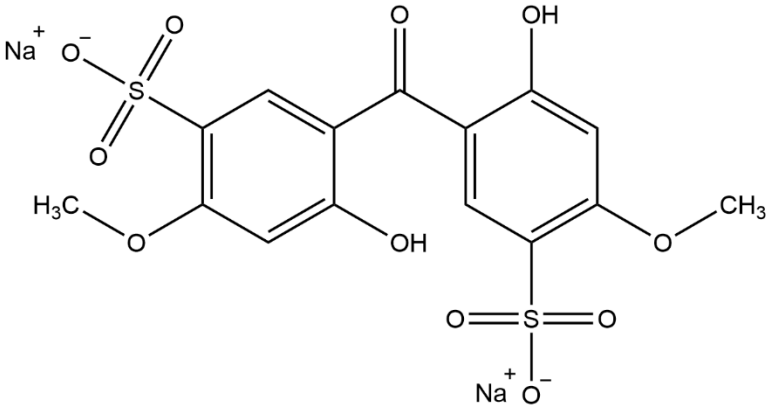
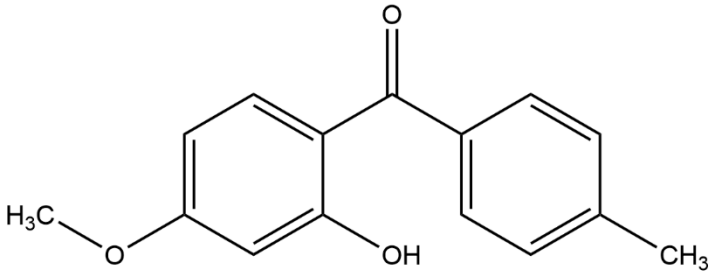
Ingredient /CAS No.	Definition & Structures	Function(s)
Benzophenone-6 131-54-4	Benzophenone-6 is a benzophenone derivative that conforms to the structure: 	Fragrance Ingredients; Light Stabilizers
Benzophenone-7 85-19-8	Benzophenone-7 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers
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

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

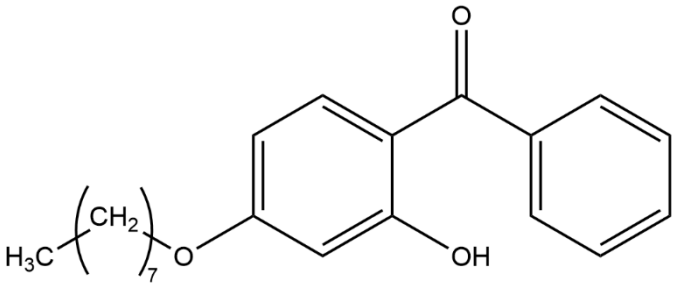
Ingredient /CAS No.	Definition & Structures	Function(s)
Benzophenone-12 1843-05-6	Benzophenone-12 is a benzophenone derivative that conforms to the structure: 	Light Stabilizers

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-7		
Molecular weight (g/mol)	232.66	1
log K_{ow}	4.09 (estimated)	10
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

Property	Value/Results	Reference
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**			
	2020[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2020[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**595**	**142**	**0.009-1.1**	**0.1-1**	**103**	**299**	**NR**	**0.1-5**
Duration of Use								
Leave-On	*566*	*128*	*0.05-1.1*	*0.1-1*	*95*	*254*	*NR*	*0.1-5*
Rinse-Off	*28*	*11*	*0.009-0.15*	*0.1*	*7*	*31*	*NR*	*0.1-1*
Diluted for (Bath) Use	*1*	*3*	*NR*	*0.1*	*1*	*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	42;2[c]	8;5[a];2[c]	NR	0.1;0.1-1[a];0.1[c]	81;4[a];2[c]	157;39[a];8[c]	NR	0.1-5;0.1[a];0.1[c]
Incidental Inhalation-Powder	2[c]	2[c]	NR	0.1[c]	2[c]	8[c]	NR	0.1[c]
Dermal Contact	55	25	0.15-0.5	0.1-1	101	274	NR	0.1-5
Deodorant (underarm)	NR	NR	NR	NR	1[a]	NR	NR	NR
Hair - Non-Coloring	NR	14	NR	0.1-1	2	25	NR	0.1-1
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	540	96	0.009-1.1	0.1-1	NR	NR	NR	NR
Mucous Membrane	1	10	0.05	0.1-1	5	15	NR	0.1-1
Baby Products	2	NR	NR	NR	NR	NR	NR	NR
	Benzophenone-3				**Benzophenone-4**			
	2020[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2020[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**989**	**47**	**0.001-0.5**	**0.1-1**	**2259**	**240**	**0.000035-1.6**	**0.1-10**
Duration of Use								
Leave-On	*853*	*43*	*0.014-0.5*	*0.1-1*	*625*	*102*	*0.0001-1.6*	*0.1-10*
Rinse-Off	*100*	*3*	*0.001-0.5*	*0.1*	*1562*	*121*	*0.000035-0.5*	*0.1-5*
Diluted for (Bath) Use	*36*	*1*	*NR*	*0.1*	*72*	*17*	*0.15*	*0.1*
Exposure Type								
Eye Area	5	NR	NR	NR	10	1	0.2	0.1-1
Incidental Ingestion	101	NR	0.5	NR	10	NR	NR	NR
Incidental Inhalation-Spray	390;110[a];78[b]	2;1[a]	0.014-0.05;0.1-0.5[b]	0.1-1;0.1[a]	91;290[a];87[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	4;78[b]	NR	0.3-0.35[b]	NR	87[c]	9[c]	0.1-0.2[b]	0.1[c]
Dermal Contact	752	10	0.0092-0.5	0.1-1	1694	104	0.005-0.5	0.1-10
Deodorant (underarm)	4[a]	NR	0.08 (spray)	NR	NR	NR	NR	NR
Hair - Non-Coloring	71	1	0.014-0.5	0.1	503	133	0.000035-1.6	0.1-5
Hair-Coloring	14	NR	0.15	NR	39	1	0.05-0.1	0.1-1
Nail	51	36	0.001-0.4	0.1-1	6	2	0.2	0.1
Mucous Membrane	157	1	0.05-0.5	0.1	1196	19	0.15-0.2	0.1-1
Baby Products	1	NR	0.05-0.25	NR	2	2	NR	0.1
	Benzophenone-5				**Benzophenone-6**			
	2020[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2020[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**14**	**10**	**0.06**	**0.1**	**NR**	**90**	**NR**	**0.1-1**
Duration of Use								
Leave-On	*12*	*10*	*NR*	*0.1*	*NR*	*84*	*NR*	*0.1-1*
Rinse-Off	*2*	*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	2	NR	0.06	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a];7[c]	NR;3[a];7[c]	NR	NR;0.1[a];0.1[c]	NR	3	NR	0.1-1
Incidental Inhalation-Powder	7[c]	7[c]	NR	0.1[c]	NR	NR	NR	NR
Dermal Contact	12	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

*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 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**			
	2020[11]	**1983[1]**	**2020[12]**	**1983[1]**	**2020[11]**	**1983[1]**	**2020[12]**	**1983[1]**
Totals*	**NR**	**4**	**NR**	**0.1-1**	**71**	**123**	**NR**	**0.1-1**
Duration of Use								
Leave-On	*NR*	*1*	*NR*	*0.1*	*41*	*41*	*NR*	*0.1-1*
Rinse-Off	*NR*	*2*	*NR*	*0.1-1*	*29*	*27*	*NR*	*0.1-1*
Diluted for (Bath) Use	*NR*	*1*	*NR*	*0.1-1*	*1*	*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]	13;5[a];15[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	15[c]	14[c]	NR	0.1-1[c]
Dermal Contact	NR	2	NR	0.1-1	68	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	0.35	0.1-1
Hair-Coloring	NR	NR	NR	NR	NR	1	NR	0.1
Nail	NR	NR	NR	NR	1	3	NR	0.1
Mucous Membrane	NR	1	NR	0.1-1	28	55	NR	0.1
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Benzophenone-11							
	2020[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

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2

Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11

Benzophenones-1 to -12 are substituted derivatives of 2-hydroxybenzophenone. They are used as photostabilizers in cosmetics and have a photoprotective effect on the skin.

When ingested and absorbed, Benzophenones were primarily conjugated and excreted in the urine. Benzophenones were practically nontoxic when chronically administered orally to rats, and Benzophenones-3 and -4 were nontoxic when applied to the skin of rabbits at doses of > 5 g/kg. Subchronic oral ingestion of Benzophenone-3 at 1% was nontoxic to rats; however, another study showed Benzophenone-3 at 0.5% was toxic. Benzophenone-1 elicited toxic effects in rats at 0.6 g/kg.

Benzophenones were nonirritating or mildly irritating to rabbit skin at concentrations of up to 100% and practically nonirritating to the eyes of rabbits. A subchronic skin irritation test indicated that Benzophenone-4 was capable of causing minimal irritation in rabbits at a concentration of 10%. Benzophenone-3 was reported to be nonsensitizing and nonphototoxic in guinea pigs and rabbits.

Benzophenones-1, -3, -4, -5, and -9 were nonmutagenic both with and without metabolic activation in the Ames test.

Skin irritation and sensitization in humans indicated that Benzophenones were mildly irritating and sensitizing at concentrations greater than those used in cosmetics.

On the basis of the available animal data and clinical human experience, it is concluded that Benzophenone-1, -3, -4, -5, -9, and -11 are safe for topical application to humans in the present practices of use and concentration in cosmetics.

INTRODUCTION

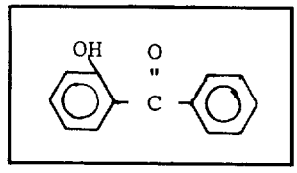
This document reviews the published and unpublished information on Benzophenones-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12. On the basis of

the information within this report, a safety assessment has been made concerning Benzophenones-1, -3, -4, -5, -9, and -11. Relevant chemical, use, toxicological, and clinical data on the other Benzophenones have been included. Benzophenones-7, -10, and -12 are not used in cosmetics; therefore, a safety recommendation is not included on these three ingredients. A separate determination of safety was made for Benzophenones-2, -6, and -8.

CHEMISTRY

General Structure

Benzophenones-1 to -12 are substituted derivatives of 2-hydroxybenzophenone, which conforms to the structure:



Substituents include hydroxy, methoxy, octyloxy, sulfonyl, methyl, and chloride groups. Benzophenones* may be mono- di-, tri-, or tetra-substituted.

Among the many preparative methods for individual Benzophenones, the most common is the Friedel–Crafts reaction.[1-4]

Benzophenones-3, -6, and -8 are components of and can be extracted from certain flower pigments.[5]

General Properties

The most important property of the Benzophenones is their ability to absorb and dissipate ultraviolet (UV) radiation. When UV light passes through a Benzophenone solution, certain frequencies or wavelengths are selectively absorbed. Electromagnetic energy is transferred to the Benzophenone molecule; as a result, outer electrons are promoted from their lowest-energy ground state to higher-energy excited states. Since only certain states are possible in any given molecule, and since the energy difference between any ground state and excited state must equal the energy added by the quantum, only certain frequencies of radiation can be absorbed by a particular Benzophenone. Excited molecules are relatively short-lived and tend to return to their ground states after approximately

*Throughout this report the term "Benzophenone(s)" is used although all compounds reviewed are 2-hydroxybenzophenones.

10^{-8} seconds. Under usual circumstances, the excited molecule loses its energy and returns to the ground state through a series of collisions with other molecules in the system; the net effect of this process is that the absorbed energy is converted to heat. If an excited molecule is slow to lose its excess energy through collision, it may return to the ground state by emitting radiation of lower frequency than the absorbed radiation. The net effect of this process is fluorescence. Benzophenones are used to protect photodegradable compounds. The Benzophenones form intermolecular hydrogen bonds with the photodegradable molecules; these bonds serve as bridges to transfer energy from the electronically excited, vulnerable molecules to the Benzophenone molecule.[6-8]

In a study that determined the effect of substituent addition and substitution on the photostabilizing property of Benzophenones, alkylation of the hydroxyl group at the para position reduced the photostabilizing potential of the molecule. Addition of a methoxy group to the second benzene nucleus also reduced the molecule's photoprotecting effect.[9]

Most Benzophenones are solid at room temperature, soluble in organic solvents, and insoluble in water.

General Reactions

Owing to the variety of substituents in these ingredients, many Benzophenone derivatives can be prepared. Benzophenones can undergo etherification and reactions typical of ketones. Via the Grignard reaction, alcohols can be prepared from Benzophenones.[10] Benzophenones can take part in photopinacolization reactions in which a reduction of two ketones produces a bond between the carbons.[11] Benzophenones are reduced by sodium hydroborates.[12]

Although Benzophenones are frequently incorporated into plastics and films, normally they do not react with the polymer itself. However, Kamogawa[13] described an acid-catalyzed reaction between N-(hydroxymethyl)-acrylamide and Benzophenones. The resulting product was a polymeric phenolic UV absorber.

General Analysis

Thin-layer chromatography and gas chromatography are frequently employed to determine the Benzophenone content in plastics, polymers, and films.[14-21] Spectroscopic methods including mass spectroscopy, spectrofluorometry, phosphorimetry, nuclear magnetic resonance (NMR), and infrared (IR) spectroscopy are also used to identify Benzophenones.[22-27]

Since 1978, reverse phase high performance liquid chromotography (HPLC) has been recommended for the analysis of Benzophenones. In the case of the Benzophenone sulfonic acids (Benzophenone-4, -5, and -9), a μ Bondapak CN column and water-methanol (95:5) mobile phase are used; in the case of the other Benzophenones, a μ Bondapak C_{18} column and a water-methanol (40:60) plus 1 to 2 volumes acetic acid mobile phase are used.[28]

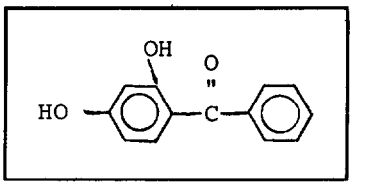
Infrared spectra for the individual Benzophenones have been reported by CTFA.[29]

Individual Benzophenone Ingredients

Benzophenone-1

Structure

Benzophenone-1 is a dihydroxy Benzophenone conforming to the structure:[3]



Other names include:

2,4-Dihydroxybenzophenone
Benzoresorcinol
4-Benzoyl Resorcinol
(2,4-Dihydroxyphenyl) phenylmethanone
Resbenzophenone

Three methods of Benzophenone-1 preparation from resorcinol are reported. Stephen[30] and Zilberman and Rybakova[31] prepared Benzophenone-1 from the Hoesch reaction of resorcinol and either a substituted imido chloride (to form an imido-ester intermediate) or a corresponding aromatic nitrile and a metal halide catalyst. Shaw and Mehta[32] described the condensation of benzamide with resorcinol in the presence of phosphorous oxychloride and zinc chloride to Benzophenone-1. Additionally, Benzophenone-1 can be prepared in low yield by the Fries rearrangement from phenyl-2-methoxy-benzoate.[1]

Properties

Benzophenone-1 (MW 214.21) is a light-yellow powder with a melting point of 144°C. It is soluble in methanol, ethanol, ethyl acetate, methyl ethyl ketone, acetone, ether, and acetic acid; slightly soluble in benzene; and insoluble in water.[3,29] Tables 1 and 2 describe other physical and chemical data for this compound.

Reactivity

Benzophenone-1 reacts with a variety of organic and inorganic compounds. Head[33] reported an etherification of the 4-hydroxy group of Benzophenone-1 to 4-(β-aryioxymethyl), 4-(β-arylethoxymethyl), and 4-[β-(aryloxymethoxy)ethyl] derivatives. In the presence of bromide, phenyl nitrate, or nitric acid, Benzophenone-1 can react to form a number of bromo-and nitrobenzenes.[34] Benzophenone-1 and methyl acrylate can combine to form a product that can polymerize with other compounds to form a photostable polymer.[35] Benzophenone-1 is highly reactive with diphenylpicrylhydrazyl.[36]

TABLE 1. Chemical and Physical Properties.[a]

Ingredient	Specific gravity (at 25°C)	pH (10%/25°C)	Moisture (% max.)	Impurities (ppm max.) Pb	As
Benzophenone-1	1.2743	2.0–3.0	2	18	1
Benzophenone-2	–[b]	4.0	5.0	8	1
Benzophenone-3	–	–	2	13	1
Benzophenone-4	–	2.0 (1%)	10–16 (trihydrate)	18	1
Benzophenone-6	1.3448	4.0–5.0	0.5	13	1
Benzophenone-8	–	–	2	–	–
Benzophenone-9	–	6.8–7.2	5.0	8	1
Benzophenone-11	1.3843	3.0–4.0	5.0	13	1

[a]Data from Refs. 3, 6, and 29.
[b]No data available.

Impurities

The maximum recommended levels of arsenic and lead in Benzophenone-1 are reported in Table 1.[3]

Benzophenone-2

Structure

Benzophenone-2 is a tetrahydroxy-substituted derivative of Benzophenone conforming to the structure:[3]

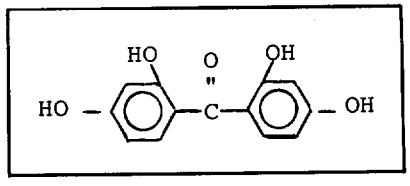


TABLE 2. UV Absorption Spectra Data for Benzophenones.

Benzophenone	λ max (nm)	log ε	λ max (nm)	log ε	λ max (nm)	log ε	Ref.
–1	242	3.94	290	3.96	338	4.12	37
–2	242	3.80	283	3.96	352	4.17	37
–3	–	–	289	4.13	322	3.96	37
–4	242	4.11[a]	288	4.14[a]	333	3.92[a]	38
–6	–	–	281	4.11	339	4.12	3,29
–8	242	4.18[b]	285	4.31[b]	330	4.18[b]	38
–9	–	–	284	3.85	333	–	3,29
–10	250	3.89	300	4.27	–	–	37
–11	–	–	285	4.10	341	4.12[c]	3,29

[a]Assuming cell path length = 1 cm.
[b]Assuming cell path length = 10 cm.
[c]Assuming average molecular wt. of BP-11 is approx. that of BP-6.

Other names include:

2,2', 4, 4'-Tetrahydroxybenzophenone

Benzophenone-2 is prepared either by the reaction of hydroxybenzenes with benzyl hydroxide in the presence of a metal halide catalyst,or by the condensation of resorcinol with 2,4-dihydroxybenzoic acid in the presence of $POCl_3$ and $ZnCl_2$.[2,3,39,40]

Properties

Benzophenone-2 (MW 302.33) is a yellow crystalline solid with a melting point of 195°C. It is soluble in methanol, ethanol, methyl ethyl ketone, and only slightly soluble in water and toluene.[29,41] Tables 1 and 2 list other physicochemical data of Benzophenone-2.

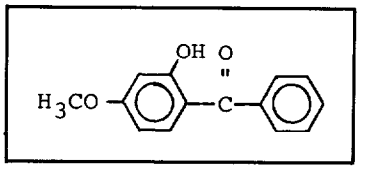
Impurities

The maximum recommended levels of lead and arsenic in Benzophenone-2 are listed in Table 1.[3]

Benzophenone-3

Structure

Benzophenone-3 is a monomethoxylated derivative of the parent compound, and it conforms to the structure:[3]



Other names include:

2-Hydroxy-4-methoxybenzophenone
Oxybenzone

Benzophenone-3 is prepared by the Friedel-Crafts reaction of benzoyl chloride with 3-hydroxyanisole. The product is then recrystallized from water/methanol and dried.[3]

Properties

Benzophenone-3 (MW 228.26) is a light cream-colored powder that melts at 66°C and has low volatility. It is soluble in most organic solvents and insoluble in water.[3,42] Tables 1 and 2 list other physical and chemical data for this compound.[3,6]

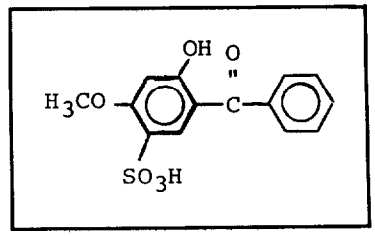
Impurities

The maximum recommended levels of lead and arsenic impurities in Benzophenone-3 are listed in Table 1.[3]

Benzophenone-4

Structure

Benzophenone-4 is a sulfonic acid derivative of Benzophenone-3. It conforms to the structure:[3]



Other names include:

2-Hydroxy-4-Methoxybenzophenone-5-Sulfonic Acid
Sulisobenzone

Benzophenone-4 is prepared via sulfonation of Benzophenone-3. The product is purified by precipitation from aqueous HCl, isolated by centrifugation, washed with acidic water, and dried.[3]

Properties

Benzophenone-4 (MW 318.39) is a pale ivory-colored powder that is soluble in water (33.4 g/100 ml H_2O), methanol, and ethanol.[43] It has a melting point of 147°C.[44,45] Tables 1 and 2 list other data for Benzophenone-4.[3,6]

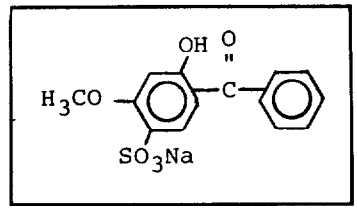
Impurities

The maximum recommended levels of lead and arsenic impurities in Benzophenone-4 are reported in Table 1.[3]

Benzophenone-5

Structure

Benzophenone-5 is the sodium salt of Benzophenone-4. It conforms to the structure:[3]



Other names include:

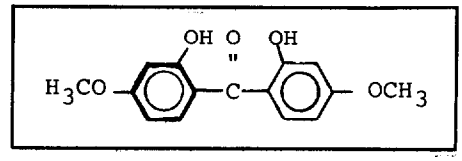
2-Hydroxy-4-Methoxybenzophenone-5-Sodium Sulfonate

No data on properties, reactivity, or impurities of Benzophenone-5 were available.

Benzophenone-6

Structure

Benzophenone-6 is a tetra-substituted Benzophenone conforming to the structure:[3]



Other names include:

2,2'-Dihydroxy-4,4'-Dimethoxybenzophenone
Bis (2-Hydroxy-4-Methoxyphenyl)-Methanone

For the synthesis of Benzophenone-6, 1,3-dimethoxybenzene is reacted with oxalyl chloride. The resulting 2,2',4,4'-tetramethoxy-benzophenone is demethylated to Benzophenone-6 with $AlCl_3$.[46] The same compound is also formed by the condensation of 3-methoxyphenol with 2-hydroxy-4-methoxybenzoic acid in the presence of phosphorous oxychloride and zinc chloride.[2] A proprietary method has been reported in which the Friedel–Crafts reaction is used.[3]

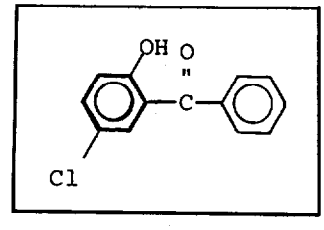
Properties

Benzophenone-6 (MW 274.26) is a light yellow solid with a melting point of 124°C. It is soluble in methanol, ethanol, ethyl acetate, methyl ethyl ketone, and toluene, but is insoluble in water.[3,29] Tables 1 and 2 contain additional information regarding Benzophenone-6.

Benzophenone-7

Structure

Benzophenone-7 is a chlorinated derivative of hydroxybenzophenone. It conforms to the structure:[3]



Other names include:

5-Chloro-2-Hydroxybenzophenone
2-Hydroxy-5-Chlorobenzophenone

Benzophenone-7 is prepared via the Friedel–Crafts reaction of chloromethoxybenzene with benzoyl chloride in the presence of aluminum chloride.[4]

Reactivity

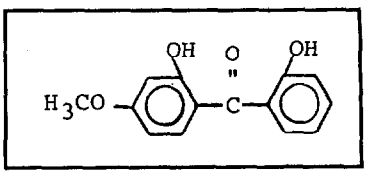
Benzophenone-7 reacts with phosphorous pentachloride to give 4-chloro-2-

$(C_6H_5CCl_2)$-$C_6H_3OPOCl_2$. It will also combine with salicylaldehyde and cobalt to form a series of cobalt (II) complexes of cyclic ligands.[47,48] Benzophenone-7 is reactive in the presence of diphenylpicrylhydrazyl.[36]

Benzophenone-8

Structure

Benzophenone-8 is the 2'-hydroxy derivative of Benzophenone-3, and it conforms to the structure:[3]



Other names include:

2,2'-Dihydroxy-4-Methoxybenzophenone
Dioxybenzone

No information regarding the manufacturing process of Benzophenone-8 was available.

Properties

Benzophenone-8 (MW 244.24) is a yellow crystalline solid. A product of 93% purity had a melting range of 73.5°–74.5°C and a boiling point at 1 mm Hg of 164°–166°C.[49] It is soluble in methanol, ethanol, ethyl acetate, isopropanol, ether, and acetone, and slightly soluble in water. Benzophenone-8 is stable to moisture at temperatures up to 200°C.[3,29,41,50] Tables 1 and 2 list other physical and chemical data for this compound.[3,6]

Reactivity

Ismail[51] reported that Benzophenone-8 reacts with organometallic compounds to give preparations which, when used in polyvinyl chloride, stabilize this polymer against ultraviolet radiation damage.

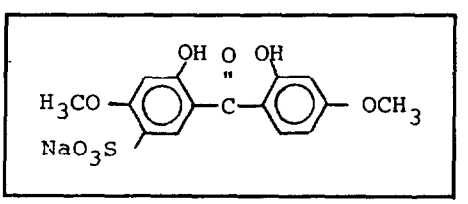
Impurities

The starting materials used or the by-products formed in the preparation of Benzophenone-8 may be present at a total concentration of up to 7% in the final product. These materials include: resorcinol dimethyl ether, resorcinol monomethyl ether, trihydroxybenzophenone, xanthone, free sulfur, or sulfur compounds.[52]

Benzophenone-9

Structure

Benzophenone-9 conforms to the structure:[3]



Other names include:

Sodium 2,2'-Dihydroxy-4,4'-Dimethoxy-5-Sulfobenzophenone

Benzophenone-9 is prepared by the sulfonation of Benzophenone-6.[3]

Properties

Benzophenone-9 is a light yellow powder with a melting point of 350°C. It is soluble in water and slightly soluble in methanol and ethanol, and insoluble in ethyl acetate and benzene. Benzophenone-9 is diluted with sodium sulfate to 67% when supplied from the manufacturer.[3,29] Tables 1 and 2 list other physiochemical data for this compound.

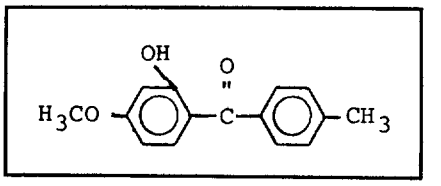
Impurities

The maximum recommended levels of lead and arsenic impurities in Benzophenone-9 are reported in Table 1.[3]

Benzophenone-10

Structure

Benzophenone-10 is a 4'-methyl derivative of Benzophenone-3. It conforms to the structure:[3]



Other names for Benzophenone-10 include:

2-Hydroxy-4-Methoxy-4'-Methylbenzophenone

Mexenone

No other chemical data regarding Benzophenone-10 were available.

Benzophenone-11

Structure

Benzophenone-11 is a mixture of 2,2'-Dihydroxy-4,4'-dimethoxybenzophenone (Benzohenone-6) and other tetra-substituted benzophenones.[3] Benzophenone-11 is manufactured by a proprietary Friedel-Crafts reaction.[3]

Properties

Benzophenone-11 is a yellow or tan powder that has a melting range of 85°–105°C.[3] It is soluble in methanol, ethanol, ethyl acetate, and methyl ethyl ketone, and insoluble in water.[45] Other properties of Benzophenone-11 are listed in Tables 1 and 2.[3,6]

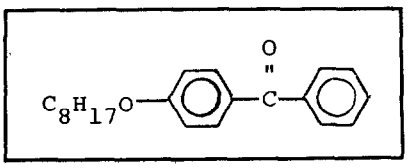
Impurities

The maximum recommended levels of lead and arsenic impurities in Benzophenone-11 are reported in Table 1.[3]

Benzophenone-12

Structure

Benzophenone-12 conforms to the structure:[3]



Other names include:

2-Hydroxy-4-(octyloxy)benzophenone
2-Hydroxy-4-(octyloxy)phenyl phenylmethanone
Octabenzone

Properties

Benzophenone-12 (MW 326.42) has a melting point of 46°C.[3] In a study of the volatility at 200°C of various antioxidants used in polymers, this ingredient was one of the least volatile.[53]

USE

Cosmetic

Benzophenones are used in cosmetics as ultraviolet light absorbers (photostabilizers). Each Benzophenone has its own characteristic absorption spectrum (Table 2). Benzophenones-2, -3, -4, -6, -8, and -9 are used in suntan lotions and hair sprays because they protect the skin and hair from the harmful effects of the sun.[54-63] These ingredients also photostabilize cosmetic dyes, creams, and lotions.[61,64-66] Although most Benzophenones are water insoluble, the presence of the sulfonic acid group in Benzophenones-4, -5, and -9 makes these ingredients soluble in water.[3]

According to the industry's voluntary submission to the Food and Drug Administration (FDA) in 1976, Benzophenones are used in over a thousand cosmetic formulations, typically in concentrations up to 1%. Benzophenones are supplied undiluted from the manufacturer, with the exceptions of Benzophenone-9, which is diluted with sodium sulfate to 67%, and Benzophenone-8, which is supplied as 93% active. The following are the maximum reported product concentrations for each Benzophenone: Benzophenone-1, 1%; Benzophenone-2, 5%; Benzophenone-3, 1%; Benzophenone-4, 10%; Benzophenone-5, $\leq$0.1%; Benzophenone-6, 1%; Benzophenone-8, 1%; Benzophenone-9, 1%; Benzophenone-11, 5%. Benzophenones-7, -10, and -12 have no current cosmetic use. Product formulation data for Benzophenones are listed in Table 3.[67,68]

The cosmetic product formulation computer printout, which is made available by the FDA, is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations (1979). Ingredients are

TABLE 3. Product Formulation Data.[a]

Product category[b]	Total no. containing ingredient	No. product formulations within each concentration range (%)[b]				
		Unreported concentration	>5-10	>1-5	>0.1-1	≤0.1
Benzophenone-1						
Bath oils, tablets, and salts	1	–	–	–	–	1
Bubble baths	2	–	–	–	–	2
Colognes and toilet waters	3	–	–	–	–	3
Other fragrance preparations	5	–	–	–	–	5
Hair shampoos (noncoloring)	7	–	–	–	–	7
Tonics, dressings, and other hair grooming aids	2	–	–	–	1	1
Wave sets	4	–	–	–	–	4
Other hair preparations (noncoloring)	1	–	–	–	–	1
Blushers (all types)	1	–	–	–	1	–
Lipstick	7	–	–	–	7	–
Nail basecoats and undercoats	5	–	–	–	3	2
Nail polish and enamel	87	–	–	–	2	85
Other manicuring preparations	4	–	–	–	1	3
Aftershave lotions	6	–	–	–	–	6
Beard softeners	2	–	–	–	–	2
Face, body, and hand skin care preparations (excluding shaving preparations)	2	–	–	–	–	2
Moisturizing skin care preparations	3	–	–	–	–	3
1976 TOTALS	142	–	–	0	15	127
1979 TOTALS[c]	113	–	–	1	21	91
Benzophenone-2						
Bath oils, tablets, and salts	3	–	–	–	–	3
Bubble baths	5	–	–	–	1	4
Other bath preparations	6	–	–	–	–	6
Colognes and toilet waters	120	–	–	1	27	92
Perfumes	22	–	–	–	1	21
Sachets	4	–	–	–	–	4
Other fragrance preparations	15	–	–	–	5	10
Hair conditioners	2	–	–	–	–	2
Hair rinses (noncoloring)	4	–	–	–	–	4
Hair shampoos (noncoloring)	14	–	–	–	2	12
Tonics, dressings, and other hair grooming aids	2	–	–	–	–	2
Wave sets	3	–	–	–	3	–
Blushers (all types)	3	–	–	–	–	3
Rouges	1	–	–	–	–	1

TABLE 3. (Continued.)

Product category[b]	Total no. containing ingredient	No. product formulations within each concentration range (%)[b]				
		Unreported concentration	>5–10	>1–5	>0.1–1	≤0.1
Makeup fixatives	1	–	–	–	–	1
Other makeup preparations (not eye)	4	–	–	–	–	4
Feminine hygiene deodorants	1	–	–	–	–	1
Aftershave lotions	30	–	–	–	6	24
Preshave lotions (all types)	1	–	–	–	–	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	6	–	–	–	2	4
Face, body, and hand skin care preparations (excluding shaving preparations)	7	–	–	–	–	7
Moisturizing skin care preparations	8	–	–	–	–	8
Paste masks (mud packs)	1	–	–	–	–	1
Skin lighteners	1	–	–	–	–	1
Skin fresheners	27	–	–	–	–	27
Wrinkle smoothers (removers)	1	–	–	–	–	1
Skin care preparations	5	–	–	–	1	4
Suntan gels, creams, and liquids	1	–	–	–	–	1
Other suntan preparations	1	–	–	–	–	1
1976 TOTALS	299	–	–	1	48	250
1979 TOTALS[c]	321	80	–	2	32	207
Benzophenone-3						
Bath oils, tablets, and salts	1	–	–	–	–	1
Colognes and toilet waters	1	–	–	–	1	–
Perfumes	1	–	–	–	–	1
Hair shampoos (noncoloring)	1	–	–	–	–	1
Makeup preparations (not eye)	1	–	–	–	1	–
Nail polish and enamel	36	–	–	–	36	–
Aftershave lotions	3	–	–	–	3	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	2	–	–	–	–	2
Skin fresheners	1	–	–	–	–	1
1976 TOTALS	47	–	–	–	41	6
1979 TOTALS[c]	62	–	–	10	45	7
Benzophenone-4						
Baby shampoos	2	–	–	–	–	2
Bath oils, tablets, and salts	11	–	–	–	–	11

TABLE 3. (Continued.)

Product category[b]	Total no. containing ingredient	No. product formulations within each concentration range (%)[b]				
		Unreported concentration	>5–10	>1–5	>0.1–1	≤0.1
Bubble baths	2	–	–	–	–	2
Other bath preparations	4	–	–	–	–	4
Eye shadow	1	–	–	–	1	–
Colognes and toilet waters	8	–	–	–	–	8
Other fragrance preparations	11	–	–	–	–	11
Hair conditioners	29	–	–	1	2	26
Hair sprays (aerosol fixatives)	1	–	–	–	–	1
Permanent waves	2	–	–	–	–	2
Hair rinses (noncoloring)	7	–	–	–	–	7
Hair shampoos (noncoloring)	45	–	–	1	16	28
Tonics, dressings, and other hair grooming aids	7	–	–	–	1	6
Wave sets	27	–	–	–	1	26
Other hair preparations (noncoloring)	13	–	–	–	–	13
Hair shampoos (coloring)	1	–	–	–	1	–
Blushers (all types)	6	–	–	–	2	4
Makeup foundations	1	–	–	–	–	1
Leg and body paints	1	–	–	–	–	1
Makeup bases	1	–	–	–	1	–
Other makeup preparations (not eye)	2	–	–	–	2	–
Cuticle softeners	2	–	–	–	–	2
Bath soaps and detergents	2	–	–	–	2	–
Aftershave lotions	2	–	–	–	–	2
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	6	–	–	–	1	5
Face, body, and hand skin care preparations (excluding shaving preparations)	9	–	–	–	–	9
Moisturizing skin care preparations	21	–	–	–	–	21
Skin fresheners	5	–	–	–	–	5
Other skin care preparations	9	–	–	–	2	7
Suntan gels, creams, and liquids	2	–	1	–	–	1
1976 TOTALS	240	–	1	2	32	205
1979 TOTALS[c]	251	67	1	1	19	163
Benzophenone-5						
Face, body, and hand skin care preparations (excluding shaving preparations)	7	–	–	–	–	7

TABLE 3. (Continued.)

Product category[b]	*Total no. containing ingredient*	*No. product formulations within each concentration range (%)*[b]				
		Unreported concentration	*>5–10*	*>1–5*	*>0.1–1*	*≤0.1*
Night skin care preparations	3	–	–	–	–	3
1976 TOTALS	10	–	–	–	–	10
1979 TOTALS[c]	11	–	–	–	–	11
Benzophenone-6						
Bath oils, tablets, and salts	2	–	–	–	–	2
Colognes and toilet waters	1	–	–	–	–	1
Perfumes	2	–	–	–	1	1
Hair shampoos (noncoloring)	1	–	–	–	–	1
Tonics, dressings, and other hair grooming aids	1	–	–	–	–	1
Wave sets	2	–	–	–	–	2
Cuticle softeners	1	–	–	–	–	1
Nail polish and enamel	77	–	–	–	77	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	1	–	–	–	–	1
Moisturizing skin care preparations	2	–	–	–	1	1
1976 TOTALS	90	–	–	–	79	11
1979 TOTALS[c]	106	–	–	–	93	13
Benezophenone-8						
Bath oils, tablets and salts	1	–	–	–	1	–
Hair conditioners	2	–	–	–	2	–
Moisturizing skin care preparations	1	–	–	–	–	1
1976 TOTALS	4	–	–	–	–	4
1979 TOTALS[c]	3	–	–	1	1	1
Benzophenone-9[d]						
Bubble baths	20	–	–	–	–	20
Bath capsules	1	–	–	–	–	1
Other bath preparations	34	–	–	–	–	34
Colognes and toilet waters	2	–	–	–	1	1
Perfumes	1	–	–	–	1	–
Other fragrance preparations	1	–	–	–	–	1
Hair conditioners	9	–	–	–	1	8
Hair rinses (noncoloring)	3	–	–	–	–	3
Hair shampoos (noncoloring)	8	–	–	–	3	5
Tonics, dressings, and other hair grooming aids	1	–	–	–	–	1
Wave sets	2	–	–	–	–	2

TABLE 3. (Continued.)

Product category[b]	Total no. containing ingredient	No. product formulations within each concentration range (%)[b]				
		Unreported concentration	>5–10	>1–5	>0.1–1	≤0.1
Other hair preparations (noncoloring)	1	–	–	–	–	1
Blushers (all types)	1	–	–	–	–	1
Makeup bases	1	–	–	–	1	–
Rouges	1	–	–	–	–	1
Nail basecoats and undercoats	1	–	–	–	–	1
Cuticle softeners	1	–	–	–	–	1
Nail creams and lotions	1	–	–	–	–	1
Aftershave lotions	3	–	–	–	1	2
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	4	–	–	–	2	2
Face, body, and hand skin care preparations (excluding shaving preparations)	14	–	–	–	1	13
Moisturizing skin care preparations	2	–	–	–	–	2
Skin fresheners	9	–	–	–	1	8
Other skin care preparations	1	–	–	–	–	1
Suntan and sunscreen preparations	1	–	–	–	1	–
1976 TOTALS	123	–	–	–	13	110
1979 TOTALS[c]	85	38	–	–	9	38
Benzophenone-11						
Bath oils, tablets, and salts	4	–	–	–	2	2
Bubble baths	4	–	–	–	–	4
Other bath preparations	1	–	–	–	–	1
Colognes and toilet waters	59	–	–	–	6	53
Perfumes	14	–	–	–	–	14
Sachets	7	–	–	–	–	7
Other fragrance preparations	8	–	–	–	–	8
Hair sprays (aerosol fixatives)	4	–	–	1	2	1
Hair shampoos (noncoloring)	13	–	–	–	–	13
Tonics, dressings, and other hair grooming aids	2	–	–	–	2	–
Wave sets	2	–	–	–	–	2
Blushers (all types)	1	–	–	–	–	1
Nail polish and enamel	3	–	–	–	–	3
Bath soaps and detergents	3	–	–	–	–	3
Aftershave lotions	16	–	–	–	–	16
Preshave lotions (all types)	1	–	–	–	–	1

TABLE 3. (Continued.)

Product category[b]	Total no. containing ingredient	No. product formulations within each concentration range (%)[b]				
		Unreported concentration	>5–10	>1–5	>0.1–1	≤0.1
Face, body, and hand skin care preparations (excluding shaving preparations)	2	–	–	–	–	2
Moisturizing skin care preparations	12	–	–	–	–	12
Skin fresheners	11	–	–	–	–	11
Other skin care preparations	1	–	–	–	–	1
1976 TOTALS	168	–	–	1	12	155
1979 TOTALS[c]	103	65	–	1	10	27

[a]Data from Ref. 67.

[b]Preset product categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4); see Scope and Extent of Use in Cosmetics.

[c]Data from Ref. 73.

[d]Benzophenone-9 is supplied as a 67% solution; use concentration values may or may not have been adjusted accordingly by manufacturers when submited to the FDA.

listed in prescribed concentration ranges under specific product-type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the true, effective concentration found in the finished product; the effective concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to tenfold error in the assumed ingredient concentration.

Benzophenones-1, -3, and -6 are most frequently found in nail polishes (in concentrations up to 1%); Benzophenones-2 and -11 are most frequently used in fragrance preparations (in concentrations up to 1%); and Benzophenones-4, -5, and -9 are generally used in hair, skin, and bath preparations, respectively (in concentrations up to 1%).

Benzophenones are used in at least nine major cosmetic categories. Formulations containing Benzophenones may come into contact with the face, hair and scalp, nails, lips, mucosa, and skin. Products containing Benzophenones are used daily or occasionally; their use may extend over a period of years. Frequency and duration of application may be continuous.

Certain Benzophenones reduce the harmful effects of UV radiation on the skin. The maximum absorption wavelengths for specific Benzophenones are

listed in Table 2. Ultraviolet light is divided into three distinct bands: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm). Exposing unprotected skin to UV light (primarily in the UV-B range) can induce sunburn and, over a long period of time, promote premature aging of the skin and skin cancer. The harmful effects of UV radiation on the skin have been reviewed.[44,56,69]

Benzophenone sunscreens, applied topically, protect the skin from these harmful effects of ultraviolet light by chemically absorbing light energy (photons). As this occurs, the Benzophenone molecule becomes excited to higher energy levels. As the excited molecule returns to its ground state, the energy is released in the form of thermal energy. The hydroxyl group in the ortho position to the carbonyl group is believed to be a structural requirement for the Benzophenones' absorption of UV light. This structural arrangement also contributes to the electronic stability of the molecule. Thus, a surface coating of Benzophenones decreases the amount of UV radiation absorbed by the skin by limiting the total amount of energy that reaches the skin. Benzophenones absorb energy throughout the UV range, though maximum absorbance is between 284 and 287 nm for the 2-hydroxybenzophenones and between 333 and 345 nm for the 2,2'-dihydroxybenzophenones. The effectiveness of any Benzophenone as a sunscreen is determined by its concentration on the skin, the pH of the skin and chemical environment, and the solvent system; a change in either of the latter two conditions can cause the peak absorbance of the Benzophenone to vary.[6,56,69]

Benzophenones also protect patients using drugs that have the side effect of eliciting from the individual a photosensitization reaction either phototoxic or photoallergic in nature. A phototoxic reaction occurs when a drug absorbs UV light and transfers energy from it to the vulnerable cell organelles; the damage caused is characterized by a sunburn-like reaction. Photoallergic reactions, on the other hand, involve an immunologic mechanism between the photosensitizing drug and skin proteins. The reaction is characterized by eczematous or polymorphic dermatitis of delayed onset, which will recur with each subsequent exposure to UV light.[56] Phototoxic reactions to long-wavelength UV radiation (320–380 nm) occur in patients who used topically applied psoralen for the treatment of vitiligo, a condition in which pigment is lost. Topical application of Benzophenone-4 or Benzophenone-8 (10%) controlled photosensitivity in these individuals.[70] Chlorpromazine, a drug frequently used to treat schizophrenia, often produces photosensitivity. Topically applied Benzophenone-4 (10% in a cream base) protected ten such chlorpromazine photosensitized individuals.[71] In addition, photosensitivity resulting from the use of chlortetracycline was effectively controlled when patients applied a skin cream containing 10% Benzophenone-4.[56] Oleniacz et al.[72] reported photosensitivity to UV light in the 300–425 nm range in individuals who used the topical antibacterial agent 3,3',4',5-tetrachlorosalicylanilide (TCSA). A TCSA in vitro study revealed the disruption of lysosomal and mast cell membranes as a primary photosensitizing event, and that TCSA enhanced the light sensitivity of lysosomes, resulting in concomitant edema and erythema. Benzophenone-4 protected TCSA-treated cells from UV radiation. Emmett et al.[74] reported that workers who handled absorbers used in the production of UV-cured inks became sensitized to UV light. This reaction induced pruritic dermatitis on sun-exposed surfaces of the body. Topical use of a

cream containing 10% Benzophenone-4 was effective in controlling this sensitization.

Benzophenones are also used as topical agents for the treatment of photodermatoses such as solar urticaria (a vascular reaction of the skin marked by wheals) and polymorphous light eruption (a skin eruption confined to sun-exposed surfaces and not attributable to medications or systemic disease).[75]

The FDA Panel on Review of Topical Analgesics has proposed that Benzophenones-3, -4, and -8 are safe and effective as active ingredients in sunscreens for over-the-counter (OTC) use at the following concentrations: Benzophenone-3, 2%–6%; Benzophenone-4, 5%–10%; and Benzophenone-8, 3%. The Panel proposed these concentration limits on a combined safety and efficacy basis (a concentration limit may reflect maximum efficacy and not necessarily an indication of toxicity at a higher concentration).[69]

Noncosmetic

Owing to their photostabilizing properties, Benzophenones are used in food and agricultural products, as well as in packaging materials. At maximum concentrations of 0.01% and 0.05%, respectively, Benzophenone-12 is a food stabilizer in petroleum wax and an antioxidant/stabilizer in olefin polymers.[76,77] Benzophenone-7 is a commercial grain fungicide, whereas Benzophenone-9 protects the insect pathogens, *Bacillus thuringensis* (spores) and spruce budworm nuclear polyhedrosis virus, from sunlight's harmful effects.[78,79] Benzophenone-2 is used in herbicides, and Benzophenone-3 is added to agricultural films (such as polyvinyl chloride), where it serves as a photostabilizer.[20,80,81] When used in packaging materials, Benzophenone-12 prevents UV radiation from reaching the stored product and increases the stability of the container.[82-84] Table 4 lists other noncosmetic uses of Benzophenones.

When studying Benzophenones as light stabilizers in packaging, Marcincin and Pikler[85] reported that an increase in the number of hydroxyl groups and a decrease in the carbon chain length of the Benzophenone substituents resulted in increased diffusion and extraction of the Benzophenone from the polymer. It was reported that when Benzophenone photostabilizers are used in packaging material, they migrate into aqueous, acidic, or dilute alkaline media, including food.[86]

BIOLOGICAL PROPERTIES

General Effects

Benzophenone-2 had an insignificant effect when tested for antitumor and antimicrobial activity. When assayed with Sarcoma 180 tumor cell cultures, this ingredient had an ID50 (dose for 50% inhibition of growth) of 17 μg/ml; tumor inhibition was considered to be insignificant. Antimicrobial activity of Benzophenone-2 against *Esherichia coli* and *Streptococcus fecalis* was also reported to be insignificant (Median inhibitory dose [ID50] = $>10^3$ *M*/l).[93]

Benzophenone-7 is a grain fungicide and was detected in starlings throughout the U.S. at concentrations up to 3.33 ppm.[78]

TABLE 4. Noncosmetic Use of Benzophenones as Light Stabilizers.

Benzophenone	*Substances used in*	*Product use*	*Ref.*
−3	Polyethylene tereptithalate	Fabrics, films, magnetic tape	87,88
−3	Polyvinyl butyral	Interlayer safety glass in autos and airplanes	89
−1, −3	Cellulose acetate	Rubber and celluloid subst., films, varnish, lacquer, fabric, records	9
−3, −6, −8	General	Adhesives, lacquers, plastics	5
−1, −3, −6	Polyvinyl chloride	Rubber subst., films, textile finishes, shoe soles, raincoats, insulation, tubing	90
−1, −2, −3, −6, −7, −11	Toluidene Red	Dye	91
−1, −2, −3, −6, −7, −11	Polyester	Tires, rubber subst., clothing, protective coatings, magnetic tapes	92
−1, −2, −3, −6, −7, −11	Acrylic acid resin	In plastics	92
−1, −2, −3, −6, −7, −11	Nitrocellulose	Celluloids, textile fibers, lacquers, rocket propellant	92
−1, −2, −3, −6, −7, −11	Polyvinyl chloride	Celluloids, textile fibers, lacquers, rocket propellant	92
−1, −2, −3, −6, −7, −11	Polystyrene	Packaging, cabinets, containers, refrigerator doors, toys	92

Absorption, Metabolism, and Excretion

Patel et al.[94] studied absorption and excretion of Benzophenone-12 incorporated in the rat diet. Preliminary short-term feeding studies indicated that most of the compound was unabsorbed and passed in the feces; the remainder was absorbed, conjugated, and excreted as a glucuronide in the urine. Long-term absorption and excretion of Benzophenone-12 was studied in 18 male albino rabbits that were maintained on diets containing 0%, 1.25%, or 5.0% Benzophenone-12 for 35 days. Daily food consumption was measured for each animal, and the individual intake of Benzophenone-12 was calculated. Daily samples of the animals' feces and urine were analyzed by paper chromatography for Benzophenone-12 or the glucuronic acid. Two animals from each dietary level were sacrificed for liver and kidney examination after 11, 22, and 35 days of feeding. Urinary excretion of Benzophenone-12 as a glucuronide in animals at both dietary levels of Benzophenone-12 was approximately 10% of the daily dose, whereas the recovery of unchanged Benzophenone-12 from the feces was about 90%. These results indicated that the animals did not retain measurable amounts of Benzophenone-12 even when the compound was ingested over a long period of time.

Patel et al.[94] conducted limited metabolism studies on Benzophenone-3 (in which a methoxy group replaces the octyloxy chain of Benzophenone-12). Preliminary results suggested that Benzophenone-3 was absorbed and conjugated to a greater extent than Benzophenone-12, indicating that the length of the alkoxy side-chain influences the degrees to which these compounds are absorbed from the intestine.

Animal Toxicology

Acute Toxicity

Oral

The Benzophenones have been tested for acute oral toxicity in rats. The animals were weighed and dosed after a one-week observation; the test material was then administered by gastric intubation. Rats were observed daily for 7 to 14 days, during which time food and water were allowed ad libitum; in some instances, animals were sacrificed and autopsied for gross pathology. Results, listed in Table 5, indicate that in acute oral toxicity tests, Benzophenones-1, -3, -6, -8, -9, and -12 are practically nontoxic, whereas Benzophenones-2, -4, and -11 are slightly toxic.

Dermal

The acute dermal toxicity of Benzophenones-3, -4, -8, and -12 was tested in albino rabbits. The test substance was applied at various dosages to the epilated skin of the back or flanks and held in contact for 18–24 hours; it was then washed off. Observations were made daily for signs of toxicity and irritation. Animals were autopsied following the 5- to 7-day observation period. Benzophenone-3 had an acute dermal LD50 >16.0 g/kg when applied to rabbits in doses of 2.0–16.0 g/kg. Local skin reactions, consisting of mild to moderate erythema, were observed in two animals at the 2.0 g/kg dose 24 and 48 hours following the exposure period. No significant pathology was revealed upon autopsy.[95] Ten rabbits dosed at 5 g/kg Benzophenone-4 had an acute dermal LD50 >5 g/kg. There were no gross signs of toxicity or irritation throughout the observation period; autopsy revealed one animal with congested kidneys.[96] The acute dermal LD50 of Benzophenone-8 was determined to be >10 g/kg; ten rabbits dosed at 10 g/kg developed no systemic toxicity, skin irritation, or pathology attributable to dermal application of this compound.[49] Benzophenone-12 had an acute dermal LD50 of >10 g/kg when tested on five rabbits; animals developed no systemic toxicity or skin irritation.[94] Results of these tests indicate that Benzophenone-8 is relatively harmless and causes no systemic toxicity when applied dermally.

Subchronic Oral Toxicity

Benzophenones-1, -3, -8, and -12 were tested for subchronic oral toxicity, the results of which appear in Table 6. Benzophenone-1, fed to 40 rats at doses of 0–1.9 g/kg for 90 days, produced depressed growth and liver and kidney lesions in animals at doses of 0.6 and 1.9 g/kg.[97] Benzophenone-3 caused no toxic effect in rats when incorporated into their diets (up to 1%) for 27 days; however, in a 90-day study, rats fed 0.5% or 1.0% Benzophenone-3 displayed depressed growth, leucocytosis, anemia, reduced organ weights, and degenerative nephrosis.[95,98] When Benzophenone-8 was fed to rats at dietary concentrations of 0%–10%, gross hematuria was occasionally noted at the two highest dose levels (5% and 10%). Upon autopsy, kidney discoloration and liver enlargement (in direct proportion to dose levels of Benzophenone-8) were observed. Hematuria

TABLE 5. Acute oral toxicity.

Benzophenone	*No. of rats*	*Conc. (%)*	*Vehicle*	*Dose*	*LD50*	*Comments*[a]	*Ref.*
−1	50	25	Corn oil	8–32 ml/kg	24.4 ml/kg	Relatively harmless	99
−1		–[b]	Olive oil	–	8.8 g/kg	Practically nontoxic	97
−2	100	5	Corn oil	1–3.5 g/kg	1.22 g/kg	Slightly toxic (convulsion and immediate death at highest dosage)	100
−2		–	Olive oil	–	7.0g/kg	Practically nontoxic	97
−3	25	25	Corn oil	6.25–16 g/kg	11.6 g/kg	Practically nontoxic	100
−3	14	15	Methyl Cellulose	4.5–6 g/kg	>6 g/kg	Pale livers and kidneys, gastrointestinal irritation	101
−3		–	Olive oil	–	7.4 g/kg	Practically nontoxic	97
−3		–	–	–	>12.8 g/kg	Practically nontoxic	98
−4	30	5	Water	0.2–6.4 g/kg	>6.4 g/kg	Practically nontoxic	102
−4	15	0.2 g/ml	Water/agar/tween	2.5–10 g/kg	6.15 g/kg	Practically nontoxic	96
−4	20	20	Agar/tween	1.25–10 g/kg	3.53 g/kg	Slightly toxic (ataxia)	96
−6	25	25	Corn oil	1–16 g/kg	>16 g/kg	Practically nontoxic	103
−8	10	0.2 g/ml	Water	10 g/kg	>10 g/kg	Practically nontoxic	49
−9	25	26.8	Water	6.14–16 g/kg	9.0 g/kg	Practically nontoxic	104
−11	100	5	Corn oil	1.5–3.75 g/kg	3 g/kg	Slightly toxic	103
−12[c]		–	Olive oil	–	>12 g/kg	Practically nontoxic	97
−12[c]	10	20	Water	10 g/kg	>10 g/kg	Practically nontoxic	94

[a]According to Hodge and Sterner.
[b]No data available.
[c]BP-12 has no reported use in cosmetics.

TABLE 6. Subchronic and chronic oral toxicity data.

Benzophenone	*No. animals/ Species*	*Dose*	*No. days on diet*	*No. deaths*	*No effect level*	*Comments*	*Ref.*
−1	40 albino rats	0, 0.19, 0.6, 1.9 g/kg	90	0	0.19 g/kg	Depressed growth, liver and kidney lesions at 0.6 and 1.9 g/kg	97
−3	40 albino rats	0%, 0.01%, 0.1%, 1%	27	0	>1.0%	No toxic effect	95
−3	120 albino rats	0%, 0.02%, 0.1%, 0.5%, 1.0%	90	0	0.1%	Depressed growth, leucocytosis, anemia, reduced organ weight, nephrosis at 0.5% and 1.0%	98
−8	40 albino rats	0%, 2.5%, 5.0%, 10%	36	0	2.5%	Gross hematuria at 5% and 10%	49
−12	40 albino rats	0, 0.19, 0.6, 1.9 g/kg	90	0	0.6 g/kg	Depressed growth, liver and kidney lesions at 1.9 g/kg	97
−12	160 albino rats	0%, 0.2%, 0.6%, 1.8%	90	2[a]	>1.8%	Nontoxic	94
−12	16 beagle dogs	0%, 0.2%, 0.4%, 0.6%	120	0	>0.6%	Nontoxic	94

[a]Unrelated to ingestion of BP-12.

was explained by the deposition of an insoluble glucuronic acid conjugate of Benzophenone-8 in the kidney tubules.[49] Benzophenone-12, fed to 160 rats at concentrations up to 1.8% (approximately 0.9 g/kg) for 90 days, was practically nontoxic at all dose levels.[94] In another 90-day study, however, rats dosed at 1.9 g/kg Benzophenone-12 exhibited depressed growth as well as liver and kidney lesions; in this study, 0.6 g/kg was reported to be the "no-effect" level.[97]

Chronic Oral Toxicity

Four groups of beagle dogs, consisting of two males and two females each, were placed on 120-day diets containing 0%, 0.2%, 0.6%, or 1.8% Benzophenone-12 (Table 6). The highest dietary concentration of Benzophenone-12 was lowered from 1.8% to 0.4% after the 14th day because the dogs rejected their food. No significant differences were observed between control and test animals in body and organ weights, hemoglobin, hematocrit, leucocyte counts, and plasma levels of urea nitrogen and alkaline phosphatase. Benzophenone-12 was considered to be nontoxic when ingested as 0.6% in the diet over a period of four months.[94]

Acute Irritation

Skin

Irritation: Procedures outlined by the Federal Hazardous Substances Labeling Act (FHSLA) were used to test Benzophenones for acute skin irritation. An occlusive patch containing 0.5 ml or 0.5 mg of the test ingredient was applied to the intact and abraded skin of albino rabbits. Patches remained in place for 24 hours and were then removed and scored for irritation according to the Draize method. Sites were again scored 24 hours after patch removal. Benzophenones-2, -3, -9, and -11 were nonirritating to intact and abraded skin when tested at concentrations from 4% to 100%. Benzophenones-1, -4, and -6 were minimally irritating (PII = 0.25–0.50) when applied as 16% solutions in dimethyl phthalate (DMP). However, these ingredients were nonirritating at 8% in DMP and at 16% in petrolatum.[96,104-107] Table 7 summarizes the results of Benzophenone skin irritation studies.

Phototoxicity and Photosensitization

A sunscreen containing 3% Benzophenone-8 was tested for potential phototoxicity in guinea pigs. A 0.1 ml dose of the undiluted lotion was applied to four areas of skin on each of three animals. Fifteen to 20 minutes later, two of the sites were exposed to UV-A light (maximum at 360 nm) from four F40BL bulbs at a distance of 12 inches for 60 minutes. The other two sites were nonirradiated controls. All sites were scored 24 hours after application of the test material. The sunscreen containing 3% Benzophenone-8 did not induce erythema at control or irradiated sites indicating a lack of phototoxicity.[113]

A sunscreen containing 6% Benzophenone-3 was tested for photosensitization in six albino rabbits. A 0.4 ml dose of the lotion was applied to the clipped dorsal skin of each animal. Skin sites were then irradiated with UV light from a sunlamp. This procedure was repeated five times weekly for two weeks (ten applications total). Sites were scored 24 hours following each irradiation. Mild

TABLE 7. Primary Skin Irritation (FHSLA Procedures).

Benzophenone	No. of albino rabbits	Conc. (%) Vehicle	Primary Irritation Index(PII)[a]	Comments	Ref.
-1	6	16,8,4/Petrolatum	0.00	Nonirritating	107
-1	6	16,8,4/DMP[b]	0.25 (16%)	Minimally irritating (16%)	107
-2	6	100	0.00	Nonirritating	105
-2	6	16,8,4/Petrolatum	0.00	Nonirritating	107
-2	6	16,8,4/DMP	0.00	Nonirritating	107
-3	6	100	0.00	Nonirritating	106
-3	6	100	0.00	Nonirritating	108
-4	6	16,8,4/Petrolatum	0.00	Nonirritating	107
-4	6	16,8,4/DMP	0.50 (16%)	Minimally irritating (16%)	107
-6	6	16,8,4/Petrolatum	0.00	Nonirritating	107
-6	6	16,8,4/DMP	0.25 (16%)	Minimally irritating (16%)	107
-9	6	10.72,5.36,2.68/ Petrolatum	0.00	Nonirritating	107
-9	6	10.72,5.36,2.68/DMP	0.00	Nonirritating	107
-9	6	5.36/Water	0.00	Nonirritating	104,106,109-112
-11	6	16,8,4/Petrolatum	0.00	Nonirritating	107
-11	6	16,8,4/DMP	0.00	Nonirritating	107

[a]Maximum score = 8.
[b]Dimethyl Phthalate.

erythema, mild edema, and desquamation were observed in both test and irradiated control animals; however, no photosensitization occurred in any of the test animals.[69]

Eye

A number of studies have determined the potential irritancy of Benzophenones to the eyes of rabbits. The test material (0.1 ml or 0.1 g) was instilled into one eye of each rabbit; the other eye served as an untreated control. Eyes were examined and scored for irritation daily for a period of three to ten days. Some test procedures included washing of the treated eyes with water four seconds after instillation of the test material. Results of eye irritation tests revealed that most Benzophenones at concentrations of 5%-100% were nonirritating when instilled into the eyes of rabbits. 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. Table 8 summarizes eye irritation data for the Benzophenones.

Subchronic Skin Irritation and Sensitization

Irritation

Marzulli and Maibach[114] used a 16-day cumulative test on rabbits to study the irritation potential of Benzophenone-4. An alcohol solution containing either

TABLE 8. Primary Eye Irritation.

Benzophenone	Method	No. of albino rabbits	Eye wash Y/N	Test Conc. (%)	Dose	Average score per day[a] 1	2	3	4	5	6	7	Comments	Ref.
−1	Draize	6	N	100	100 mg	20	7.00	0	–	–	–	–	Mildly irritating (conjunctiva and cornea)	115
−1	FHSLA	6	N	16,8,4/DMP[b]	0.1 ml	0	0	0	–	–	–	–	Nonirritating	107
−1	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	0	0	0	–	–	–	–	Nonirritating	107
−2	Draize	6	N	100	100 mg	17	15	10.3	3.0	0	–	–	Moderately irritating (conjunctiva and cornea)	110
−2	FHSLA	6	N	16,8,4/DMP	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−2	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−3	FHSLA	6	N	16,8,4/DMP	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−3	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−3	Draize	6	N	100	100 mg	0	0	0	0	–	–	0	Nonirritating	111
−3	Mod. FHSLA	3	N	100	3 mg	0	0	0	0	0	0	–	Nonirritating	101
−3	Mod. FHSLA	6	N	100	100 mg	0	0	0	–	–	–	0	Nonirritating	108
−4	FHSLA	6	N	16,8,4/DMP	0.1 ml	–	–	–	–	–	–	–	Irritating (Cornea, conjunctiva−16%; conjunctiva−8%)	107
−4	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	–	–	–	–	–	–	–	Irritating (Cornea, conjunctiva−16%; conjunctiva−8%)	107

−4	Draize	9	Y−3 rabbits	5/water	0.1 ml	0	0	0	0	–	–	0	Nonirritating	116
−4	Draize	6	N	100	100 mg	2.58	2.38	2.05	–	–	–	–	Irritating to iris and conjunctiva	96
−6	FHSLA	6	N	16,8,4/DMP	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−6	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−6	Draize	6	N	100	100 mg	0	0	0	0	–	–	0	Nonirritating	109
−8	–	5	N	100	100 mg	0	0	0	–	–	–	0	Nonirritating	49
−9	FHSLA	6	N	10.72,5.36,2.68/ DMP	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−9	FHSLA	6	N	10.72,5.36,2.68/ Petrolatum	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−9	Draize	6	N	5.36/water	0.1 ml	0	0	0	0	–	–	0	Nonirritating	112
−11	FHSLA	6	N	16,8,4/DMP	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−11	FHSLA	6	N	16,8,4/Petrolatum	0.1 ml	0	0	0	–	–	–	0	Nonirritating	107
−11	Draize	9	N	5/DMP	0.1 ml	2.89	0.67	0	0	0	0	0	Slightly irritating to conjunctiva in all rabbits	104
−12	Draize	5	N	100	100 mg	0	0	0	0	0	0	0	Nonirritating	94

[a]Maximum score = 110.
[b]Dimethyl phthalate.

10% or 1% Benzophenone-4 was applied uncovered to the depilated backs of six New Zealand albino rabbits. Twenty-four hours later the sites were scored for irritation, and the solution was reapplied. This procedure was repeated every other day for five weeks, until a total of 16 applications of Benzophenone-4 had been made. The average cumulative irritation score was then calculated (maximum score = 64); applications of Benzophenone-4 (10%) and Benzophenone-4 (1%) produced scores of 3.6 and 0.3, respectively.

Sensitization

Benzophenone-3 was tested for sensitizing potential using the Kligman Maximization Procedure. A 0.05 ml intradermal injection of 5% Benzophenone-3 in corn oil or 50% Benzophenone-3 in aqueous Freund's Adjuvant was administered to the shaved back of each of ten female albino guinea pigs per solution. Seven days following injection, a topical booster patch containing 10% Benzophenone-3 in petrolatum was applied for 48 hours. Two weeks later, a challenge test of 0.1 ml of 2.5% Benzophenone-3 in petrolatum was applied under an occlusive patch to a virgin site for 24 hours. Sites were scored 24 and 48 hours after patch removal. Results of this test indicated that Benzophenone-3 was not a skin sensitizer.[117]

Special Studies

Mutagenesis

The Ames *Salmonella*/Mammalian-Microsomal Assay was used to test Benzophenones-1, -2, -3, -4, -6, -8, -9, and -11 for mutagenicity. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were used; all tests were performed in the presence and absence of Aroclor-induced rat liver microsomal S-9 cell fraction to observe the mutagenic effect of each compound following metabolic activation. Preliminary cytotoxicity studies determined the dose range of each compound to be used. The results of these tests appear in Table 9. All Benzophenones were nonmutagenic when assayed directly. All but three Benzophenones (-2, -6, and -8) were nonmutagenic with metabolic activation. Benzophenone-8 was weakly mutagenic in *Salmonella* strain TA1537; whereas, Benzophenone-6 was determined to be mutagenic at three doses in the same strain (TA1537). Benzophenone-2, in the presence of rat liver microsomes, induced a "small but fairly consistent increase in the number of mutants" in four *Salmonella* strains tested. At doses of 100–300 μg, Benzophenone-2 induced mutant increases of 50–100% in TA100 and 200%–500% in TA1537. A mutant increase of 50% was observed in strains TA98 and TA1535, but these strains had not been tested enough times to provide conclusive results. The investigator suggested that "the small and somewhat erratic nature of the (mutagenic) response we have seen raises the possibility that the observed effect may be due to the presence of an impurity." The purity of the test sample was 99% (lab-grade) and was assumed to be purer than that of the cosmetic-grade. Additional tests using lab-grade Benzophenone-2 found this ingredient to be mutagenic in TA1537 at doses of 200 and 750 μg when activated by Aroclor-induced hamster liver enzymes. Preliminary assays of cosmetic-grade Benzophenone-2 revealed mutagenic activity not differing significantly from that of the purer lab-grade.[118-122]

TABLE 9. Ames *Salmonella* Mutagenesis Assay.[a]

Benzophenone	Dose range (μg) (Solvent)	Results[b] without S-9 metabolic activation					Results[b] with S-9 metabolic activation					Comment
		TA98	TA100	TA1535	TA1537	TA1538	TA98	TA100	TA1535	TA1537	TA1538	
−1	0.1–500 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation
−2	0.1–10,000 (DMSO)	(−)	(−)	(−)	(−)	−[c]	(+)	(+)	(+)	(+)	−[c]	Mutagenic with S-9 activation in all strains (see text)
−2	10–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation
−3	1.0–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation
−4	1.0–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation
−6	1.0–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	Mutagenic only in TA1537 with S-9 activation at 10 and 100 μg. Toxic to TA1537 at 500 and 1000 μg with S-9
−8	7.0–700 (ETOH)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	Dose-dependent, weak but significant mutagen in TA1537 with S-9 activation only
−9	1.0–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation
−11	10–1000 (DMSO)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Nonmutagenic with and without S-9 activation

[a]Data from Refs. 118–122.
[b](−) = Nonmutagenic; (+) = mutagenic.
[c]No data.

An in vitro cytogenic assay was used to evaluate the ability of Benzophenone-2 to induce sister chromatid exchange (SCE) and chromosome aberrations (CA) in L5178Y mouse lymphoma cells. Assays were performed in the presence and absence of Aroclor-induced rat liver microsomal enzymes (S-9). The solubility of Benzophenone-2 in DMSO and its cytotoxicity were first determined. For the mutagenesis assays, doses of 6.250–200.00 μg Benzophenone-2 per plate were used. When assayed in the absence of S-9, Benzophenone-2 induced small but "biologically insignificant" increases in SCE frequency at 100 and 200 μg; CA frequencies were not elevated at any dose. With metabolic activation, however, Benzophenone-2 produced "statistically and biologically significant" increases in SCE frequency at the three highest dose levels, indicating a dose-response relationship. The author noted that Benzophenone-2 was more toxic to cells under the activation system; only 17 scorable cells were located at the 100 μg dose. The investigator reported that 10 CAs (including a quadriradial, a translocation, and two triradials) were observed among the 67 cells scored at the two highest doses with activation. He concluded that Benzophenone-2 does not directly induce significant SCE or CA increases but does, under metabolic activation, induce these changes.[123]

A Mouse Lymphoma Forward Mutation Assay was used to test Benzophenones-2 and -8 for mutagenesis. The L5178Y TK+/− cell line was used; assays were performed in the presence and absence of an Aroclor-induced rat liver microsomal preparation (S-9). Materials were dissolved in DMSO and tested for preliminary cytotoxicity to determine doses to be used in the assays.

Without activation, Benzophenone-2 was mutagenic at "highly toxic" doses. In the presence of S-9, Benzophenone-2 became more toxic. An increase in the mutant frequency (3.0–6.8 times) was observed with the three most toxic doses. A dose-response relationship was not demonstrated. The investigator suggested that Benzophenone-2 "appears to react with microsomal system to yield a mutagenic product that induces mutants at lower applied concentrations and toxicities than under nonactivation conditions." It was concluded that Benzophenone-2 induced an increase in mutations at the TK locus in L5178Y mouse lymphoma cells only for highly toxic doses with or without metabolic activation and that this material is weakly mutagenic under the conditions of the test.[123] These findings need to be reconfirmed since there was no dose-response pattern of toxicities over the preferred relative growth range in any of the trials; increases in mutant frequency in the assays occurred only at levels bordering total lethality; and the lethal dose was poorly reproduced from one trial to another with metabolic activation.

When assayed directly, Benzophenone-8 did not induce mutant frequencies significantly greater than those of controls. With metabolic activation, however, Benzophenone-8 induced dose-dependant mutant frequency increases of 3.8 and 2.0 times for the two highest doses (32 and 24 μg, respectively). The investigator concluded that, under the test conditions, Benzophenone-8 is nonmutagenic when assayed directly, but under metabolic activation it induces a significant, dose-dependent increase in mutant frequency.[118]

Other

No information was available on any of the Benzophenones with respect to teratogenesis and carcinogenesis.

Clinical Assessment of Safety

Skin Irritation and Sensitization

Benzophenones were tested for potential irritation and sensitization to human skin. In general, these ingredients were reported to be nonirritating and nonsensitizing at concentrations higher than those found in cosmetics. Table 10 summarizes the results of these studies.

Four studies reported irritation and/or sensitization to Benzophenones. Benzophenone-2 was applied to 50 subjects in a Shelanski repeated insult patch test (RIPT). Induction patches were applied to the subject's skin for 24 hours every other day for a total of 15 patches. The initial nine patches contained a 4.9% Benzophenone-2 solution; the remaining six patches contained a 2.45% Benzophenone-2 solution. Two weeks after removal of the last induction patch, a challenge patch containing 2.45% Benzophenone-2 was applied to the original test site. All sites were scored upon patch removal. Sixteen of 50 subjects reacted to one or more of the induction patches; responses of 1+ to 3+ (maximum score = 4+) were observed. Four subjects reacted to the challenge patch with responses of 1+ and one with 2+. The investigators concluded that at 4.5%, Benzophenone-2 is not a primary irritant but is a "fatiguing agent" and possibly a sensitizer.[124]

A Modified Draize/Shelanski RIPT was used to study the irritancy and sensitizing potential of a sunscreen containing a 3.0% Benzophenone-3 in 57 subjects. One subject displayed erythema (1+ response) to the final induction and challenge patches; no reaction was elicited by a challenge patch on a virgin site. This subject was repatched 11 months later with each component of the sunscreen. Benzophenone-3 in ethyl alcohol was applied under occlusion to her upper arm for 24 hours. The site was scored at 48 and 72 hours. Spreading erythema and mild edema (2+ response) was elicited at 48 hours; by 72 hours only erythema (1+ response) was observed. It was concluded that this test subject was sensitized to Benzophenone-3. The investigators concluded that the product containing 3% Benzophenone-3 may have a "minimum potential for inducing sensitization under the exaggerated conditions of the test."[125]

Benzophenone-8 was tested for irritation and sensitization using a Modified Draize/Shelanski RIPT. Ten induction patches containing 25% Benzophenone-8 in petrolatum were applied to each of 100 subjects. Following a one-week rest, a challenge patch containing 10% Benzophenone-8 in petrolatum was applied to a fresh skin site. Seven subjects reacted to both induction and challenge patches; these results indicated contact sensitivity. Moreover, one subject exhibited 2+ reactions for induction patches 8–10 and a 4+ reaction to the challenge patch. The authors concluded that Benzophenone-8 (25%) is a moderate sensitizer.[50]

Benzophenones-4 and -11 were tested for potential skin irritation in separate single insult patch tests. Each ingredient was applied at concentrations of 16, 8, and 4% in DMP and in petrolatum to the skin of each of 14 subjects. At a concentration of 16% in either base, Benzophenones-4 and -11 were irritating to four and two subjects, respectively. Neither ingredient was irritating at concentrations of 4% or 8% in either vehicle.[107]

Marzulli and Maibach[114] tested the potential irritancy of Benzophenone-4 on six adult white humans. Patches containing 1% or 10% Benzophenone-4 in

TABLE 10. Human Patch Test Data.

Benzophenone	*Test method*[a]	*No. of subjects*	*Effective conc. (%)*	*No. of reactions*	*Comments*	*Ref.*
−1	Shelanski RIPT	100	1 in butyl carbitol	0	Nonirritating/nonsensitizing	126
−1	SIPT	14	16,8,4/DMP			
			16,8,4/Petrolatum	0	Nonirritating	107
−2	Shelanski RIPT	50	2.45 and 4.9/H_2O	17/50−induction 5/50−challenge	Evidence of fatiguing and possible sensitization at 5%; none at 2.5%	124
−2	SIPT	14	16,8,4/DMP			
			16,8,4/Petrolatum	"Mild reactions similar to toilet soap"	Nonirritating	107
−3	SIPT	14	16,8,4/DMP			
			16,8,4/Petrolatum	0	Nonirritating	107
−3	Mod. D/S RIPT	100	25−induction in petrolatum			
			10−challenge in petrolatum	0	Nonirritating/nonsensitizing	50
−3(3% in a lotion)	Mod. Draize RIPT	203	3.0	0	Nonirritating/nonsensitizing	113
−3(3% in a sunscreen)	SIPT (48 hr)	100	3.0	0	Nonirritating	69
−3(3% in a sunscreen)	Mod. D/S RIPT	150	3.0	"several nonspecific reactions"	"Not a primary irritant"; nonsensitizing	69
−3(3% in a sunscreen)	Mod. Draize RIPT	150	3.0	Mild irritation (but no sensitization) challenge patches	Nonsensitizing	69
−3(3% in a sunscreen)	Mod. D/S RIPT	57	3.0	1 sensitized reaction	Minimum sensitizing potential	125
−4	SIPT	14	16,8,4/DMP	Four subjects reacted to 16% BP-4 in DMP and petrolatum.	Irritating at 16% in DMP and petrolatum	107
			16,8,4/Petrolatum	One subject reacted to 8% BP-4 in DMP and Petrolatum.		

−4	Shelanski RIPT	50	5−in H_2O	0	Nonirritating/nonsensitizing	127
−4	Mod. D/S RIPT	100	25−induction/ Petrolatum 10−challenge/ Petrolatum	0	Nonirritating/nonsensitizing	50
−6	SIPT	14	16,8,4/DMP 16,8,4/Petrolatum	0	Nonirritating	107
−6	Shelanski RIPT	50	100	0	Nonirritating/nonsensitizing	109
−8	Mod. D/S RIPT	100	25−induction/ Petrolatum 10−challenge/ Petrolatum	Seven cases of irritation to induction and challenge patches	Contact sensitizing	50
−8(2% in a lotion)	Mod. Draize RIPT	205	2	0	Nonirritating/nonsensitizing	113
−9	SIPT	14	10.72,5.36,2.68/ DMP 10.72,5.36,2.68/ Petrolatum	0	Nonirritating	107
−11	SIPT	14	16,8,4/DMP 16,8,4/Petrolatum	Two subjects reacted to 16% BP-11 in DMP and petrolatum.	Nonirritating at 8 and 4%	107
−11	Shelanski RIPT	50	20−in butyl carbitol acetate	0	Nonirritating/nonsensitizing	128
−12	Mod. D/S RIPT	50	25−induction/ Petrolatum 10−challenge/ Petrolatum	0	Nonirritating/nonsensitizing	94

[a]Shelanski double insult patch test−120-hr patch/3 wk rest/48-hr patch (challenge) to original site.
Single Insult Patch Test (SIPT)−24-hr patch.
Shelanski repeated insult patch test (RIPT)−15 (24-hr patch/24-hr rest)/2-wk rest/24-hr patch (challenge) to original site.
Modified Draize/Shelanski RIPT−10 (21-hr patch/24-hr rest)/1-wk rest/24-hr patch (challenge) to virgin site.
Modified Draize RIPT−10 (24 hr-patch/24 hr-rest)/2-wk rest/72-hr patch (challenge) to virgin site.

alcohol were applied to the subjects for 24 hours, after which time the patches were removed, the sites scored, and fresh patches applied. This procedure was repeated every other day, three days per week for seven weeks, until a total of 21 patches had been made. The mean cumulative irritation scores for 1% and 10% solutions were 8.6 and 53.1, respectively (maximum score = 84). The latter value is indicative of a primary irritant.

Fisher[129] reported that "in the past ten years of patch testing on hundreds of patients [at his practice], only two patients have been allergic to Benzophenone-4." He concluded that this indicates "very low sensitivity" in the population, and that this ingredient is safe for general use.

No data were available regarding the clinical assessment of Benzophenone-5.

The scientific literature generally confirms the clinical safety of topically used Benzophenones; however, several cases of contact sensitivity to these ingredients have been reported. Pariser[130] reported contact dermatitis caused by topical use of a sunscreen containing 3% Benzophenone-3 and 3% Benzophenone-8. "Standard" patch tests of the sunscreen lotion, 2% Benzophenone-8 (in petrolatum), or 2% Benzophenone-3 (in petrolatum), revealed irritation at 48 and 72 hours to the first two and mild irritation at 72 hours to the last. Ramsey et al.[131] reported a case of contact sensitivity resulting from topical use of a suntan lotion containing 10% Benzophenone-4. A scratch test of a 1% Benzophenone-4 solution resulted in a 2+ response; a 1% Benzophenone-3 solution also elicited a 2+ reaction. A single 24-hour patch test of a 5% Benzophenone-4 solution (aqueous) revealed a 2+ papular reaction at 24 and 48 hours. Thompson et al.[132] reported that a 62-year-old man with a history of photosensitivity developed contact dermatitis after he used a sunscreen containing Benzophenones-3 and -8 (no concentrations given). Patch tests of the lotion or of the individual Benzophenones at product concentrations resulted in 2+ reactions at 48 hours.

Photosensitivity

Phototoxicity

Cosmetic products containing Benzophenones-2, -3, or -4 (0.1%–3.5%) were tested for phototoxicity in humans (Table 11). In each study, the test material was applied under occlusion to the subject's skin for 24 hours. Sites were then scored, exposed to UV radiation, and then scored daily for up to seven days. Nonirradiated/treated and nontreated/irradiated controls were frequently used. Products containing Benzophenones-2, -3, and -4 were nonphototoxic in all studies; however, a number of subjects experienced slight irritation (usually a 1+ response) to the test material.

Photoallergenicity

Cosmetic products containing up to 3.5% Benzophenone-3 were tested for photoallergenicity potential in humans (Table 11). In each study, the protocol was similar to that for phototoxicity except that the procedure was repeated three times weekly until 10 or 12 induction applications had been made. Following a 10- to 14-day rest, 24-hour challenge patches were applied to the original site

TABLE 11. Clinical Photosensitivity.

Benzophenone (Product)	*Benzophenone Test conc. (%)*	*Test*	*No. of subjects*	*UV-light source*[a]	*Reactors*[b]*/ Photosens.*	*Conclusion/Comments*	*Ref.*
−2(bath prep)	0.0005	Phototox.[c]	22	Sunlight	9(1+;c,i)/0	Nonphototoxic (reactions due to primary irritation)	134
−2(bath prep)	0.0005	Phototox.[c]	22	Sunlight	6(1+;c,i)/0	Nonphototoxic (reactions due to primary irritation)	134
−2(bath prep)	0.0005	Phototox.[c]	22	Sunlight	5(1+;c,i)/0	Nonphototoxic (reactions due to primary irritation)	134
−3(face lotion)	2	Phototox.	10	BL	0/0	Nonphototoxic	125
−3(suntan lotion)	3.5	Phototox.	10	BL	0/0	Nonphototoxic	125
−3(eye cream)	2	Phototox.	10	BL	0/0	Nonphototoxic	133
−3(lotion)	3.5	Phototox.	28	BL/HQML	0/0	Nonphototoxic	40
−3(sunscreen)	1	Phototox.	10	XASS	10/0	Nonphototoxic (minimal irritation due to presence of UV-B light)	135
−3(sunscreen)	3	Phototox.	12	BL	1(1+;c,i)/0	Nonphototoxic (slightly irritating to 1 subject)	125
−3(sunscreen)	3	Phototox.	26	–	0/0	Nonphototoxic	69
−4(skin prep)	0.1	Phototox.	25	Sunlight	0/0	Nonphototoxic	136
−3(suntan lotion)	3.5	Photoall.	27	BL	3(1+;c,i−induct)/ 1(1+;c,i,o,v)/0	Nonphotoallergenic (primary irritation/1 subject sensitized)	125
−3(face lotion)	2	Photoall.	27	BL	2(1+;i) 1(1+;c)	Nonphotoallergenic (primary irritation)	125
−3(eye cream)	2	Photoall.	28	BL	1(c,i,o)/0	Nonphotoallergenic (1 subject sensitized)	133
−3(lotion)	3.5	Photoall.	28	BL	0/0	Nonphotoallergenic	40
−3(sunscreen)	3	Photoall.	30	BL	1(2+;c,i,o)/0	Nonphotoallergenic (1 subject sensitized to BP-3)	125
−3(sunscreen)	3	Photoall.	25	–	0/0	Nonphotoallergenic	69

[a]sunlight (UV-A, UV-B); XASS−Xenon arc solar simulator (UV-A); BL−F40 black lights (UV-A); HQML−Hot quartz mercury lamp (UV-B).
[b]c = control site; i = irradiated site; o = original site (challenge); v = virgin site (challenge).
[c]Procedure repeated daily for five consecutive days.

and/or a fresh site. Sites were scored upon patch removal and daily for up to four days. The products containing Benzophenone-3 were nonphotoallergenic in all studies; however a number of subjects experienced irritation or sensitization to the test material. One of 30 subjects reacted (2+ response) to one induction patch and the challenge patches, each containing 3.0% Benzophenone-3 sunscreen. Irritation persisted throughout the 72-hour observation period. When this subject was rechallenged six weeks later, similar reactions were observed at original and virgin sites. Eleven months later, the subject was patched with each component of the sunscreen. Benzophenone-3 induced sensitization in this individual. The investigators concluded, however, that the product containing 3% Benzophenone-3 is nonphotoallergenic.[125]

One subject with a history of contact sensitivity to cosmetics reacted to a challenge patch containing 2% Benzophenone-3 applied to the original site. The reaction occurred at both control and irradiated sites. However, no irritation resulted from patches applied to a fresh site.[133] Three of ten subjects reacted to a total of five induction patches containing 2% Benzophenone-3 in a face lotion. No irritation resulted from challenge patch application.[133] The suntan lotion (3.5% Benzophenone-3) caused several reactions to induction patches concurrently at control and irradiated sites. Although one subject developed irritation (1+) to the challenge patches at original and virgin sites, the response occurred at both control and irradiated sites.[125]

Other Clinical Experience

Benzophenones-3 (2%–10%); -4 (1%–10%); -8 (2%–10%); and -10 (0.5%–10%) have been tested for sunscreen efficacy in more than 121, 167, 130, and 295 human subjects, respectively, and under various sources of UV radiation (Table 12). In all tests combined, there was no report of irritancy or phototoxic reaction to these ingredients.

SUMMARY

Benzophenones-1 to -12 are substituted derivatives of 2-hydroxybenzophenone. These ingredients have similar chemical and physical properties. Benzophenones-7, -10, and -12 have no current use in cosmetics; however, they are used noncosmetically as fungicides, pharmaceutical sunscreens, and antioxidant/photostabilizers. In addition to their widespread use as photostabilizers in cosmetics, Benzophenones, have a photoprotective effect on the skin. Benzophenones are typically used in cosmetic formulations at concentrations up to 1%; however, concentrations of up to 5 or 10% are reported for certain Benzophenones.

Benzophenones-3, -4, and -8 are registered with the FDA as safe and effective sunscreen ingredients (at concentrations up to 10%) for OTC use and as indirect food additives (Benzophenone-12, up to 0.01%). These sunscreens play an active role in protecting individuals who have photodermatoses, especially drug-mediated.

When ingested, absorbed Benzophenones were primarily conjugated and

TABLE 12. Benzophenone Sunscreen Efficacy Tests.

Benzophenone	Conc. tested (%)	No. of subjects	UV radiation source	Ref.
−3	3	9–17	Solar simulator	69
	3	18	Solar simulator	69
	3	20	Sunlight	55
	2	10	Xenon arc solar simulator	63
	5	10	Xenon arc solar simulator	63
	10	10	Xenon arc solar simulator	63
	3	12	Germicidal mercury lamp	38
	3	23	Prism grating monchrom.	45
	3	9	Sunlight	137
−4	1–3	10	Solar simulator	69
	10	5	Sunlight	69
	10	10	Ultraviolet lamp	71
	10	10	Hot quartz lamp	62
	10	20	Sunlight	55
	5	10	Xenon arc solar simulator	63
	5	12	Germicidal mercury lamp	38
	10	12	Germicidal mercury lamp	38
	10	16 normal 10 photosens.	Quartz mercury lamp	138
	10	30	Sunlight	139
	10	6	Mercury UV lamp	45
	10	16	Prism grating monochrom.	45
−8	3	9	Sunlight	69
	3	33	Sunlight	69
	3	20	Sunlight	55
	2	10	Xenon arc solar simulator	63
	5	10	Xenon arc solar simulator	63
	10	10	Xenon arc solar simulator	63
	3	12	Germicidal mercury lamp	38
	3	17	Prism grating monochrom.	45
	3	9	Sunlight	137
−10	10	86	Sunlight	140
	0.5	104 normal 28 photosens.	Xenon arc monochromator	57
	–	77	–	58

excreted in the urine, while the unabsorbed material passed out with the feces. Benzophenones were practically nontoxic when administered orally to rats, and Benzophenones-3, -4, -8, and -12 were nontoxic when applied to the skin of rabbits at doses of > 5 g/kg. In subchronic oral toxicity studies, Benzophenones-3 and -12, at 1% and 1.8% in the diet, respectively, were nontoxic to rats. Benzophenones-1 and -12 elicited toxic effects in rats at 0.6 and 1.9 g/kg, respectively, when fed for 90 days. In the same time period, Benzophenone-3, fed at 0.5% in the diet, and Benzophenone-8, fed at 5%, produced toxic effects. In a 120-day feeding study, Benzophenone-12 was nontoxic to dogs at a concentration of 0.6% in the diet.

Benzophenones were nonirritating or mildly irritating when applied to rabbit skin at concentrations of up to 100%. Benzophenones were practically nonirritating to the eyes of rabbits, even when instilled undiluted. A subchronic skin irritation test revealed that Benzophenone-4 was capable of causing minimal irritation (in rabbits) at a concentration of 10%. When Benzophenone-3 was tested for potential sensitization through the Kligman Guinea Pig Maximization procedures, it was reported to be nonsensitizing. Benzophenone-8 (3%) and Benzophenone-3 (6%) were nonphototoxic in guinea pigs and rabbits, respectively.

Benzophenones-2, -6, and -8 were reported to be weakly mutagenic with metabolic activation in the Ames test. Benzophenones-6 and -8 were mutagenic in one *Salmonella* strain only. In a Mouse Lymphoma Forward Mutation Assay and a cytogenic assay, Benzophenone-2 was weakly mutagenic at high concentrations and with metabolic activation. All other Benzophenones were nonmutagenic both with and without metabolic activation in the Ames test.

Benzophenones were tested for skin irritation and sensitization in humans. In general, these ingredients were mildly irritating and sensitizing at concentrations greater than those used in cosmetics. The published scientific literature reports isolated incidences of contact sensitization to Benzophenones-3, -4, and -8.

Sunscreens and other cosmetic products containing Benzophenones-2, -3 and -4 (at concentrations of 0.1%–3.5%) were tested for phototoxicity and/or photosensitivity in a number of studies. All products were reported to be nonphototoxic and nonphotoallergenic, although instances of primary irritation and contact sensitization to these products were observed.

Benzophenones-3, -4, -8, and -10 were tested extensively for sunscreen efficacy; no instances of irritation or phototoxicity were reported.

DISCUSSION

Benzophenones-1, -2, -3, -4, -5, -6, -8, -9, and -11 are photostabilizers in cosmetics. Benzophenones-7, -10, and -12 have no reported cosmetic use, yet they are used noncosmetically as fungicides, pharmaceutical sunscreens, and antioxidant/photostabilizers, respectively. Relevant chemical, use, toxicological, and clinical data on Benzophenones-2, -6, and -8 have been included in this report. The mutagenicity data on these three ingredients are available in a subsequent Addendum to the Final Report.

Benzophenones-3, -4, and -8 are approved by the FDA for use as safe and effective OTC sunscreen ingredients at concentrations equal to or greater than those used in cosmetics.

Although there are no animal toxicology or clinical data for Benzophenone-5, this ingredient is simply the sodium salt of Benzophenone-4. It would be expected that Benzophenones-4 and -5 have similar biological properties (i.e., toxicity, irritancy potential, etc.). At high concentrations, Benzophenone-5 may be an eye irritant; however, it would not be expected to induce significant ocular irritation at cosmetic use concentration ($\leq$0.1%).

All Benzophenones were tested for mutagenesis under the Ames *Salmonella* test. All Benzophenones were nonmutagenic when assayed directly, and all but

Benzophenones-2, -6, and -8 were nonmutagenic following metabolic activation. Benzophenones-6 and -8 were weakly mutagenic at high doses in only one strain (TA1537). Benzophenone-2 was nonmutagenic with and without activation in one Ames test, but in another test it was mutagenic under activation in four *Salmonella* strains. Benzophenone-2 was also reported to be weakly mutagenic under activation conditions in a cytogenic assay and in a forward mutation assay.

Benzophenones-1, -5, -6, -9, and -11 lack photosensitivity data, but because of their conditions of use, as well as similarities in chemical structure and UV-absorption spectra to other Benzophenones, these five ingredients would not be expected to induce phototoxicity or photoallergenicity.

CONCLUSION

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.

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

INTRODUCTION

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

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

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

SHORT-TERM TESTS

Data from the Final Report

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

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

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

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

TABLE 9A. Ames *Salmonella* Mutagenesis Assay.

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

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

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

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

New Data

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

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

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

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

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

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

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

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

CONCLUSION

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

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makeup, makeup and skin care preparations. Currently Lanolin Acid is used in 44 products at a maximum use concentration of 3% in mascara. Table 1 provides the available use information.

Lanolin Alcohols are a mixture of organic alcohols obtained from the hydrolysis of Lanolin (q.v.). These are used as emulsion stabilizer agent, hair conditioning agent, binder, and nonaqueous viscosity increasing agents. These were used in 738 cosmetic products in 1976, with the highest concentration range of >0.1% to 50% in skin care preparations. Currently Lanolin Alcohols are used in 337 products at a maximum use concentration of 4% in hair coloring preparations (other). Table 1 provides the available use information.

Lanolin Oil is the liquid fraction obtained by physical means from whole Lanolin which is used as a skin-conditioning agent-emollient and hair conditioning agent. It was used in 1256 cosmetic products in 1976, with the highest concentration range of >0.1% to >50% in makeup preparations. Currently Lanolin Oil is used in 532 products at a maximum use concentration of 65% in lipsticks. Table 1 provides the available use information.

Lanolin Wax is the semisolid fraction obtained by physical means from whole Lanolin (Pepe et al. 2002). It is used as hair conditioning agent, skin-conditioning agents—emollient, nonaqueous viscosity-increasing agents, and binder. It was used in 157 cosmetic products in 1976, with the highest concentration range of ≤0.1% to 50% in makeup preparations. Currently Lanolin Wax is used in 97 products at a maximum use concentration of 23% in lipsticks. Table 1 provides the available use information.

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BENZOPHENONE AND BENZOPHENONE-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, AND -12

A safety assessment of Benzophenone-1, -3, -4, -5, -9, and -11 was published in 1983 with the conclusion "safe for topical application to humans in the present practices of use and

concentration in cosmetics" (Elder 1983a). An addendum to this safety assessment, addressing the mutagenicity/genotoxicity of Benzophenone-2, -6, and -8, was also published in 1983 with a conclusion stating that they are not mutagenic or genotoxic, but are safe for topical application to humans in the present practices of use and concentration in cosmetics (Elder 1983b). Studies available since the safety assessment and addendum were completed (listed at the end of this section), along with the updated information regarding uses and use concentrations, were considered. The Panel determined to not reopen this safety assessment.

The CIR Expert Panel's discussion focused on National Toxicology Program (NTP) carcinogenicity studies on Benzophenone and Benzophenone-3. An NTP 2-year carcinogenicity study on Benzophenone was initiated in 1999, and the pathology quality assessment for this study is ongoing. Benzophenone-3 is listed among the chemicals that NTP has assigned to a laboratory for toxicology/carcinogenesis testing. The Panel determined to not reopen its safety assessment until results from the NTP carcinogenicity studies are available.

In 1976, Benzophenones were used in 1044 cosmetic products, typically at concentrations ≤0.1%. Currently, there are uses reported in 1008 products, typically at concentrations <1%. Table 2 presents the available use information.

TABLE 2
Historical and current cosmetic product uses and concentrations for Benzophenones

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
		Benzophenone-1		
Bath oils, tablets, and salts	1	—	≤0.1	—
Bubble baths	2	—	≤0.1	—
Colognes and toilet waters	3	1	≤0.1	0.2–1
Perfumes	—	1	—	0.1–1
Fragrance preparations (other)	5	—	≤0.1	1
Shampoos (noncoloring)	7	—	≤0.1	0.05
Hair tonics, dressings, etc.	2	1	≤0.1–1	—
Wave sets	4	—	≤0.1	—
Hair preparations (other)	1	3	≤0.1	—
Blushers (all types)	1	—	>0.1–1	—
Lipstick	7	1	>0.1–1	0.2
Nail basecoats and undercoats	5	23	≤0.1–1	0.4–0.8
Nail creams and lotions	—	2	—	—
Nail polish and enamel	87	75	≤0.1–1	0.1–2
Nail polish and enamel removers	—	8	—	—
Nail care preparations (other)	4	11	≤0.1–1	0.1
Aftershave lotions	6	—	≤0.1	0.2–0.5
Beard softeners (category omitted in 2002)	2	—	≤0.1	—
Total uses/ranges for Benzophenone-1	**113**	**127**	**≤0.1–1**	**0.05–2**
		Benzophenone-2		
Bath oils, tablets, and salts	3	—	≤0.1	0.1–0.2
Bubble baths	5	5	≤0.1–1	0.05–0.2
Bath preparations (other)	6	4	≤0.1	0.07–0.2
Eye makeup remover	—	1	—	—
Colognes and toilet waters	120	193	≤0.1–5	0.1–0.4
Perfumes	22	53	≤0.1–1	0.005–0.3
Powders	—	1	—	—
Sachets	4	1	≤0.1	0.2
Other fragrance preparations	15	18	≤0.1–1	—
Hair conditioners	2	3	≤0.1	0.1
Hair sprays (aerosol fixatives)	—	1	—	0.05

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TABLE 2
Historical and current cosmetic product uses and concentrations for Benzophenones *(Continued)*

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
Rinses (noncoloring)	4	—	≤0.1	0.05
Shampoos (noncoloring)	14	4	≤0.1–1	0.1
Hair tonics, dressings, etc.	2	—	≤0.1	0.1
Wave sets	3	—	>0.1–1	—
Hair preparations (other)	—	2	—	0.1
Blushers (all types)	3	—	≤0.1	—
Lipsticks	—	3	—	—
Rouges	1	1	≤0.1	—
Makeup fixatives	1	—	≤0.1	—
Makeup preparations (other)	4	1	≤0.1	—
Nail polish and enamel removers	—	1	—	—
Bath soaps and detergents	—	1	—	0.05–0.2
Underarm deodorants	—	1	—	0.1
Douches	—	—	—	0.1
Feminine deodorants	1	—	≤0.1	—
Personal cleanliness products (other)	—	—	—	0.1
Aftershave lotions	30	45	≤0.1–1	0.2
Preshave lotions	1	1	≤0.1	0.03–0.1
Shaving preparation products (other)	—	6	—	0.1
Skin cleansing creams, lotions, liquids, and pads	6	6	≤0.1–1	0.05–0.1
Face and neck skin care preparations		—		—
Body and hand skin care preparations	7*	3	≤0.1*	0.07–0.2
Moisturizers	9	2	≤0.1	0.1
Night skin care preparations	—	—	—	0.05
Paste masks (mud packs)	1	3	≤0.1	0.05
Skin lighteners	1	N/A**	≤0.1	N/A**
Skin fresheners	27	9	≤0.1	0.2%
Skin care preparations (other)	5	11	≤0.1–1	0.1–0.2
Suntan gels, creams, and liquids	—	—	—	6
Total uses/ranges for Benzophenone-2	**321**	**380**	**≤0.1–5**	**0.005–6**
	Benzophenone-3			
Baby shampoos	—	—	—	0.01
Baby lotions, oils, powders, and creams	—	—	—	3
Bath oils, tablets, and salts	1	11	≤0.1	0.2–0.4
Bubble baths	—	1	—	0.1
Bath preparations (other)	—	18	—	0.2
Eyeliner	—	1	—	—
Eye shadow	—	—	—	0.5
Eye makeup (other)	—	1	—	0.2–0.4
Colognes and toilet waters	1	9	>0.1–1	0.1–2
Perfumes	1	12	≤0.1	0.2–2
Fragrance preparations (other)	—	4	—	0.2–2
Hair conditioners	—	21	—	0.05–0.5
Hair sprays	—	13	—	0.01
Shampoos	1	13	≤0.1	0.01–0.3
Hair tonics, dressings, etc.	—	18	—	0.3–0.5
Hair preparations (other)	—	5	—	0.3

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TABLE 2
Historical and current cosmetic product uses and concentrations for Benzophenones *(Continued)*

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
Blushers	—	1	—	1–6
Face powders	—	2	—	1–4
Foundations	—	28	—	0.1–3
Lipsticks	—	85	—	1–4
Makeup bases	—	3	—	1–2
Makeup (other)	1	4	>0.1–1	0.1–3
Nail basecoats and undercoats	—	2	—	0.4–3
Nail creams and lotions	—	1	—	—
Nail polish and enamel	36	10	>0.1–1	0.2–1
Nail polish and enamel removers	—	2	—	0.2–0.4
Nail care preparations (other)	—	1	—	0.2
Bath soaps and detergents	—	2	—	0.05–0.06
Personal cleanliness products (other)	—	1	—	—
Aftershave lotions	3	9	>0.1–1	2–3
Shaving cream	—	—	—	0.2
Shaving products (other)	—	—	—	3
Skin-cleansing creams, lotions, liquids, and pads	2	1	≤0.1	0.05–0.2
Face and neck skin care preparations		17		0.3–5
Body and hand skin care preparations	—*	20	—*	2–5
Moisturizers	—	59	—	0.5–7
Night skin care preparations	—	1	—	1–2
Skin fresheners	1	—	≤0.1	0.005
Skin care preparations (other)	—	12	—	1–6
Suntan gels, creams, and liquids	—	47	—	1–6
Indoor tanning preparations	—	9	—	1–5
Suntan preparations (other)	—	8	—	3–6
Total uses/ranges for Benzophenone-3	**62**	**451**	**≤0.1–1**	**0.005–7**
	Benzophenone-4			
Baby shampoos	2	1	≤0.1	0.01
Baby lotions, oils, powders, and creams	—	1	—	0.2
Bath oils, tablets, and salts	11		≤0.1	0.1
Bubble baths	2	4	≤0.1	0.1–0.2
Bath capsules	—	1	—	—
Bath preparations (other)	4	19	≤0.1	0.1–0.2
Eye shadow	1	—	>0.1–1	—
Eye lotion	—	1	—	—
Eye makeup remover	—	—	—	0.05
Eye makeup (other)	—	1	—	0.01
Colognes and toilet waters	8	—	≤0.1	0.3
Perfumes	—	3	—	0.1
Powders	—	1	—	—
Fragrance preparations (other)	11	4	≤0.1	0.1–0.2
Hair conditioners	29	37	≤0.1–5	0.01–0.5
Hair sprays (aerosol fixatives)	1	18	≤0.1	0.1
Hair straighteners	—	—	—	0.01
Permanent waves	2	1	≤0.1	—

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TABLE 2
Historical and current cosmetic product uses and concentrations for Benzophenones *(Continued)*

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
Rinses (noncoloring)	7	4	≤0.1	—
Shampoos (noncoloring)	45	48	≤0.1–5	0.01–0.5
Hair tonics, dressings, etc.	7	51	≤0.1–1	0.01–0.9
Wave sets	27	6	≤0.1–1	0.4
Hair preparations (other)	13	38	≤0.1	0.05–0.1
Shampoos (coloring)	1	—	>0.1–1	—
Hair-coloring preparations (other)	—	2	—	0.04
Blushers (all types)	6	—	≤0.1–1	1
Foundations	1	—	≤0.1	—
Leg and body paints	1	—	≤0.1	—
Makeup bases	1	—	>0.1–1	—
Makeup (other)	2	1	>0.1–1	0.05–0.1
Cuticle softeners	2	1	≤0.1	—
Nail polish and enamel removers	—	2	—	—
Nail care products (other)	—	1	—	—
Bath soaps and detergents	2	18	>0.1–1	0.05–0.5
Underarm deodorants	—	2	—	0.1–0.7
Personal cleanliness products (other)	—	6	—	0.05–0.3
Aftershave lotion	2	5	≤0.1	0.05–1
Shaving products (other)	—	2	—	0.1
Skin-cleansing creams, lotions, liquids, and pads	6	33	≤0.1–1	0.01–0.3
Face and neck skin care preparations	9*	4	≤0.1*	0.05–2.5
Body and hand skin care preparations		20		0.05–0.2
Foot powders and sprays	—	2	—	0.05
Moisturizers	21	33	≤0.1	0.1–1
Night skin care	—	1	—	—
Paste masks (mud packs)	—	1	—	0.1–0.2
Skin fresheners	5	16	≤0.1	0.05–2
Skin care preparations (other)	9	10	≤0.1–1	0.05–0.5
Suntan gels, creams, and liquids	2	1	≤0.1–10	2
Suntan preparations (other)	—	—	—	0.005
Total uses/ranges for Benzophenone-4	**251**	**402**	**≤0.1–10**	**0.005–2.5**
	Benzophenone-5			
Eye shadow	—	1	—	—
Colognes and toilet waters	—	—	—	0.2
Shaving products (other)	—	1	—	—
Hair tonics, dressings, etc.	—	—	—	0.2
Skin-cleansing creams, lotions, liquids, and pads	—	2	—	—
Face and neck skin care preparations	7*	—	≤0.1*	—
Body and hand skin care preparations		6		0.05
Moisturizers	—	3	—	—
Night skin care preparations	3	1	≤0.1	0.3
Paste masks (mud packs)	—	1	—	—
Skin fresheners	—	4	—	—
Total uses/ranges for Benzophenone-5	**11**	**20**	**≤0.1**	**0.05–0.3**

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TABLE 2

Historical and current cosmetic product uses and concentrations for Benzophenones *(Continued)*

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
	Benzophenone-6			
Bath oils, tablets, and salts	2	1	≤0.1	—
Eye makeup remover	—	1	—	—
Colognes and toilet waters	1	1	≤0.1	0.2
Perfumes	2	3	≤0.1–1	0.2
Shampoos (noncoloring)	1	—	≤0.1	—
Hair tonics, dressings, etc.	1	2	≤0.1	—
Wave sets	2	—	≤0.1	—
Cuticle softeners	1	—	≤0.1	—
Nail polish and enamel	77		>0.1–1	0.3
Aftershave lotion	—	1	—	0.1
Skin-cleansing creams, lotions, liquids, and pads	1	—	≤0.1	—
Face and neck skin care preparations		—		0.07
Body and hand skin care preparations	—*	1	—*	—
Moisturizers	2	—	≤0.1–1	—
Skin care preparations (other)	—	1	—	—
Total uses/ranges for Benzophenone-6	**106**	**11**	**≤0.1–1**	**0.07–0.3**
	Benzophenone-8			
Bath oils, tablets, and salts	1	—	>0.1–1	—
Bubble baths	—	2	—	—
Bath preparations (other)	—	1	—	—
Colognes and toilet waters	—	2	—	—
Hair conditioners	2	—	>0.1–1	—
Personal cleanliness products (other)	—	2	—	—
Moisturizers	1	—	≤0.1	—
Skin care preparations (other)	—	—	—	0.2
Total uses/ranges for Benzopheone-8	**3**	**7**	**≤0.1–1**	**0.2**
	Benzophenone-9			
Baby products (other)	—	—	—	0.2
Bath oils, tablets, and salts	—	—	—	0.1
Bubble baths	20	6	≤0.1	0.1–0.2
Bath capsules	1	—	≤0.1	—
Bath preparations (other)	34	14	≤0.1	0.1–0.2
Colognes and toilet waters	2	4	≤0.1–1	0.1–0.2
Perfumes	1	—	>0.1–1	—
Sachets	—	—	—	0.1
Fragrance preparations (other)	1	1	≤0.1	0.2
Hair conditioners	9	1	≤0.1–1	0.1–0.2
Rinses (noncoloring)	3	—	≤0.1	0.05
Shampoos (noncoloring)	8	3	≤0.1–1	0.1
Hair tonics, dressings, etc.	1	1	≤0.1	0.03
Wave sets	2	—	≤0.1	0.2
Hair preparations (other noncoloring)	1	—	≤0.1	—
Hair-coloring preparations (other)	—	—	—	0.4
Blushers	1	—	≤0.1	—
Makeup bases	1	1	>0.1–1	—

(Continued on next page)

TABLE 2
Historical and current cosmetic product uses and concentrations for Benzophenones *(Continued)*

Product category	1976 use (Elder 1983)	2002 use (FDA 2002)	1976 concentrations (Elder 1983) (%)	2002 concentrations (CTFA 2002) (%)
Rouges	1	—	≤0.1	—
Makeup (other)	—	—	—	0.05
Nail basecoats and undercoats	1	—	≤0.1	—
Cuticle softeners	1	—	≤0.1	—
Nail creams and lotions	1	—	≤0.1	—
Bath soaps and detergents	—	8	—	0.05–0.2
Underarm deodorants	—	—	—	0.1
Personal cleanliness products (other)	—	—	—	0.1
Aftershave lotion	3	2	≤0.1–1	0.1–0.2
Shaving preparations (other)	—	—	—	0.09
Skin-cleansing creams, lotions, liquids, and pads	4	1	≤0.1–1	—
Face and neck skin care preparations		—	≤0.1–1*	0.3
Body and hand skin care preparations	14*	2		0.1–0.3
Moisturizers	2	4	≤0.1	0.05
Night skin care preparations	—	—	—	0.05
Skin fresheners	9	2	≤0.1–1	0.1
Skin care preparations (other)	1	1	≤0.1	0.1
Suntan gels, creams, and liquids	1	—	≤0.1–1	—
Suntan preparations (other)	—	2	—	—
Total uses/ranges for Benzophenone-9	**85**	**53**	**≤0.1–1**	**0.05–0.4**
	Benzophenone-11			
Bath oils, tablets, and salts	4	2	≤0.1–1	—
Bubble baths	4	—	≤0.1	—
Bath preparations (other)	1	—	≤0.1	—
Colognes and toilet waters	59	3	≤0.1–1	0.2
Perfumes	14	2	≤0.1	0.1
Sachets	7	—	≤0.1	—
Fragrance preparations (other)	8	—	≤0.1	—
Hair sprays (aerosol fixatives)	4	—	≤0.1–5	—
Shampoos	13	—	≤0.1	—
Hair tonics, dressings, etc.	2	—	>0.1–1	0.2
Wave sets	2	—	≤0.1	—
Blushers	1	—	≤0.1	—
Nail polish and enamel	3	—	≤0.1	—
Bath soaps and detergents	3	—	≤0.1	—
Underarm deodorants	—	—	—	0.1
Aftershave lotion	16	1	≤0.1	0.2
Preshave lotions (all types)	1	—	≤0.1	0.1
Face and neck skin care preparations	2*	—	≤0.1*	—
Body and hand skin care preparations		—		—
Moisturizers	12	—	≤0.1	—
Skin fresheners	11	—	≤0.1	—
Skin care preparations (other)	1	—	≤0.1	—
Total uses/ranges for Benzophenone-11	**103**	**8**	**≤0.1–5**	**0.1–0.2**

*These categories were combined when the original safety assessment was performed and are now separate categories.
**No longer considered a cosmetic product category.

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BUTOXYETHANOL

A safety assessment of Butoxyethanol was published in 1996 with the conclusion "safe in hair and nail products at concentrations up to 10.0%" (Andersen 1996). Studies available since that safety assessment was completed, along with the updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

2020 FDA VCRP Data

Benzophenone-1

Other Baby Products	01C	2
Bath Oils, Tablets, and Salts	02A	1
Cologne and Toilet waters	04A	33
Perfumes	04B	7
Other Fragrance Preparation	04E	2
Basecoats and Undercoats	08A	64
Nail Creams and Lotions	08C	5
Nail Extenders	08D	1
Nail Polish and Enamel	08E	411
Nail Polish and Enamel Removers	08F	26
Other Manicuring Preparations	08G	33
Aftershave Lotion	11A	6
Preshave Lotions (all types)	11D	1
Cleansing	12A	1
Body and Hand (exc shave)	12D	2
Total		**595**

Benzophenone-2

Bath Oils, Tablets, and Salts	02A	1
Cologne and Toilet waters	04A	60
Perfumes	04B	18
Other Fragrance Preparation	04E	3
Shampoos (non-coloring)	05F	1
Tonics, Dressings, and Other Hair Grooming Aids	05G	1
Bath Soaps and Detergents	10A	4
Deodorants (underarm)	10B	1
Aftershave Lotion	11A	6
Cleansing	12A	1
Body and Hand (exc shave)	12D	2
Moisturizing	12F	3
Paste Masks (mud packs)	12H	1
Other Skin Care Preps	12J	1
Total		**103**

Benzophenone-3

Other Baby Products	01C	1
Bath Oils, Tablets, and Salts	02A	3
Bubble Baths	02B	32
Other Bath Preparations	02D	1
Eye Lotion	03D	5
Cologne and Toilet waters	04A	222
Perfumes	04B	94
Other Fragrance Preparation	04E	68
Hair Conditioner	05A	23
Hair Spray (aerosol fixatives)	05B	6

Hair Straighteners	05C	5
Rinses (non-coloring)	05E	2
Shampoos (non-coloring)	05F	12
Tonics, Dressings, and Other Hair Grooming Aids	05G	7
Wave Sets	05H	2
Other Hair Preparations	05I	14
Hair Dyes and Colors (all types requiring caution statements and patch tests)	06A	5
Hair Shampoos (coloring)	06D	8
Other Hair Coloring Preparation	06H	1
Face Powders	07B	4
Foundations	07C	34
Lipstick	07E	101
Makeup Bases	07F	2
Makeup Fixatives	07H	1
Other Makeup Preparations	07I	36
Basecoats and Undercoats	08A	7
Cuticle Softeners	08B	2
Nail Creams and Lotions	08C	2
Nail Polish and Enamel	08E	21
Nail Polish and Enamel Removers	08F	10
Other Manicuring Preparations	08G	9
Bath Soaps and Detergents	10A	11
Deodorants (underarm)	10B	4
Feminine Deodorants	10D	4
Other Personal Cleanliness Products	10E	5
Aftershave Lotion	11A	16
Shaving Cream	11E	1
Cleansing	12A	15
Face and Neck (exc shave)	12C	49
Body and Hand (exc shave)	12D	25
Moisturizing	12F	58
Night	12G	3
Skin Fresheners	12I	2
Other Skin Care Preps	12J	16
Suntan Gels, Creams, and Liquids	13A	30
Indoor Tanning Preparations	13B	5
Other Suntan Preparations	13C	5
Total		**989**

Benzophenone-4

Other Baby Products	01C	2
Bath Oils, Tablets, and Salts	02A	7
Bubble Baths	02B	51
Other Bath Preparations	02D	14
Eye Lotion	03D	4
Eye Makeup Remover	03E	3

Other Eye Makeup Preparations	03G	3
Perfumes	04B	2
Other Fragrance Preparation	04E	6
Hair Conditioner	05A	85
Hair Spray (aerosol fixatives)	05B	70
Hair Straighteners	05C	3
Rinses (non-coloring)	05E	1
Shampoos (non-coloring)	05F	97
Tonics, Dressings, and Other Hair Grooming Aids	05G	150
Wave Sets	05H	9
Other Hair Preparations	05I	88
Hair Rinses (coloring)	06C	2
Hair Shampoos (coloring)	06D	6
Hair Color Sprays (aerosol)	06E	13
Other Hair Coloring Preparation	06H	18
Foundations	07C	3
Lipstick	07E	10
Other Makeup Preparations	07I	3
Cuticle Softeners	08B	2
Other Manicuring Preparations	08G	4
Bath Soaps and Detergents	10A	1010
Other Personal Cleanliness Products	10E	111
Aftershave Lotion	11A	7
Shaving Cream	11E	1
Shaving Soap	11F	2
Other Shaving Preparation Products	11G	3
Cleansing	12A	206
Face and Neck (exc shave)	12C	44
Body and Hand (exc shave)	12D	43
Moisturizing	12F	118
Night	12G	9
Paste Masks (mud packs)	12H	5
Skin Fresheners	12I	9
Other Skin Care Preps	12J	31
Indoor Tanning Preparations	13B	2
Other Suntan Preparations	13C	2
Total		**2259**

Benzophenone-5

Eye Makeup Remover	03E	1
Shampoos (non-coloring)	05F	1
Tonics, Dressings, and Other Hair Grooming Aids	05G	1
Makeup Bases	07F	2
Cleansing	12A	1
Face and Neck (exc shave)	12C	7
Moisturizing	12F	1
Total		**14**

Benzophenone-6 - No FDA Data

Benzophenone-7 - No FDA Data

Benzophenone-8 - No FDA Data

Benzophenone-9

Bubble Baths	02B	1
Cologne and Toilet waters	04A	6
Perfumes	04B	2
Other Fragrance Preparation	04E	5
Lipstick	07E	2
Nail Creams and Lotions	08C	1
Bath Soaps and Detergents	10A	21
Other Personal Cleanliness Products	10E	4
Aftershave Lotion	11A	1
Cleansing	12A	3
Face and Neck (exc shave)	12C	8
Body and Hand (exc shave)	12D	7
Moisturizing	12F	3
Night	12G	2
Paste Masks (mud packs)	12H	1
Other Skin Care Preps	12J	4
Total		**71**

Benzophenone-10 - No FDA Data

Benzophenone-11 - No FDA Data

Benzophenone-12 - No FDA Data

1983 FDA VCRP Data

Benzophenone-1

Bath Oils, Tablets, and Salts	02A	1
Bubble Baths	02B	2
Cologne and Toilet waters	04A	3
Other Fragrance Preparation	04E	5
Shampoos (non-coloring)	05F	7
Tonics, Dressings, and Other Hair Grooming Aids	05G	2
Wave Sets	05H	4
Other Hair Preparations	05I	1
Blushers	07A	1
Lipstick	07E	7
Basecoats and Undercoats	08A	5
Nail Polish and Enamel	08E	87

Other Manicuring Preparations	08G	4
Aftershave Lotion	11A	6
Beard Softeners	11B	2
Body and Hand (exc shave)	12D	2
Moisturizing	12F	3
Total		**142**

Benzophenone-2

Bath Oils, Tablets, and Salts	02A	3
Bubble Baths	02B	5
Other Bath Preparations	02D	6
Cologne and Toilet waters	04A	120
Perfumes	04B	22
Sachets	04D	4
Other Fragrance Preparation	04E	15
Hair Conditioner	05A	2
Rinses (non-coloring)	05E	4
Shampoos (non-coloring)	05F	14
Tonics, Dressings, and Other Hair Grooming Aids	05G	2
Wave Sets	05H	3
Blushers	07A	3
Rouges	07G	1
Makeup Fixatives	07H	1
Other Makeup Preparations	07I	4
Feminine Deodorants	10D	1
Aftershave Lotion	11A	30
Preshave Lotions (all types)	11D	1
Cleansing	12A	6
Body and Hand (exc shave)	12D	7
Moisturizing	12F	8
Paste Masks (mud packs)	12H	1
Skin Fresheners	12I	27
Other Skin Care Preps	12J	7
Suntan Gels, Creams, and Liquids	13A	1
Other Suntan Preparations	13C	1
Total		**299**

Benzophenone-3

Bath Oils, Tablets, and Salts	02A	1
Cologne and Toilet waters	04A	1
Perfumes	04B	1
Shampoos (non-coloring)	05F	1
Other Makeup Preparations	07I	1
Nail Polish and Enamel	08E	36
Aftershave Lotion	11A	3
Cleansing	12A	2
Skin Fresheners	12I	1

Total		**47**
Benzophenone-4		
Baby Shampoos	01A	2
Bath Oils, Tablets, and Salts	02A	11
Bubble Baths	02B	2
Other Bath Preparations	02D	4
Eye Shadow	03C	1
Cologne and Toilet waters	04A	8
Other Fragrance Preparation	04E	11
Hair Conditioner	05A	29
Hair Spray (aerosol fixatives)	05B	1
Permanent Waves	05D	2
Rinses (non-coloring)	05E	7
Shampoos (non-coloring)	05F	45
Tonics, Dressings, and Other Hair Grooming Aids	05G	7
Wave Sets	05H	27
Other Hair Preparations	05I	13
Hair Shampoos (coloring)	06D	1
Blushers	07A	6
Foundations	07C	1
Leg and Body Paints	07D	1
Makeup Bases	07F	1
Other Makeup Preparations	07I	2
Cuticle Softeners	08B	2
Bath Soaps and Detergents	10A	2
Aftershave Lotion	11A	2
Cleansing	12A	6
Body and Hand (exc shave)	12D	9
Moisturizing	12F	21
Skin Fresheners	12I	5
Other Skin Care Preps	12J	9
Suntan Gels, Creams, and Liquids	13C	2
Total		**240**
Benzophenone-5		
Body and Hand (exc shave)	12D	7
Night	12G	3
Total		**10**
Benzophenone-6		
Bath Oils, Tablets, and Salts	02A	2
Cologne and Toilet waters	04A	1
Perfumes	04B	2
Shampoos (non-coloring)	05F	1
Tonics, Dressings, and Other Hair Grooming Aids	05G	1
Wave Sets	05H	2

Cuticle Softeners	08B	1
Nail Polish and Enamel	08E	77
Cleansing	12A	1
Moisturizing	12F	2
Total		**90**

Benzophenone-8

Bath Oils, Tablets, and Salts	02A	1
Hair Conditioner	05A	2
Moisturizing	12F	1
Total		**4**

Benzophenone-9

Bubble Baths	02B	20
Bath Capsules	02C	1
Other Bath Preparations	02D	34
Cologne and Toilet waters	04A	2
Perfumes	04B	1
Other Fragrance Preparation	04E	1
Hair Conditioner	05A	9
Rinses (non-coloring)	05E	3
Shampoos (non-coloring)	05F	8
Tonics, Dressings, and Other Hair Grooming Aids	05G	1
Wave Sets	05H	2
Other Hair Coloring Preparation	06H	1
Blushers	07A	1
Makeup Bases	07F	1
Rouges	07G	1
Basecoats and Undercoats	08A	1
Cuticle Softeners	08B	1
Nail Creams and Lotions	08C	1
Aftershave Lotion	11A	3
Cleansing	12A	4
Body and Hand (exc shave)	12D	14
Moisturizing	12F	2
Skin Fresheners	12I	9
Other Skin Care Preps	12J	1
Other Suntan Preparations	13C	1
Total		**123**

Benzophenone-11

Bath Oils, Tablets, and Salts	02A	4
Bubble Baths	02B	4
Other Bath Preparations	02D	1
Cologne and Toilet waters	04A	59
Perfumes	04B	14
Sachets	04D	7

Other Fragrance Preparation	04E	8
Hair Spray (aerosol fixatives)	05B	4
Shampoos (non-coloring)	05F	13
Tonics, Dressings, and Other Hair Grooming Aids	05G	2
Wave Sets	05H	2
Blushers	07A	1
Nail Polish and Enamel	08E	3
Bath Soaps and Detergents	10A	3
Aftershave Lotion	11A	16
Preshave Lotions (all types)	11D	1
Body and Hand (exc shave)	12D	2
Moisturizing	12F	12
Skin Fresheners	12I	11
Other Skin Care Preps	12J	1
Total		**168**

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 14, 2020

SUBJECT: Concentration of Use by FDA Product Category: Benzophenones (non-sunscreen use)

Concentration of Use by FDA Product Category – Benzophenones (does not include sunscreen use)*

Benzophenone-1
Benzophenone-2
Benzophenone-3
Benzophenone-4
Benzophenone-5
Benzophenone-6
Benzophenone-7
Benzophenone-8
Benzophenone-9
Benzophenone-10
Benzophenone-11
Benzophenone-12

Ingredient	FDA Product Category	Maximum Concentration of Use
Benzophenone-1	Lipstick	0.05%
Benzophenone-1	Other makeup preparations	0.5%
Benzophenone-1	Basecoats and undercoats (manicuring preparations)	0.4-0.5%
Benzophenone-1	Nail creams and lotions	0.05%
Benzophenone-1	Nail polish and enamel	0.2-1.1%
Benzophenone-1	Nail polish and enamel removers	0.009-0.1%
Benzophenone-1	Aftershave lotions	0.15%
Benzophenone-1	Preshave lotions	0.15%
Benzophenone-3	Baby shampoos	0.2%
Benzophenone-3	Baby lotions, oils and creams Not powder	 0.25%
Benzophenone-3	Other baby products	0.05%
Benzophenone-3	Colognes and toilet waters	0.1-0.5%
Benzophenone-3	Perfumes	0.48%
Benzophenone-3	Other fragrance preparations	0.3%
Benzophenone-3	Hair conditioners	0.05-0.17%
Benzophenone-3	Hair sprays Aerosol Pump spray	 0.014% 0.05%
Benzophenone-3	Rinses (noncoloring)	0.1%
Benzophenone-3	Shampoos (noncoloring)	0.1-0.3%
Benzophenone-3	Tonics, dressings and other hair grooming aids	0.1-0.5%
Benzophenone-3	Hair shampoos (coloring)	0.15%
Benzophenone-3	Lipstick	0.5%
Benzophenone-3	Other makeup preparations	0.5%
Benzophenone-3	Basecoats and undercoats (manicuring preparations)	0.4%
Benzophenone-3	Nail polish and enamel	0.2-0.54%
Benzophenone-3	Nail polish and enamel removers	0.001-0.18%
Benzophenone-3	Other manicuring preparations	0.2%
Benzophenone-3	Bath soaps and detergents	0.05-0.5%
Benzophenone-3	Deodorants Pump spray	 0.08%
Benzophenone-3	Aftershave lotions	0.15%
Benzophenone-3	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0092-0.3%
Benzophenone-3	Body and hand products	

	Not spray	0.3-0.35%
Benzophenone-3	Moisturizing products Not spray	 0.03-0.25%
Benzophenone-3	Other skin care preparations	0.3%
Benzophenone-4	Other bath preparations	0.15%
Benzophenone-4	Other eye makeup preparations	0.2%
Benzophenone-4	Other fragrance preparations	0.02%
Benzophenone-4	Hair conditioners	0.000035-0.8%
Benzophenone-4	Hair sprays Aerosol Pump spray	 0.015% 0.001-0.1%
Benzophenone-4	Shampoos (noncoloring)	0.000035-0.1%
Benzophenone-4	Tonics, dressings and other hair grooming aids	0.0001-0.5%
Benzophenone-4	Wave sets	0.05%
Benzophenone-4	Other hair preparations (noncoloring)	0.01-1.6%
Benzophenone-4	Hair dyes and colors	0.1%
Benzophenone-4	Hair tints	0.05%
Benzophenone-4	Hair shampoos (coloring)	0.05%
Benzophenone-4	Other manicuring preparations	0.2%
Benzophenone-4	Bath soaps and detergents	0.2%
Benzophenone-4	Aftershave lotions	0.015%
Benzophenone-4	Shaving cream	0.14%
Benzophenone-4	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.05-0.5%
Benzophenone-4	Face and neck products Not spray	 0.1-0.2%
Benzophenone-4	Moisturizing products Not spray	 0.005%
Benzophenone-4	Other skin care preparations	0.03-0.5%
Benzophenone-5	Eye makeup removers	0.06%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2020
Table prepared: April 14, 2020