Safety Assessment of Benzaldehyde as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	May 19, 2023
Panel Meeting Date:	June 12-13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.S., Senior Scientific Analyst/Writer, CIR.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Preethi S. Raj, M.Sc.,
Senior Scientific Analyst/Writer, CIR
Date: May 19, 2023
Subject: Re-Review of the Safety Assessment of Benzaldehyde

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a Final Report on the Safety Assessment of Benzaldehyde in 2006 (identified in the pdf as *originalreport_Benzaldehyde_062023*). On the basis of data presented in the report, the Panel concluded that Benzaldehyde is safe as used in cosmetic products.

Because it has been at least 15 years since the previous review was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of Benzaldehyde should be re-opened. An extensive search of the world's literature was performed as of April 2023 for studies dated 2001 forward. Data on the daily intake, FEMA GRAS status, concentration limits in finished cosmetic products, acute oral toxicity, acute inhalation toxicity, oral developmental and reproductive toxicity, in vitro genotoxicity, acute dermal irritation, and 2 guinea pig maximization tests were found. None of the newly found data were notably different from data in the 2006 report. An historical overview, comparison of original and new use data, and the search strategy used are included herein (*newdata_Benzaldehyde_062023*).

Also included for your review is a table of current and historical use data (*usetable_Benzaldehyde_062023*). According to 2023 FDA VCRP data, Benzaldehyde has 6 reported uses, a minor change from the 7 reported uses in 2001. Reported use categories have not changed significantly and concentrations of use have remained constant over time. In 2023, the maximum reported concentration of use for Benzaldehyde is 0.2% in non-spray face and neck products, and, in 2001, Benzaldehyde was reported to be used at a maximum concentration of 0.5% in perfumes.

If upon review of the updated use data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

Re-Review - Benzaldehyde - History and New Data

(Preethi Raj – June 2023 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Existing Data	Results	Notes
Benzaldehyde *Changes to Original List* *none*	IJT 25(S1):11-28, 2006	safe as used	frequency of use (2023) conc of use (2022)	6 ≤ 0.2%	frequency of use (2001) conc of use (2001)	7 ≤ 0.5%	Frequency of use has remained constant Concentrations of use have not significantly changed No new use categories of interest

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Non-Cosmetic			
EFSA Contact Materials, Enzymes, Flavorings, and Processing Aids (CEF). Scientific opinion on flavoring group evaluation 20, revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. EFSA J 2012; 10(12): 2994. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2012.2994	Flavoring regulation, Europe	According to the default maximized survey-derived daily intake approach, 41 flavoring substances allocated to structural class I (including Benzaldehyde) have intakes in Europe from 0.001 - 610 µg/capita/d, which are below the threshold of concern value for structural class I (1800 µg/person/d).	ADI is not in the original report
Adams et al. The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients. Food Chem Toxicol 2005; 43: 1207 – 1240.	Flavoring/food additive designation	Benzaldehyde, a benzyl derivative, has FEMA GRAS status as a flavoring agent.	FEMA GRAS status not in the original report
Api et al. RIFM fragrance ingredient safety assessment, benzaldehyde, CAS Registry Number 100-52-7. Food Chem Toxicol 2019; 134: 110878. https://fragrancematerialsafetyresource.elsevier.com/sites/default/files/100-52-7_0.pdf https://ifrafragrance.org/standards/IFRA_STD_007.pdf	limits in finished products based on assessment as a fragrance ingredient	IFRA maximum acceptable concentrations in finished products for Benzaldehyde are: Products applied to the lips: 0.045% Products applied to the axillae: 0.014% Products applied to the face/body using fingertips: 0.27% Products related to fine fragrances: 0.25% Body lotion products applied to the face and body using the hands (palms), primarily leave-on: 0.064% Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on: 0.064% Hand cream products applied to the face and body using the hands (palms), primarily leave-on: 0.064% Baby cream, oil, talc : 0.021% Products with oral and lip exposure: 0.15% Products applied to the hair with some hand contact: 0.52% Products with significant ano-genital exposure (tampon): 0.021% Products with body and hand exposure, primarily rinse-off (bar soap): 0.49% Household care products with mostly hand contact (hand dishwashing detergent): 0.49% Aerosol air freshener 1.8 %	Accepted concentrations in personal care products are not in the original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
		Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad): 0.021% Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin: 100%	
IFRA Standard for Benzaldehyde: 49th Amendment https://ifrafragrance.org/standards/IFRA_STD_007.pdf	Benzaldehyde content (%) as a component of natural complex substances	Benzaldehyde content in natural complex substances ranges from 0.01% in Tolu balsam gum to 99% in bitter almond oil and cherry bark wild extract.	Content in natural complex substances is not in the original report
SCCS Opinion on fragrance allergens in cosmetic products (June 2012 meeting) *https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf*	Contact allergen status	Benzaldehyde is identified in Annex I as being a mild contact allergen (+, to indicate up to 10 positive test reactions)	study in original report showed that it did not cause contact allergy
Toxicity Studies			
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Acute toxicity, oral	OECD TG 471. Male Wistar rats (10/group) were administered 0.8, 1.0, 1.1, 1.2, 1.3, 1.5, or 1.8 ml/kg undiluted Benzaldehyde, via gavage. LD_{50} was determined to be approximately 1430 mg/kg bw	no
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Acute toxicity, oral	Similar to OECD TG 401. Male Wistar rats (10/group) were administered 1.5, 2, 2.2, 2.3, 2,5, or 3.1 ml/kg undiluted Benzaldehyde, via gavage. LD_{50} was determined to be 2170 mg/kg	no
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Acute toxicity, inhalation	OECD TG 436. Male and female Wistar rats (3/sex/group in a limit test) Animals were exposed for 4 h, nose-only, to an aerosol of Benzaldehyde at 1 mg/l or 5 mg/l. All females and 1 male were found dead within 2 or 24 h after exposure to the 5 mg/l Benzaldehyde; no mortality occurred at 1 mg/l. Flat hunched posture, ventro-lateral recumbency, labored/ shallow respiration, rales, piloerection, ptosis and/or chromodacryorrhoea were observed in the animals exposed to 5 mg/l Benzaldehyde. Mean body weight gain was within the expected range for both sexes. Isolated dark red foci were noted in the lungs of 2 females found dead after being exposed to the higher dose. One male that was killed from the 5 mg/l group showed small intestines distended with gas. MMAD at 5 mg/l exceeded the recommended range of 1 – 4 µm, possibly due to agglomeration of the aerosol particles; at this concentration, the LC_{50} was considered to be within the range of 1 – 5 mg/l under the conditions of this study.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Acute toxicity, inhalation	Male Fischer rats were pre-treated with a vapor of 15 ppm formaldehyde 6/h/d for 9 d, and on day 10 were challenged with the test article. Groups of 4 animals were exposed, head only, to varying concentrations of Benzaldehyde, with a maximal ambient vapor concentration of approximately 1600 ppm, for 10 min. Control animals were treated identically without formaldehyde pre-treatment. At the 1600 ppm concentration, the decrease in respiratory rate was similar in both pre-treated and control animals (33%); the RD_{50} was determined to be > 1600 ppm.	no; head-only exposure, lower decrease in respiratory rate than described in the original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Developmental and reproductive toxicity, oral	OECD TG 414. Prenatal developmental toxicity study. Groups of female Wistar Han rats (22/group) received 0, 100, 300, or 600 mg/kg bw/d Benzaldehyde, in 0.5% methylcellulose with 0.5% Tween-80 in purified water, via gavage, from day 6 to day 20 of gestation. Dams were sacrificed on day 21 of gestation; ovaries, uteri, and thyroid glands were extracted for gross and histopathological examination, along with blood samples. Pups were examined for skeletal, soft, and head tissue, and anogenital distance. No treatment-related deaths or changes in bodyweight were observed. Mean adjusted body weight on day 21 of gestation and mean adjusted body weight gain in the 300 or 600 mg/kg groups were statistically significantly higher than controls. There was no clear effect of treatment on gravid uterine weight in the 100 or 300 mg/kg bw/d groups. Mean gravid uterine weight was slightly lower than controls in the 600 mg/kg group, which was not statistically significant. Statistically significantly low mean serum triiodothyronine and thyroxine concentrations were observed at 600 mg/kg bw/day when compared to control animals (reduction of 24% and 26%, respectively). These differences were not considered to be adverse in the absence of any microscopic changes of the thyroid and with no effect of treatment on thyroid stimulating hormone concentration. Pups from the 600 mg/kg bw group were observed to have major eye and skeletal abnormalities, compared to controls, which were considered treatment-related. The NOAEL values for maternal and fetal toxicity were determined to be 600 mg/kg bw/d and 300 mg/kg bw/d, respectively.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Genotoxicity Studies			
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Genotoxicity, in vitro	OECD TG 487. In vitro mammalian cell micronucleus test. Human peripheral blood lymphocytes were treated with 0, 100, 200, 400, 500, 600, 650, 700, 750, 800, 850, 950, or 1062 µg/ml Benzaldehyde, in DMSO, with metabolic activation (for 3 h) or without metabolic activation (for 24 h). Cyclophosphamide, mitomycin C, and vinblastine were used as positive controls. The test material did not induce biologically relevant increases in the frequency of micronuclei.	no; human cell line not used in original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Genotoxicity, in vitro	OECD TG 490. In vitro mammalian cell gene mutation test, using thymidine kinase gene. Mouse lymphoma L5178Y cells were tested with 0, 100, 200, 400, 500, 600, 700, and 800 µg/ml. Benzaldehyde, in DMSO, the presence and absence of metabolic activation for 3 h. The test material did not induce mutation at the *tk* locus of mouse lymphoma L5178Y cells, under these study conditions.	no; positive signal seen for study in original report
Demir et al. Assessment of genotoxic effects of benzyl derivatives by the comet assay. Food Chem Toxicol 2010; 48: 1239 - 1242	Genotoxicity, in vitro	The potential for 4 benzyl derivatives used as flavoring agents (Benzaldehyde, benzyl alcohol, benzyl acetate, and benzoic acid) to cause single strand DNA breaks was evaluated in an vitro comet assay. Benzaldehyde was dissolved in distilled water (solvent control) to the following concentrations 1, 5, 10, 25, or 50 mM. 5mM ethyl methane sulfonate was used as a positive control. A significant increase in the % tail DNA was observed for the 10, 25, and 50 mM concentrations of benzaldehyde, while a significant increase in the tail moment was observed at the 10 and 25 mM Benzaldehyde concentrations.	no
Dermal Irritation and Sensitization			
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Dermal sensitization, animal	OECD TG 406. Guinea pig maximization test. Groups of 10 guinea pigs received a single, intradermal injection of 2.7% Benzaldehyde, in paraffin oil. Data on the time between the induction and the challenge applications was not available. Three occlusive, 24-h, challenge applications of 0.24%, 0.64%, and 2.4% Benzaldehyde, in petroleum, were made. Test sites were evaluated 24 and 48 h after application. Isoeugenol was used as the positive control; positive controls gave an expected response. No reactions were observed for any dose at each observation timepoint; the test substance was considered non-sensitizing.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/registration-dossier/-/registered-dossier/15940	Dermal sensitization, animal	OECD TG 406. Guinea pig maximization test. Similar to test procedure described above; 2.7% Benzaldehyde, in paraffin oil, was used for the intradermal injection, and three occlusive, 48-h challenge applications of 2.1%, 2.1%, and 0.64% Benzaldehyde, in petroleum, were made. Data on the time between the induction and the challenge applications was not available. Test sites were evaluated 24 and 48 h after application. Isoeugenol was used as the positive control; positive controls gave an expected response. One animal from the first 2.1% group, and 3 animals from the second 2.1% group had reactions. The test substance was considered non-sensitizing.	no

ADI – accepted daily intake; DMSO – dimethyl sulfoxide; DNA – deoxyribonucleic acid; FEMA – Flavor Extract Manufacturers Association; GRAS – generally recognized as safe; IFRA – International Fragrance Research Association; LD_{50} – lethal dose; MMAD – mean mass aerodynamic diameter; NOAEL – no-observed-adverse-effect-level; OECD – Organisation for Economic Cooperation and Development; RD_{50} – sensory irritation threshold; RIFM – Research Institute for Fragrance Materials; TG – test guideline

Search (from 2001 on)
PubMed
(benzaldehyde) OR (100-52-7) AND (2001:2023[pdat]) – 7588 hits/ 3 useful

((((((((((benzaldehyde) OR (100-52-7) AND (2001:2023[pdat])) OR (artificial almond oil)) OR (benzaldehyde (HCPA))) OR (benzaldehyde (RIFM))) OR (benzenecarbonal)) OR (benzenecarboxaldehyde)) OR (benzoic aldehyde)) OR (phenylformaldehyde)) OR (phenylmethanol aldehyde)) OR (OriStar BZAH) – 8343 hits/5 useful

((((((((((benzaldehyde) OR (100-52-7) AND (2001:2023[pdat])) OR (artificial almond oil)) OR (benzaldehyde (HCPA))) OR (benzaldehyde (RIFM))) OR (benzenecarbonal)) OR (benzenecarboxaldehyde)) OR (benzoic aldehyde)) OR (phenylformaldehyde)) OR (phenylmethanol aldehyde)) OR (OriStar BZAH)) AND (toxicity) – 596 hits/ 5 useful

Table 1. Frequency (2023/2001) and concentration (2022/2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[1]	**2001[2]**	**2022[3]**	**2001[2]**
Totals*	**6**	**7**	**0.000001 - 0.2**	**0.0001-0.5**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*3*	*1*	*0.000001 - 0.2*	*0.0002 - 0.5*
Rinse-Off	*3*	*4*	*0.00033 - 0.068*	*0.0001 - 0.003*
Diluted for (Bath) Use	*NR*	*2*	*0.015 - 0.05*	*NR*
Exposure Type				
Eye Area	1	NR	NR	NR
Incidental Ingestion	NR	NR	0.04	NR
Incidental Inhalation-Spray	1; 1[a]	NR	0.00008 - 0.1; 0.000003 - 0.0055[b]	0.5; 0.0002[b]
Incidental Inhalation-Powder	1[a]	NR	0.0003 - 0.2[c]	NR
Dermal Contact	6	5	0.000001 - 0.2	0.0001 - 0.5
Deodorant (underarm)	NR	NR	spray: 0.00008; not spray: 0.0002	0.001[b]
Hair-Non-Coloring	NR	2	0.00008 - 0.012	0.0006 - 0.003
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	2	0.0016 – 0.068	0.0001 - 0.003
Baby Products	NR	NR	NR	NR
as reported by product category				
Bath Preparations (diluted for use)				
Bath Oils, Tablets, and Salts	NR	2	NR	NR
Bubble Baths	NR	NR	0.015	NR
Other Bath Preparations	NR	NR	0.05	NR
Eye Makeup Preparations				
Eye Lotion	1	NR	NR	NR
Fragrance Preparations				
Perfumes	NR	NR	NR	0.5
Other Fragrance Preparation	1	NR	NR	NR
Hair Preparations (non-coloring)				
Hair Conditioner	NR	NR	0.00033 - 0.012	0.0006
Hair Spray (aerosol fixatives)	NR	NR	0.00008 - 0.00042	NR
Shampoos (non-coloring)	NR	1	0.001 - 0.01	0.003
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	0.005 - 0.0055	NR
Other Hair Preparations	NR	1	NR	NR
Makeup Preparations				
Lipstick	NR	NR	0.04	NR
Personal Cleanliness Products				
Bath Soaps and Detergents	NR	NR	0.0016 - 0.068	0.0001
Deodorants (underarm)	NR	NR	spray: 0.00008 not spray: 0.0002	0.001
Other Personal Cleanliness Products	NR	NR	0.02	0.003
Shaving Preparations				
Aftershave Lotion	NR	NR	0.006	NR
Other Shaving Preparations	1	NR	0.0091	NR
Skin Care Preparations				
Cleansing	NR	1	NR	NR
Face and Neck (exc shave)	1	NR	not spray: 0.0003 - 0.2	NR
Body and Hand (exc shave)	NR	NR	not spray: 0.025	NR
Foot Powders and Sprays	NR	NR	spray: 0.1	NR
Night	NR	NR	NR	0.0002
Paste Masks (mud packs)	2	2	NR	NR
Other Skin Care Preparations	NR	NR	0.075	0.004
Suntan Preparations				
Suntan Gels, Creams, and Liquids	NR	NR	not spray: < 0.000001	NR
Indoor Tanning Preparations	NR	NR	0.000003	NR

NR – not reported

*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.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] 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

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

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

REFERENCES

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023.)

2. Andersen A. Final report on the safety assessment of benzaldehyde. *Int J Toxicol.* 2006;25 Suppl 1:11-27.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Benzaldehyde. (Unpublished data submitted by the Personal Care Products Council on July 7, 2022.)

International Journal of Toxicology, 25(Suppl. 1):11–27, 2006

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Final Report on the Safety Assessment of Benzaldehyde[1]

Benzaldehyde is an aromatic aldehyde used in cosmetics as a denaturant, a flavoring agent, and as a fragrance. Currently used in only seven cosmetic products, its highest reported concentration of use was 0.5% in perfumes. Benzaldehyde is a generally regarded as safe (GRAS) food additive in the United States and is accepted as a flavoring substance in the European Union. Because Benzaldehyde rapidly metabolizes to Benzoic Acid in the skin, the available dermal irritation and sensitization data demonstrating no adverse reactions to Benzoic Acid were considered supportive of the safety of Benzaldehyde. Benzaldehyde is absorbed through skin and by the lungs, distributes to all well-perfused organs, but does not accumulate in any specific tissue type. After being metabolized to benzoic acid, conjugates are formed with glycine or glucuronic acid, and excreted in the urine. Little acute toxicity was seen. The oral LD_{50} of Benzaldehyde in rats and mice ranged from 800 to 2850 mg/kg. The intraperitoneal LD_{50} in white rats was 3265 mg/kg. In short-term oral studies, the no observed adverse effect level (NOAEL) was 400 mg/kg in rats and mice. In subchronic oral studies, the NOAEL was 400 mg/kg in rats and 600 mg/kg in mice. In a 16-week feeding study, rats given up to 10,000 ppm showed no signs of toxicity. Repeated inhalation of volatilized Benzaldehyde produced ocular and nasal irritation at 500 ppm and death in rabbits at 750 ppm. Undiluted Benzaldehyde was irritating to rabbit eyes, causing edema, erythema, and pain. Benzaldehyde was determined not to be a contact sensitizer, but did produce allergic reactions in a maximization test. Clinical reports of allergy to Benzaldehyde are rare. Benzoic Acid did not produce irritation or sensitization reactions in human clinical studies. Benzoic Acid also failed to produce reactions in phototoxicity and photosensitization tests. Neither Benzaldehyde, Benzoic Acid, nor Sodium Benzoate are reproductive or developmental toxicants at doses that are nontoxic to the mother. In a behavioral study, blood levels of 0.12 ng/ml Benzaldehyde produced a 44% reduction in motor activity in mice. Benzaldehyde did not produce mutations in bacterial assays, but did produce chromosomal abnormalities in Chinese hamster cells and increased mutations in a mouse lymphoma forward mutation assay. Benzaldehyde was evaluated by the National Toxicology Program, which found no evidence of carcinogenicity in rats, and some evidence of carcinogenicity in mice. Several studies have suggested that Benzaldehyde can have carcinostatic or antitumor properties. Overall, at the concentrations used in cosmetics, Benzaldehyde was not considered a carcinogenic risk to humans. Although there are limited irritation and sensitization data available for Benzaldehyde, the available dermal irritation and sensitization data and ultraviolet (UV) absorption and phototoxicity data demonstrating no adverse reactions to Benzoic Acid support the safety of Benzaldehyde as currently used in cosmetic products.

INTRODUCTION

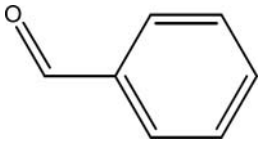
This safety evaluation compiles relevant data on the safety of Benzaldehyde (CAS no. 100-52-7) in cosmetic products.

Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate have structures similar to Benzaldehyde. In addition, Benzoic Acid is the primary product of Benzaldehyde metabolism (Bray et al. 1951). The Cosmetic Ingredient Review (CIR) Expert Panel issued a Final Report on the Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. In that report the Panel concluded that "Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate are safe for use in cosmetic formulations at concentrations up to 5%." They also concluded that "the available data are insufficient to support the safety of these ingredients in cosmetic products in which a primary route of exposure is inhalation. Benzyl Alcohol is safe for use in hair dyes at concentrations up to 10%" (Andersen 2001). Data on Benzoic Acid are included in this assessment and are considered relevant to the safety assessment of Benzaldehyde.

CHEMISTRY

Definition and Structure

Benzaldehyde is an aromatic aldehyde that conforms to the formula shown below (Pepe et al. 2002):



Synonyms include Artificial Almond Oil, Benzenecarbonal, Benzoic Aldehyde, Benzenecarboxaldehyde, Benzenemethylal, Benzene Carboxaldehyde, Bitter Almond Oil (synthetic), Phenylformaldehyde, and Phenylmethanol Aldehyde (Research Institute for Fragrance Materials [RIFM] 2001; Pepe et al. 2002).

Physical and Chemical Properties

Benzaldehyde is a clear colorless to slightly yellowish oily liquid. It has an odor of bitter almonds and a burning aromatic

Recieved 6 December 2005; accepted 2 March 2006.
[1]Reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

TABLE 1
Chemical and physical properties of Benzaldehyde

Property	Benzaldehyde	Reference
Molecular weight	106.13	NRC 1981
Relative density at 15/4°C	1.0415 (water = 1)	NRC 1981
Specific gravity at 25°C	1.041–1.046	Clark 1995
Refractive index at 20°C	1.544–1.547	Clark 1995
Melting point	−26°C	NRC 1981
Boiling point	179°C	Clark 1995
Flash point	145°F	NRC 1981
Vapor pressure at 26°C	1 mm Hg	NRC 1981
Vapor density	3.66 (air = 1)	NRC 1981
log $K^{a}_{o/w}$ (experimental)	1.48	İnel and İşeri 1997
log $K^{a}_{o/w}$ (predicted)	2.2	İnel and İşeri 1997

[1] $K_{o/w}$ is the octanol-water partition coefficient.

taste (Macht 1922). It is only slightly soluble in water, 0.3% at 20°C, but easily soluble in most organic solvents and oils (Clark 1995). Benzaldehyde readily oxidizes to Benzoic Acid when exposed to air (Bray et al. 1951; National Research Council [NRC] 1981; Clark 1995). The measurable chemical and physical properties of Benzaldehyde are summarized in Table 1.

Method of Manufacture

Benzaldehyde can be extracted from bitter almond oil (Macht 1922) or produced from amygdalin, a cyanogenic glycoside in fruit kernels (Krings and Berger 1998). The latter method produces equimolar amounts of hydrocyanic acid. Krings and Berger (1998) also reported that a cost-effective alternative method involves microbial degradation of L-phenylalanine to produce Benzaldehyde without toxic coproducts.

According to Clark (1995), from about 1900 to 1970, the method most employed commercially was to chlorinate toluene into benzal chloride which was transformed into benzal glycol. Benzal glycol was then treated with caustic soda or milk of lime or with water under pressure, and Benzaldehyde was formed. This method has not been used since 1993, because it also produced various chlorinated organic compounds, and the intermediate benzal chloride was determined to be a carcinogen. This author indicated that the most common process for producing synthetic Benzaldehyde is the direct oxidation of toluene with a cobalt catalyst. This method produces Benzaldehyde as well as Benzoic Acid.

Analytical Methods

The purity of Benzaldehyde has been determined by elemental analysis, Karl Fischer water analysis, gas chromatography, reaction of the carbonyl group with hydroxylammonium chloride in the presence of 2-dimethylaminoethanol and back-titration with perchloric acid of the hydroxylamine, and titration with sodium hydroxide to determine free Benzoic Acid content. The identity of Benzaldehyde can also be confirmed by infrared spectroscopy (National Toxicology Program [NTP] 1990). High-performance liquid chromatography has also been used to detect Benzaldehyde (Tan et al. 1991). High-resolution capillary gas chromatography–mass spectrometry (GC–MS) has been used to measure Benzaldehyde in municipal wastewater (Nguyen et al. 1994).

Impurities

Benzaldehyde readily oxidizes to Benzoic Acid with exposure to air (Bray et al. 1951). The purities of Benzaldehyde (United States Pharmacopeia [USP]-grade) samples were analyzed by gas chromatography and found to contain between 0.38% and 0.29% Benzoic Acid. No deterioration of Benzaldehyde was observed in studies lasting up to 2 years (NTP 1990).

USE

Cosmetic

According to the *International Cosmetic Ingredient Dictionary and Handbook*, Benzaldehyde is used in cosmetic products as a denaturant, a flavoring agent, and a fragrance (Pepe et al. 2002). Table 2 lists the frequency and concentrations of use of Benzaldehyde in cosmetic formulations as a function of product type as reported to the FDA (2001) and CTFA (2001). The highest concentration of Benzaldehyde use reported was 0.5% in perfumes. The low-level uses are consistent with the use of Benzaldehyde as a denaturant, flavor or fragrance.

Benzaldehyde is not included on the list of ingredients that must not be combined in cosmetic products that are marketed in Japan (Ministry of Health, Labor and Welfare [MHLW] 2001a) or on the list of restricted ingredients for cosmetic products that are marketed in Japan (MHLW 2001b).

Noncosmetic

According to Krings and Berger (1998), next to vanillin, Benzaldehyde is considered the second most important food flavoring agent. It is a key ingredient in natural fruit flavors.

Benzaldehyde is generally recognized as safe (GRAS) as a food additive in the United States in the Code of Federal Regulations (CFR) at 21CFR182.60.

Benzaldehyde appears on a list of legally accepted flavoring substances in Europe (European Commission 1999). Benzaldehyde is also approved by the U.S. Bureau of Alcohol, Tobacco, and Firearms for use as a denaturant in specially denatured alcohol at 27CFR21.151.

Because Benzaldehyde seems to have carcinostatic properties, it is used clinically in some cancer therapies (Takeuchi et al. 1978; Kochi et al. 1980).

TABLE 2
Frequency of use of Benzaldehyde in cosmetic formulations

Product category (total number of products in the category) (FDA 2001)	Current frequency of use (FDA 2001)	Current concentration of use (CTFA 2001)	Historical frequency of use (FDA 1984)	Historical concentration of use (FDA 1984)
Bath preparations				
Oils, tablets, and salts (140)	2	—	1	0.1–1%
Soaps and detergents (405)	—	0.0001%	—	—
Noncoloring hair preparations				
Hair conditioners (630)	—	0.0006%	—	—
Shampoos—noncoloring (851)	1	0.003%	—	—
Other hair preparations (276)	1	—	—	—
Cleansers (733)	1	—	—	—
Fragrance preparations				
Colognes and toilet waters (683)	—	—	1	≤0.1%
Perfumes (227)	—	0.5%	—	—
Other (173)	—	—	1	0.1–1%
Personal hygiene products				
Underarm deodorants (247)	—	0.001%	—	—
Other (307)	—	0.003%	2	≤1%
Skin care preparations				
Skin cleansers (733)	1	—	1	≤0.17%
Skin creams, lotions, etc. (1131)	—	—	2	0.1–1%
Night skin care (200)	—	0.0002%	—	—
Paste masks/mud packs (269)	2	—	2	≤0.17%
Other (715)	—	0.004%	1	5–10%
Total Benzaldehyde use in cosmetic products	7		10	

BIOLOGICAL PROPERTIES

Occurence in Nature

According to Macht (1922), Benzaldehyde occurs in a number of plants, especially in the family *Rosaceae* and in particular in the genus *Prunus*. These species include cherries, prunes, and peaches. It is usually found in the pit or seed and in the deep fruit meat around the pit. Benzaldehyde is also found in bitter almond, and the oil of bitter almonds is an important natural source of Benzaldehyde. Schade et al. (2001) noted that Benzaldehyde is one of the volatile compounds contributing to the fragrance of carnation flowers.

Feron et al. (1991) stated that Benzaldehyde naturally occurs in alcoholic beverages (0.01 to 0.8 ppm), dairy products, meat (0.03 to 0.13 ppm), poultry, fruits (0.0003 to 8.9 ppm), vegetables (up to 1.2 ppm), coffee, tea, cocoa (0.7 to 7.4 ppm), and spices.

Benzaldehyde is used by various species of insects for chemical defense and as pheromones (Opdyke 1976; Blum et al. 1969), and it is one of several antibacterial compounds found in the fruit body of some mushrooms (Beltran-Garcia et al. 1997).

Researchers at Rutgers University (Zhang et al. 1994) conducted a study to determine ambient aldehyde concentrations in and around suburban homes. Indoor and outdoor air samples were collected from 36 houses of a suburban area in Central New Jersey during the summer months of 1992. The air samples were analyzed for aldehyde content by HPLC. Of the 36 homes sampled, 14 had detectable Benzaldehyde indoors at a mean concentration 0.25 ppb, and 22 had detectable Benzaldehyde outdoors at a mean concentration of 0.38 ppb.

Antimicrobial Activity

DeGreef and Sumere (1966) reported that Benzaldehyde limited the growth of *Saccharomyces cerevisae*. Details of exposure were not reported.

A series of experiments by Bowles and Juneja (1998) demonstrate that Benzaldehyde has antimicrobial properties. Several species of common foodborne bacteria (*Yersinia enterocolitica*, *Listeria mono-cytogenes*, *Salmonella typhimurium*, *Bacillus cereus*, *Shigella flexneri*, *Aeromonas hydrophila*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Clostridium botulinum*) were preincubated with medium containing various concentrations of Benzaldehyde (0.15 to 2500 mM) for 24 h. Then the medium was changed and the bacteria were incubated for another 24 h at 37°C. Vehicle-control groups received similar

treatment without Benzaldehyde exposure. After the incubation period, the cultures were examined for turbidity.

Growth of *B. cereus* and *S. flexneri* were inhibited by as little as 1.5 mM Benzaldehyde. *L. monocytogenes* and *S. typhimurium* cultures were reduced by Benzaldehyde concentrations of 3.13 mM and higher. Exposure to a minimum of 6.25 mM Benzaldehyde impaired the growth of *Y. enterocolitica*, *A. hydrophila*, and *E. coli* O157:H7. *S. aureus* and *C. botulinum* required Benzaldehyde concentrations of 7.81 and 125 mM, respectively, to inhibit growth. A 30-min exposure to 57°C during the preincubation period reduced the minimum concentrations required to inhibit bacterial growth for most species, except *S. typhimurium*.

C. botulinum spores were aerobically exposed to 0, 0.3125, 0.625, 1.25, or 2.5 mM Benzaldehyde for 30 min at 25°C and then subjected to 0, 1, 3, or 5 KGy of radiation from a cesium-137 gamma source. Spore population densities were determined after a 24-h incubation at 37°C. Irradiation and Benzaldehyde each dose-dependently reduced bacterial growth. The combination of Benzaldehyde and irradiation appeared to have additive and/or synergistic inhibitory effects on *C. botulinum* growth (Bowles and Juneja 1998).

Cellular and Molecular Effects

Effects on Muscle Tissues

Macht (1922) reported that Benzaldehyde has been shown to induce a relaxation or antispasmodic effect in smooth muscle at concentrations as low as 0.2% in saline. This was demonstrated in isolated smooth muscle preparations from the uterus, stomach, urinary bladder, gall bladder, ureters, vas deferens, seminal vesicles, bronchi, and arteries from various animal species.

In a study of the heart muscle, James and Bear (1968) anesthetized dogs with sodium pentobarbital (30 mg/kg intravenous [IV]) and their trachea were intubated for mechanical ventilation with room air. Each dog's chest was opened to expose the heart. Electrodes were placed directly on the heart to record electrical activity. A cannula was introduced into the sinus node nutrient artery. The cannula apparently had no affect on the electrical function of the sinus node. Perfusion of the sinus nodes of four dogs with Benzaldehyde at concentrations of 0.001 μg/ml to 10 mg/ml had no significant chronotropic effect and produced no effect on the Q-T interval. Other aldehydes such as formaldehyde, acetaldehyde, and glutaraldehyde had varying effects on measurable heart functions.

Effects on Energy Metabolism

Giudicelli et al. (1973) evaluated several aldehydes for their ability to induce lipolysis and glucose metabolism in rat adipose tissue. Epididymal fat pads were obtained from albino Wistar rats. Fat tissue was placed into flasks (200 to 300 mg fat per flask) and incubated in a nutrient medium at 37°C for 3 h with gentle shaking. In five flasks, nutrient medium contained 11 mM Benzaldehyde, and another five flasks contained medium without Benzaldehyde. Lipolysis was measured by the rate of glycerol and free fatty acid (FFA) output in the medium. Glucose uptake was determined by disappearance of glucose from the medium. The presence of Benzaldehyde caused significant ($p < 0.01$) increases in glycerol, FFA, and lactate output, compared to control. Glucose uptake and pyruvate output were significantly reduced ($p < 0.01$) in the Benzaldehyde treatment. Other aldehydes had similar effects.

Petterson et al. (1980) screened 320 compounds that are found in tobacco or tobacco smoke for their ability to inhibit noradrenalin-induced oxidative metabolism in isolated brown fat cells. The fat cells were collected from adult hamsters. The rates of oxygen consumption in the cells were measured using a Clark-type oxygen electrode fitted in a 1-ml Persplex vessel. The output of the electrode was amplified and continuously recorded during the experiments.

A suspension of 10^5 cells/ml was incubated in 1 mM of each test compound dissolved in ethanol or dimethyl sulfoxide for 5 min while oxygen consumption was measured. After this 5 min of preincubation, the cells were exposed to 1 μM noradrenalin, and oxygen consumption continued to be measured for another 5 min. Vehicle-control cells were preincubated with dimethyl sulfoxide or ethanol and stimulated with noradrenalin, as described above.

The typical oxygen consumption profile in vehicle control cells showed a baseline oxygen consumption rate followed by a sharp increase in oxygen consumption immediately after exposure to noradrenaline. The effects of the screened compounds were based on oxygen consumption rates in cells preincubated with test compounds compared to that of vehicle control cells.

Preincubation of the fat cells with 1 mM Benzaldehyde caused a 47% inhibition in noradrenalin-induced oxygen consumption, compared to vehicle control. Preincubation with Benzoic Acid and Benzyl Alcohol caused a 60% and 32% inhibition in noradrenalin-induced oxygen consumption, respectively (Petterson et al. 1980).

Benzaldehyde at 300 μM inhibited pyruvate/malate-mediate respiration and at 2 to 20 mM inhibited succinate dehydrogenase activity in rat liver mitochondria (Wolf et al. 1982). Benzaldehyde has also been shown to dose-dependently deactivate the enzyme pyruvate decarboxylase in vitro at doses of 100 to 300 mM (Chow et al. 1995) and alcohol dehydrogenase at 10 mM (Bowen et al. 1986).

Effects on Detoxifying Enzymes

Furman et al. (1998) reported that toluene and its metabolites (including Benzaldehyde) were shown to inhibit mixed-function oxidase enzymes in Sprague-Dawley rat lung and liver. Benzaldehyde inhibited lung and liver cytochrome P450 2B (CYP2B) with mean IC_{50} ($\pm$ *SEM*) values of 1.28 ($\pm$ 0.05) and 2.12 ($\pm$ 0.35) mM, respectively. CYP1A1 isozymes in the lung and liver were also inhibited by Benzaldehyde, with IC_{50} ($\pm$*SEM*) values of 3.00 ($\pm$ 0.09) and 7.30 ($\pm$ 0.45) mM, respectively. Pulmonary arylhydrocarbon hydroxylase was inhibited

with an IC_{50} (± *SEM*) of 2.28 (± 0.19) mM. Benzaldehyde was less potent than toluene and more potent than Benzyl Alcohol in these enzyme inhibitory effects.

Tabatabaie and Floyd (1986) showed that Benzaldehyde inactivates the antioxidant enzyme glutathione peroxidase ($K_i \approx 15\ \mu M$) but had no effect on other antioxidant enzymes tested, catalase, superoxide dismutase, and glutathione reductase. The enzymes studied appear to be from the rat, however this was not explicitely clear. The inhibitory effect on glutathione peroxidase was specific to Benzaldehyde, as other structurally related and unrelated aldehydes did not affect this enzyme. The authors proposed this enzyme effect to be the cause of reported neurotoxicity associated with chronic Benzaldehyde and toluene exposure.

According to Mattia et al. (1991), toluene has been shown to increase lipid peroxidation and reactive oxygen species (ROS) formation in vitro and in vivo. However, this effect is blocked in vivo in the presence of mixed-function oxidase inhibitors, thus it was deduced that a metabolite of toluene must be responsible for the ROS formation and lipid peroxidation associated with toluene. Benzyl Alcohol, Benzoic Acid, and Benzaldehyde were evaluated in an in vitro system for their ability to catalyze reactive oxygen formation. Although Benzoic Acid and Benzyl Alcohol were found to quench free radicals, Benzaldehyde exhibited induction of ROS formation. Thus, Benzaldehyde was proposed as the agent responsible for toluene-induced lipid peroxidation and ROS generation, which has in turn been associated with the neurotoxic effects of toluene and Benzaldehyde.

Effects on Gastric Enzymes

Benzaldehyde (0.1%) inhibited pepsin activity by 20% in an in vitro experiment using artificial gastric fluids and by 87% in an in vivo study of ulcer patients and normal people. The author proposed that Benzaldehyde is probably the compound that makes dried sweet almonds an effective antiacid and pepsin inhibitor (Kleeberg 1959).

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

Absorption

Benzaldehyde liquid absorbs through *in vitro* preparations of human cadaver skin (~0.4 mm thick) at a rate of 1970 ± 720 $\mu g/cm^2/h$ as a pure liquid and at a rate of 450 ± 70 $\mu g/cm^2/h$ as a saturated aqueous solution (Barry et al. 1985).

Distribution

The kinetics of inhaled [^{11}C]Benzaldehyde in rats were studied by Kutzman et al. (1980). Female Sprague-Dawley rats, 8 to 10 weeks old, were restrained in a closed inhalation chamber in which only the nose was exposed to the [^{11}C]Benzaldehyde for 2 min. Benzaldehyde was labeled in the carbonyl position using [^{11}C]carbon dioxide. The [^{11}C]Benzaldehyde used in this study had a specific activity of approximately 7.4 Ci/mmol and a decay half-life of 20.4 min.

The end of the 2-min exposure period was deemed the zero time point, and subsequent radioactivity calculations were adjusted to account for the rapid decay rate of [^{11}C]Benzaldehyde. After the 2-min exposure, rats were removed from the inhalation chamber. At 1.5, 5, 12, 20, and 40 min after exposure the rats were killed by cervical dislocation. Blood was collected by decapitation, and major organs were isolated and cleaned of perfused blood to determine the distribution of the radiolabel. Expired breath, urine, and contents of the small intestine were also collected. Radioactivity in the samples were counted in a Packard sodium iodide well counter.

Inhaled [^{11}C]Benzaldehyde was rapidly absorbed by the lungs. At the 1.5-min time point, only 1.2% of the administered dose was present in the respiratory tract. The ratios of radiolabel in the blood to that in well-perfused tissues (visceral organs) were consistent from the 1.5- to 40-min time points. Poorly perfused tissues (skin, muscle, and adipose) had steady increases in accumulation of radiolabel up to 12 min. After peak concentrations of [^{11}C]Benzaldehyde, removal of radiolabel from all tissues was rapid and linear over time.

The biological half-life of Benzaldehyde was about 10 min. No $^{11}CO_2$ or radiolabeled metabolites were detected in expired breath, and very low radiolabel was found in the intestinal contents. [^{11}C]Benzaldehyde was almost exclusively eliminated by the kidneys in the form of hippuric acid, with radiolabel detectable in the urine at the earliest time point, 1.5 min (Kutzman et al. 1980).

Pellizzari et al. (1982) found Benzaldehyde in the breast milk of eight out of 12 lactating women living in urban areas of New Jersey, Louisiana, and Pennsylvania. Milk samples were manually expressed and frozen until analysis by GC-MS. The origin of the Benzaldehyde was not speculated in the report; however, 8 of the 12 breast milk samples had detectible toluene, a metabolic precursor to Benzaldehyde. It was not clear whether Benzaldehyde and toluene were found in milk from the same eight women. Benzoic Acid was not found in any samples.

Metabolism

Bray et al. (1951) reported that Benzaldehyde is metabolized to Benzoic Acid by first-order kinetics at a velocity rate constant of 0.33 h^{-1} in rabbits. Benzoic Acid is then conjugated with glycine (to form hippuric acid) or with glucuronic acid and excreted in the urine.

In a study of esterase and alcohol dehydrogenase activity in skin, Boehnlein et al. (1994) reported that Benzyl Alcohol was metabolized to Benzoic Acid in viable and nonviable hairless guinea pig skin by alcohol dehydrogenase (Benzaldehyde is the presumed intermediate). In viable guinea pig skin, conjugation with glycine to form hippuric acid was also demonstrated.

Excertion/Metabolism

Zlatkis et al. (1973) determined the volatile urinary metabolites of 150 normal healthy volunteers and 40 subjects with diabetes mellitus by GC-MS. Benzaldehyde was among the detectable components in the urine of normal and diabetic subjects.

Laham and Potvin (1987) orally administered 0.4 to 1.0 g/kg Benzaldehyde to Sprague-Dawley rats for 13 consecutive days, and analyzed their urine for benzylmercapturic acid using GC–MS.

Although it was not a major metabolic product of Benzaldehyde, the concentrations of benzylmercapturic acid in the urine did increase at higher Benzaldehyde doses. No data were presented to suggest what percentage of the Benzaldehyde dose was excreted in urine as benzylmercapturic acid (Laham and Potvin 1987).

Laham et al. (1988) conducted a study in which New Zealand white rabbits were divided into three groups (3 rabbits/group) and given a single oral dose of either 0.35 g/kg or 0.75 g/kg Benzaldehyde or water (control). Urine was collected daily for 15 consecutive days and analyzed by gas chromatography, ultraviolet, infrared, and mass spectrometry.

The total urinary output values of Benzaldehyde metabolites in the 0.35 and 0.75 g/kg dose groups were 83.2% and 82.3% of the total dose administered, respectively. Hippuric acid was the most abundant metabolite at 69.9% of the lower dose and 66.7% of the higher dose. Benzoic Acid conjugated to glucuronic acid represented 8.8% and 11.2% of the total low- and high-dose groups, respectively. Benzyl glucuronide made up 2.9% of the low dose and 3.0% of the high dose. Free Benzoic Acid represented 1.6% and 1.4% of the low and high doses, respectively, and benzyl mercapturic acid was found in trace amounts, $<0.01\%$ of the total dose (Laham et al. 1988).

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Jenner et al. (1964) exposed 20 rats (10/sex) and guinea pigs (number unspecified) to various (unspecified) doses of Benzaldehyde by a single gastric intubation. The LD_{50} values (with 95% confidence limits) for rats and guinea pigs were 1300 (1110–1540) and 1000 (800–1250) mg/kg, respectively. The time lapse between dosing and death for rats was 4 to 18 h, and for guinea pigs was 1 h to 4 days. Before dying, rats displayed depression and coma at higher doses, and guinea pigs had diuresis, tremors, intestinal irritation, and hemorrhage.

Taylor et al. (1964) reported an oral LD_{50} of 1300 mg/kg Benzaldehyde in Osborne-Mendel and Sherman strain rats, weighing 180 to 350 g. Toxic signs were depression and comatose, and the time of death was 4 to 18 h after dosing.

Sporn et al. (1967) reported an oral LD_{50} for Benzaldehyde of 2850 mg/kg in white rats, but no details were provided.

Schafer and Bowles (1985) calculated the approximate lethal dose (ALD) for Benzaldehyde in the deer mouse (*Peromyscus maniculatus*) to be 470 mg/kg. According to the authors, the ALD approximates the LD_{50} of a chemical by using fewer animals than the traditional acute toxicity study. Using a single animal per dose level, each succeeding dose was 50% higher than the previous dose level until mortality occurs.

In a study by Eastman Kodak (1991), 10 rats and 10 mice (strains unspecified) were given a single oral dose of 200 to 3200 mg/kg undiluted Benzaldehyde. The LD_{50} for each species was reported to be approximately 800 to 1600 mg/kg. Clinical observations reported for dosed rats include slight to very weak, rough coat, diarrhea, and bloody urine. Time to death after dosing in rats was 1.5 to 4.5 h. In mice the clinical observations included normal to quite weak, ataxia, prostration, rough coat, and sides caved in. Time to death after dosing in mice was 4 h to 2 days.

Dermal

Undiluted Benzaldehyde was applied to the shaved skin of three guinea pigs in quantities of 5.0 to 20.0 cc/kg on a gauze patch. The patch was held on the skin for 24 h. Immediate observations included moderate to gross edema and erythema. Eschars developed with some scarring along the edge of the patch area. Necrosis was seen in the patch area at one week after exposure. Higher dose groups showed reduced weight gain at 2 weeks after exposure (Eastman Kodak 1991).

Inhalation

Swiss-Webster and $B6C3F_1$ mice were exposed to various concentrations of Benzaldehyde by inhalation for 10 min. Sensory irritation was quantified by measuring respiratory rate depression as recorded by a body plethysmograph. The RD_{50}, the concentration eliciting a 50% decrease in respiratory rate, was calculated to be 394 ppm for $B6C3F_1$ mice and 333 ppm for Swiss-Webster mice (Steinhagen and Barrow 1984).

Parenteral

Intravenous injection of Benzaldehyde emulsions (dose not reported) in rabbits induced relaxation of the intestines and urinary bladder and vasodilation of the splanchic vessels within 10 min. A 2.5-kg cat was injected intravenously with 4 cc of a 0.5% Benzaldehyde solution. There was an immediate reduction in blood pressure and respiration rate (Macht 1922).

Sporn et al. (1967) reported that the intraperioneal LD_{50} of Benzaldehyde in white rats was 3265 mg/kg.

Short-Term Toxicity

Oral

Kluwe et al. (1983) administered 7-week-old F344/N rats 12 oral doses of 0, 100, 200, 400, 800, or 1600 mg/kg Benzaldehyde in corn oil over a period of 16 days ($n = 5$/sex/dose level).

Animals were observed twice daily and weighed on days 1, 8, and 16. At study termination, gross necropsies were performed on all animals. All rats in the 1600 mg/kg group died on the 2nd day. Two of each sex in the 800 mg/kg dose group also died.

In the 800 mg/kg dose group male and female body weights were respectively 14% and 11% lower than control animals. Body weights of animals in all lower dose groups were not different from controls. No compound-related clinical signs were observed in the surviving animals. No compound-related lesions were found in any animals that survived to study termination.

These same authors administered 8-week-old B6C3F$_1$ mice 12 oral doses of 0, 200, 400, 800, 1600, or 3200 mg/kg Benzaldehyde in corn oil over a period of 16 days (n = 5/sex/dose level). Animals were observed twice daily and weighed on days 1, 8, and 16. At study termination, gross necropsies were performed on all animals.

All mice that received 1600 or 3200 mg/kg Benzaldehyde died by day 3, and one male receiving 800 mg/kg died on day 10. No compound-related clinical signs were observed in the surviving animals. Body weights of all surviving animals were not different from controls, and no compound-related lesions were found upon gross necropsy (Kluwe et al. 1983).

Inhalation

Laham et al. (1991) administered Benzaldehyde daily by inhalation to Sprague-Dawley rats, 6 hours per day for 14 consecutive days. The exposure levels were 0, 500, 750, and 1000 ppm Benzaldehyde (n = 14 rats/sex/level). Animals were observed for signs of toxicity. Body temperatures were determined by a digital thermometer equipped with a Teflon rectal probe after the 2nd, 7th, and 14th exposure. Body weights were recorded after the 2nd, 8th, and 14th exposure. After the last exposure, the rats were sacrificed and venous blood was collected for biochemical and hematological analyses, gross necropsy was performed, organs were examined and weighed, and tissues were collected for histopathological examination.

Animals in the two higher dose groups exhibited aggressive behavior and impaired central nervous system function displayed as abnormal gait, tremors, seizures, sensitivity to noise, and diuresis. There were significant reductions in weight gain and body temperature in all dosed groups, compared to controls ($p < 0.05$). The severity of nasal and ocular irritation increased with increases in concentration.

Ten females and one male in the 1000 ppm group and one female in the 750 ppm group died in the first week. Two female rats in the 750 ppm group were found moribund in the second week. The most prominent histopathological finding in exposed rats was goblet cell metaplasia of the respiratory epithelium lining the nasal septum seen in males of the 500 and 1000 ppm levels. This was not seen in the females, and no other remarkable histological findings were observed (Laham et al. 1991).

Subchronic Oral Toxicity

Hagan et al. (1967) fed young Osborne-Mendel rats powdered diet containing 0, 1000, or 10,000 ppm Benzaldehyde for 16 weeks (n = 5/sex/dose) or 27 to 28 weeks (n = 5/sex/dose). Fresh diets were made and distributed weekly. At the end of the dosing period, blood was collected for hematological examination, and the rats were killed and exanguinated. Tissues were weighed and examined. Samples of tissues were embedded in paraffin for histopathological evaluation. No pathological effects were seen in the tissues of animals exposed to Benzaldehyde.

Sporn et al. (1967) dosed white rats with 0 or 10 mg Benzaldehyde (n = 16/group) by oral gavage every 2 days for 12 weeks. There were no treatment-related effects observed in body weight gain, liver enzyme activity, liver weight, liver nitrogen or lipid content, weight of adrenal glands, or ascorbic acid content of adrenal glands.

Kluwe et al. (1983) gave 6-week-old F344/N rats daily doses of Benzaldehyde 5 days a week for 13 weeks (n = 10/sex/dose level). Dose levels were 0, 50, 100, 200, 400, and 800 mg/kg Benzaldehyde in corn oil, administered by oral gavage. Animals were observed twice daily and weighed once a week. At the end of 13 weeks, surviving animals were killed and necropsied. Complete histological examinations were performed on animals from the 0, 400, and 800 mg/kg groups.

Six males and three females from the 800 mg/kg group, and one female each from the control and 400 mg/kg groups died prior to study termination. The final mean body weight of male rats in the 800 mg/kg group was 26% lower than control males. All other treatment conditions had mean body weights similar to controls.

Compound related lesions were seen only in the 800 mg/kg dose group and included degeneration of the cerebellum and necrosis of neurons in the hippocampus; hyperplasia and/or hyperkeratosis of the forestomach; degeneration of the liver; and degeneration and necrosis of the tubular epithelium of the kidneys.

These authors also gave 8-week-old B6C3F$_1$ mice daily oral doses of Benzaldehyde 5 days a week for 13 weeks (n = 10/sex/dose level). Dose levels were 0, 75, 150, 300, 600, and 1200 mg/kg Benzaldehyde in corn oil, administered by oral gavage. Animals were observed twice daily and weighed once a week. At the end of 13 weeks, surviving animals were killed and necropsied. Complete histological examinations were performed on animals from the 0, 600, and 1200 mg/kg groups.

Nine of the 10 male mice in the 1200 mg/kg group died within the first week of dosing, and the last male mouse in that group died in the fourth week. One female mouse in the 1200 mg/kg group died in the first week.

The final mean body weight for males in the 600 mg/kg group was 9% lower than controls. All other test groups had final mean body weights similar to controls. The only compound-related lesion observed at necropsy was mild to moderate renal tubular degeneration in all males in the 1200 mg/kg

group and in one male in the 600 mg/kg group (Kluwe et al. 1983).

Chronic Oral Toxicity

Benzaldehyde was studied in a 2-year oral carcinogenicity study by the National Toxicology Program (NTP 1990). This study is included in the section on Carcinogenicity.

Ocular Irritation

In a study reported by Eastman Kodak (1991), one drop of undiluted Benzaldehyde was applied to the conjunctival sac of a rabbit. There was immediate irritation marked by moderate erythema, squealing, and blinking. At 1 h, there was erythema of the conjunctiva, lids, and nictitating membrane, and some edema of the palpebra conjunctiva and nictitating membrane.

At 24 h, the rabbit was in poor condition, appearing anemic and smelling of urine. The iris was injected and the anterior cornea was hazy. The upper orbital and lower palpebra and nictitating membrane were stained. At 48 h there was only slight erythema of the nictitating membrane and conjunctiva. The rabbit died on the sixth day, passing clear mucus in its stool, but the death was not attributed to the Benzaldehyde dosing (Eastman Kodak 1991).

Dermal Sensitization

In a comparison of methods of allergencity evaluation of 32 fragrance materials, Klecak et al. (1977) reported that Benzaldehyde was positive for allergenicity in guinea pigs in the Draize test, the maximization test, and a test with Freund's complete adjuvant. In the Draize test, a dose of 0.05 ml of a 0.1% Benzaldehyde solution was injected intradermally on day 0 and additional 0.1 ml doses on 9 alternate days (total dose = 0.95 mg). Treated and control animals were challenged with 0.05 ml of a 0.1% Benzaldehyde solution on days 35 and 49. The number of animals in the Draize test was not reported.

In the maximization test, guinea pigs were injected intradermally two times each with 0.1 ml of a 5% Benzaldehyde solution, with 0.1 ml of a 5% Benzaldehyde in Freund's complete adjuvant (FCA), or with FCA alone on day 0. On day 8, 250 mg Benzaldehyde in petrolatum was applied to clipped skin on the dorsal neck area and kept occluded for 2 days. On day 21, an occlusive patch with a subirritant concentration (not specified) in petrolatum was applied to the flank for 24 h. Reactions were read 24 to 48 h after removal of the patch. The number of animals tested was not reported.

In the Freund's complete adjuvant test, doses of 0.05 ml of undiluted Benzaldehyde and 0.05 ml of FCA were injected into the necks of guinea pigs on days 0, 2, 4, 7, and 9 (total dose = 250 mg Benzaldehyde). Control animals were treated with FCA alone. On days 21 and 35 animals were challenged with an epicutaneous dose of a subirritant concentration (not specified) in petrolatum for 24 h. Reactions were read 24 to 48 h after removal of the patch. The number of animals tested was not reported.

These authors also reported that Benzaldehyde was not a sensitizer in an open epicutaneous test (OET). In the OET assay, Himalayan white spotted guinea pigs were exposed to 0.1 ml of undiluted or 0.03%, 0.1%, 0.3%, 1%, 3%, 10%, or 30% Benzaldehyde on a 8-cm^2 area of shaved skin on the flank ($n = 6$ to 8 animals per group). The applications were repeated for 21 days. Application sites were left uncovered and were scored 24 h after each treatment. The scoring was all-or-none for signs of irritation. Results were reported as the minimum irritating concentration, which is the concentration producing a positive irritant response in at least 25% of the exposed animals.

The minimum irritating concentration after one dose was 10% Benzaldehyde and after 21 exposures was 3% Benzaldehyde. On day 35 (14 days after the 21st exposure), the animals were challenged with an exposure to 3% (the day 21 minimum irritating concentration) or an unspecified lower concentration Benzaldehyde on a 2-cm^2 area of shaved skin. The challenge application sites were scored at 24, 48, and 72 h. Benzaldehyde did not show signs of sensitization (Klecak et al. 1977).

The Environmental Protection Agency (EPA 1992) evaluated Benzaldehyde as a contact sensitizer in guinea pigs using the Magnusson-Kligman method. Guinea pigs ($n = 10$ per group) were initially exposed either by a 0.1 ml intradermal injection of 3% Benzaldehyde in paraffin oil or a topical application of 15% Benzaldehyde in petrolatum to a 2 × 4 cm patch of skin. The topical application was occluded for 48 h. Later (time duration not specified) the skin was challenged by a topical application of 7% Benzaldehyde in petrolatum on a 2 × 2 cm patch of skin. This dose was occluded for 24 h. The challenge site was then evaluated for erythema.

Benzaldehyde failed to induce erythema in either group and was not a contact sensitizer under the conditions of this test (EPA 1992).

Phototoxicity

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate

Eberlein-König et al. (1993) incubated suspensions of human erythrocytes with Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. Each material was tested at 10^{-5}, 10^{-4}, and 10^{-3} mol/L. Erythrocyte free samples were also incubated with the test materials and used as controls. Following incubation, suspensions and samples were exposed to varying amounts of ultraviolet A (UVA) light. Hemolysis was measured as a function of absorbance of 550-nm light. None of these three substances produced significant photohemolysis.

Because Benzaldehyde is presumed to be an intermediate in the metabolism of Benzyl Alcohol to Benzoic Acid (Bray et al. 1951), these data are considered relevant to the potential phototoxicity of Benzaldehyde.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Benzaldehyde

Sporn et al. (1967) gave rats 0 or 2 mg Benzaldehyde (in 0.1 ml of "oil solution") orally every other day for 32 weeks.

Rats were bred 75 and 180 days after initiation of dosing. The number of gestating females, number of live-born offspring, pup weights at birth and on postnatal days 7 and 21, and pup viability were recorded. The number of gestating females was decreased in the Benzaldehyde treatment, compared to controls. All other parameters were similar between the treatment and control groups.

Abramovici and Rachmuth-Roizman (1983) evaluated the embryotoxic effects of several fragrance aldehydes in young chick embryos. The embryos, white Leghorn × Rhode Island red strain in the third day of development, were injected suprablastodermically with a single dose of the test compound. Benzaldehyde doses administered were 0, 1.25, 12.5, and 25 μM/embryo in an olive oil vehicle ($n \geq 50$ embryos/dose level). The embryos were incubated at 37.5°C until the 12th day of development. Control embryos had a lethality rate of 17.8% and a frequency of malformations of 7.9%. The percentage of abnormal embryos in the low, mid, and high doses were 7.6%, 17.8%, and 36.6%, respectively. The majority of abnormalities were skeletal and limb malformations. Lethality rates for the low, mid, and high doses were 23.1%, 19.6%, and 48.3%, respectively. Statistical analyses of these malformation and lethality rates were not reported.

The Joint FAO/WHO Expert Committee on Food Additives reviewed the reproductive toxicity and teratogenicity of Benzaldehyde and related compounds (benzyl acetate, benzyl alcohol, and benzoic acid and its salts). "Delayed development and reduced fetal and postnatal pup body weights were observed in developmental toxicity studies in rats, mice, hamsters and rabbits, but only at doses that were toxic to the mother" (World Health Organization 1996).

Benzoic Acid and Sodium Benzoate

Because Benzaldehyde and Sodium Benzoate are both metabolized primarily to Benzoic Acid (Bray et al. 1951), data regarding the developmental toxicity of Benzoic Acid and Sodium Benzoate may be considered relevant to Benzaldehyde.

Kimmel et al. (1971) reported no evidence of teratogenicity in rats administered 510 mg/kg of Sodium Benzoate by gavage on gestational days (GDs) 9 to 11.

The Food and Drug Research Labs Inc. (1972) administered Sodium Benzoate at doses of 1.75, 8, 38, and 175 mg/kg by oral intubation to groups of at least 20 pregnant albino CD-1 outbred mice and Wistar albino rats on GDs 6 to 15. Groups of 21 to 22 pregnant hamsters were dosed with 3, 14, 65, or 300 mg Sodium Benzoate/kg on GDs 6 to 10. Groups of 10 Dutch-belted rabbits were artificially inseminated and then dosed by oral intubation with 2.5, 12, 54, or 250 mg Sodium Benzoate/kg on GDs 6 to 18. Dams were individually caged, and received feed and water ad libitum. Positive-control groups for mice, rats, and hamsters received aspirin. A positive-control group of rabbits received 6-aminonicotinamide. Sham groups for each animal type served as negative controls. Caesarean sections were performed on mice, rats, hamsters, and rabbits on days 17, 20, 14, and 29, respectively.

Neither adverse effects in maternal or fetal survival, nor significant increases in fetal abnormalities in either soft or skeletal tissues were noted in any of the animals (Food and Drug Research Labs Inc. 1972).

In a study reported by the Polish Academy of Sciences (1977), Benzoic Acid at doses of 6, 30, 60 and 600 mg/kg was administered by stomach tube to groups of 21 to 24 pregnant golden hamsters on GDs 6 to 10. Two negative-control groups were maintained, one was treated with water, the other with 0.5% carboxymethylcellulose. A positive-control group received either thalidomide or aspirin. Dams were killed on day 16. No adverse effect in maternal survival was noted. A significant number of resorptions was noted in hamsters which received ≥30 mg/kg. The incidence of fetal malformations reached statistical significance at >600 mg/kg.

In the same report, Benzoic Acid at doses of 5, 25, 50 and 500 mg/kg was administered by stomach tube to groups of 20 pregnant Wistar rats on GDs 6 to 15. Two negative-control groups were maintained, one was treated with water, the other with 0.5% carboxymethylcellulose (used to keep the Benzoic Acid in suspension). A positive-control group received either thalidomide or aspirin. Dams were killed on day 21. Maternal survival was similar for treated and control groups. A significant number of resorptions was noted in rats which received ≥25 mg/kg. The incidence of fetal malformations in Benzyl Alcohol treated rats did not reach statistical significance (Polish Academy of Sciences 1977).

BEHAVIORAL EFFECTS AND NEUROTOXICITY

In a study of sedative effects of fragrance compounds and essential oils, Buchbauer et al. (1993) reported that a 1-h period of exposure to undiluted Benzaldehyde vapors by inhalation resulted in a 43.69% reduction in motility in outbred Swiss mice, compared to control animals. For comparison, exposure to lavender oil vapors resulted in a 78.4% reduction in motility and Thymol vapor resulted in a 33.02% increase in motility. The authors concluded that some fragrance compounds and essential oils can be used as mild sedatives in aromatherapy.

GENOTOXICITY

Bacterial

Benzaldehyde at 3 μmol per plate was not mutagenic in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, or TA 1537 with or without S9 (Florin et al. 1980). In *S. typhimurium* strains TA 98 and TA 100 exposed to Benzaldehyde at concentrations of 0.05 to 500 μg/plate diluted in DMSO, the exposure failed to produce the two-fold increase in revertant colony count over vehicle control required to be considered positive for mutagenicity (Kasamaki et al. 1982). Benzaldehyde at concentrations up to 1000 μg per plate was not mutagenic in *S. typhimurium* strains TA 98, TA 100, TA 1535, or TA 1537 with or without S9 (Haworth et al. 1983).

Watanabe et al. (1988) showed that Benzaldehyde could dose-dependently prevent the mutagenic response to 4-nitroquinoline

1-oxide (4NQO) in *Escherichia coli* WP2s *uvrA trpE*. Incubation with 20 and 30 μmol/plate Benzaldehyde caused 25% and 49% reductions in the frequency of 4NQO induced mutations.

Benzaldehyde scored negative for mutagenicity in *Salmonella* strains during a screening of potential food additive aldehydes, although details such as strains, concentration, and exposure duration were not reported (Zeiger 1993). Another study using Ames test methods in *S. typhimurium* strains TA 98, TA 102, and TA 104 was negative for Benzaldehyde at doses ranging from 33 to 3333 μg/plate with and without S9 mix (Dillon et al. 1998).

Mammalian

Kasamaki et al. (1982) exposed Chinese hamster cell line B241 cells to 50 nM Benzaldehyde or DMSO vehicle for 24 h, then incubated them for 24 h without Benzaldehyde, followed by treatment with colchicine (100 nM) for 2 to 3 h. Chromosomes from the cells were prepared by Giemsa staining and evaluated for aberrations.

Out of 162 Benzaldehyde-treated cells scored, 8 displayed chromatid gaps (similar to control); 9 had chromatid breaks; 1 had a chromosome break; 0 showed ring, dicentric, or chromatid exchange aberrations; and 3 had other unspecified aberrations. In all, 21 of 162 Benzaldehyde-exposed cells (13%) were abnormal. About 3.2% to 3.7% of vehicle control cells (n = 135 to 221) were abnormal (Kasamaki et al. 1982).

McGregor et al. (1991) reported that Benzaldehyde was 1 of 27 compounds screened for mutagenic potential by using a mouse lymphoma cell forward mutation assay. In this assay, chemicals were evaluated for their ability to induce a forward mutation at the thymidine kinase locus (tk) in a colony of L5178Y clone 3.7.2C mouse lymphoma cells in the presence of 5-trifluorothymidine (TFT). The Benzaldehyde concentrations tested in this assay were 0, 50, 100, 200, 400, and 800 μg/ml. The vehicle control was DMSO, and methyl methanesulphonate was the positive control to induce the forward mutation.

The results for Benzaldehyde were reported as follows: "Single dose, statistically significant increases in mutant fraction were observed in two experiments without S9 mix. The LOED was 400 μg/ml, while 640 μg/ml was a lethal dose in one of the experiments. Clearly, concentrations which induced significant increases in the mutant fraction were very close to highly toxic doses" (McGregor et al. 1991).

DNA

Becker et al. (1996) reported that Benzaldehyde alone failed to induce single strand breaks in supercoiled PM2 DNA, but Benzaldehyde and copper (II) chloride together had a synergistic effect in causing single strand breaks. This synergistic effect was dose-dependent for both $CuCl_2$ and Benzaldehyde.

CARCINOGENICITY

Based on the studies presented below, the NTP (1990) concluded that there was no evidence of carcinogenicity of Benzaldehyde in rats, and some evidence of carcinogenicity of Benzaldehyde in mice.

Eight-week-old F344/N rats were orally dosed with 0, 200, or 400 mg/kg Benzaldehyde in corn oil daily five days a week for 103 weeks (n = 50/sex/dose level). Animals were observed twice a day. Body weights were recorded once per week for the first 13 weeks and then once per month thereafter. Necropsies were performed on all animals that died or found moribund prior to study termination. At the end of the dosing period, all rats were killed and necropsied. Tissue samples were imbedded in paraffin and examined microscopically.

The survival of male rats to study termination in the 400 mg/kg group was significantly lower ($p < 0.001$) than the survival rate of vehicle control males. Survival of other groups of each sex was similar to controls. Mean body weights at study termination for surviving rats in all doses within the sexes were similar to controls.

The incidence of adenomas of the pancreas in the 400 mg/kg dose group was slightly (9%) higher than that of the vehicle control but was well within the historical incidence of pancreatic neoplasms in corn oil vehicle controls at the laboratory where the study was performed. Slight increases in mononuclear cell leukemia and squamous cell papilloma of the forestomach were observed in the dose groups and were not considered to be related to the Benzaldehyde treatment. No other findings of the necropsies or histological examinations were remarkable.

In this same study, 8-week-old $B6C3F_1$ mice were administered 0, 200, or 400 mg/kg Benzaldehyde in corn oil daily 5 days a week for 103 weeks (n = 50/sex/dose level). Mice were observed twice a day. Body weights were recorded once per week for the first 13 weeks and then once per month thereafter. Necropsies were performed on all animals that died or were found moribund prior to study termination. At the end of the dosing period, all mice were killed and necropsied. Tissue samples were embedded in paraffin and underwent pathohistological examinations.

Mean body weights and survival rates of both dosed groups were similar to vehicle controls. No compound-related clinical observations were reported during the study.

At necropsy the incidences of focal hyperplasia in the forestomach were significantly increased in both sexes of the 400 mg/kg group ($p < 0.01$) and in females of the 200 mg/kg group ($p < 0.05$), compared to vehicle controls. Incidences of squamous cell papillomas were slightly increased in the dosed animals, but the increase was not statistically significant. There were no other remarkable findings noted in the histological examinations (NTP 1990).

Carcinostatic and Antitumor Properties

Animals

Using the premise that the fruit of the fig (*Ficus carica* L.) has been used as a traditional carcinostatic drug in many parts of the world, Takeuchi et al. (1978) implanted BFD_1 mice

subcutaneously with adenocarcinoma 755. Beginning 24 h after the carcinoma implantation, seven of the mice were given a daily intraperitoneal injection of a distillate obtained from homogenized fig fruit for 10 days. Mice were then killed and the tumors were measured.

The mice given fig distillate had a mean tumor weight 39% smaller than that of mice that did not receive the fig dose. The researchers then used a high-speed gas chromatograph to separate the components of the fig distillate.

Four main peaks appeared, and the effluent from each peak was injected into tumor-implanted mice by the same schedule described above. The fourth effluent reduced the mean tumor weight by 36%. Gas chromatography–mass spectrometry was performed on the fourth effluent, and it was found to be Benzaldehyde. This identification was confirmed by thin-layer chromatography and infrared (IR) spectra analysis.

These authors repeated the test using Benzaldehyde at 100 mg/kg day^{-1} and reported a 40% mean reduction in tumor weight. In another experiment, Swiss mice were implanted with Ehrlich carcinoma (solid type) and treated with intraperitoneal injections of 10 mg/kg of Benzaldehyde once a day for 9 days. A mean reduction in tumor weight of 44.4% was reported (Takeuchi et al. 1978).

Grier et al. (1982) used Benzaldehyde in the form of β-cyclodextrin Benzaldehyde (CDBA) as a clinical treatment against advanced or terminal solid tumors in eight dogs and one cat. Each animal received 3 mg/kg Benzaldehyde twice a day, 5 days a week for 16 weeks. Prior to treatment, the tumors were measured by x-ray or photographed. CDBA was ineffective in preventing the progression of the tumors in these animals. The cat and four dogs were euthanized prior to study completion. The four dogs that completed the 16-week trial had recurrence of original tumors and/or growth of new tumors. No CDBA-related toxicity was apparent.

MacEwen (1986) treated tumors that were deemed unacceptable for treatment by surgery or chemotherapy in 14 dogs and 11 cats orally with 10 mg/kg/day^{-1} with Benzaldehyde in the form of CDBA tablets. Success in preventing tumor growth was marginal, in that only two of the dogs had a partial response (>50% regression), and one dog had minimal response (<50% regression). All other animals had no response. No toxic effects from the CDBA treatment were observed.

In Vitro

Zundel et al. (1978) reported that Benzaldehyde was selectively cytotoxic to rat embryo fibroblast cells that had been transformed with simian virus (SV-40). Benzaldehyde had only a slight toxic effect on nontransformed normal cells at an exposure of 100 μg/ml, but not at 1.25, 5, or 25 μg/ml. Cells that had been transformed with Simian virus (SV-40) showed a dose-dependant cytotoxicity within the same range of doses. The 100 μg/ml dose produced "complete cell destruction."

Miyakawa et al. (1979) incubated rat embryo fibroblast cells transformed with 40 (SV-40) in medium containing 0, 25, or 50 μg/ml Benzaldehyde. Growth of cells exposed to 25 μg/ml Benzaldehyde was similar to growth in control cells. Growth of cells exposed to 50 μg/ml Benzaldehyde was completely inhibited. However, upon removal of Benzaldehyde the cells resumed the normal growth rate.

Nambata et al. (1980) reported that growth of C3H/He mouse embryo cells in vitro was reduced upon exposure to 1 mM but not 0.1 mM Benzaldehyde.

Nambata et al. (1981) grew HeLa human cervical carcinoma cells in flasks containing medium with 0, 0.1, 0.5, 1.0, 1.5, or 2.0 mM Benzaldehyde for two days. Cell growth was measured by counting the cells in each flask at the end of the two-day incubation period. Growth of HeLa cells was concentration-dependently inhibited by Benzaldehyde. [^{3}H] thymidine incorporation was used to demonstrate that Benzaldehyde dose-dependently slows the rate of the cell cycle in these cells, apparently caused by delays in the S and G_2 periods.

Nishimura et al. (1981) used V79, HeLa, and EJ-1 cell lines to screen Benzaldehyde and several related chemicals for cytotoxicity and ability to inhibit DNA synthesis. Cells were incubated with various doses of the test compounds for 3 days in test chambers. Cells were then fixed, stained and scored for cytotoxicity on a scale from 0 (no cellular damage) to 4 (complete cytolysis or degeneration). Benzaldehyde scored 2, 1, and 0 for exposure concentrations of 1000, 600, and 320 μg/ml, respectively.

An additional experiment was performed in which cells were exposed to Benzaldehyde with radiolabeled [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]leucine. Cells were later collected and incorporation of the radiolabel into cellular macromolecules (e.g., DNA, RNA) was detected by a liquid scintilation counter. Benzaldehyde did not affect DNA synthesis when compared to controls (Nishimura et al. 1981).

Pettersen et al. (1983a, 1983b) reported a study in which NHIK 3025 cells were exposed to Benzaldehyde. Cells derived in situ from human cervical carcinoma were cultivated in medium E2a. Populations of synchronized cells were obtained by selecting mitotic cells from populations in exponential growth. Under the growth conditions, these cells had a median cell cycle time of ~18 h, with median G_1 and S durations of ~7 and ~8 h, respectively.

Synchronized cells were divided into populations of equal number in culture flasks and seeded immediately. Two hours after seeding, the cells had attached to the bottom of the flask, and the medium was removed and replaced with medium containing different concentrations of Benzaldehyde. After the desired exposure time, the medium containing Benzaldehyde was removed, the flask was rinsed with medium, and then regular medium was restored to support cell survival. After 10 to 12 days, cells were fixed and stained with methylene blue, and the colonies were counted. Cell cycle kinetics were measured by flow cytometry of DNA histograms and microscopic examination.

Exposure to Benzaldehyde concentrations higher than 6.4 mM for 4 or 24 h caused a marked decrease in cell survival.

Administration of Benzaldehyde during G_1 or S phase had no selective effect on cell survivability. When Benzaldehyde was present only during mitosis, the cell cycle was inhibited to a greater extent than when exposure was exclusively during interphase. The mechanism for this inhibitory effect on the cell cycle in these cells is unknown (Pettersen et al. 1983a, 1983b).

Choi et al. (1992) compared Benzaldehyde and CDBA in their efficacy as antitumor agents in MCF-7 human breast adenocarcinoma and HT-29 human colon adenocarcinoma cell lines. Cells were incubated in several concentrations of Benzaldehyde or CDBA or control medium for 6 days. Cytotoxicity was reported as ED_{50}, the effective dose at which cell growth is reduced by 50% of control cultures.

The ED_{50} values for Benzaldehyde were 0.7 and 0.5 mM in the MCF-7 and HT-29 cell lines, respectively. The ED_{50} values for CDBA were 0.6 and 0.7 mM in the MCF-7 and HT-29 cell lines, respectively. These differences were not statistically significant (Choi et al. 1992).

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Benzoic Acid

Bioresearch Inc. (1992a) applied a liquid/powder foundation containing 0.2% Benzoic Acid in a 24-h occlusive patch to the back of 12 panelists. A total of three exposures occurred within 1 week. Sites were evaluated at the time of patch removal and 24 h later (i.e., prior to application of the subsequent patch). No reactions were observed.

The Education and Research Foundation, Inc. (1992) reported a study in which 48 female panelists participated in an in-use study which investigated the acnegenic and irritation potential of a liquid/powder foundation containing 0.2% Benzoic Acid. Approximately half of the test population had "mild to moderate" acne, the rest did not have significant acne. Panelists were instructed to apply the product to the entire face and neck area at least twice a day for 45 days. Acne lesions and irritation were evaluated by a dermatologist on days 0, 3, 7, 10, 28, and 45. Objective and subjective evaluations of irritation were made by a nurse or technician on days 15, 21, and 35. Panelists also maintained daily response logs.

There was no change in the acne lesion counts of the non acne subjects and the acne subjects had a decrease in acne lesions. All objective irritation grades were 0's. Transient grade 1 irritation was noted by the technician; panelists' logs recorded occasional instances of dryness, itching, and flakiness (Education and Research Foundation, Inc. 1992).

Dermal Sensitization

Benzoic Acid

Leyden and Kligman (1977) reported that Benzoic Acid (5% in petrolatum) did not elicit an allergic reaction when applied to the skin of 10 panelists who, in a previous Kligman-maximization assay, had tested positive for benzoyl peroxide sensitivity.

A RIFM report (Opdyke 1979) on Benzoic Acid cited an unpublished maximization test (Kligman 1977) that tested 2% Benzoic Acid in petrolatum using 25 volunteers (skin types: 5 Black females, 2 Black males, 5 Caucasian females, 14 Caucasian males). During induction, a total of five 48-h occlusive patches was applied to the same site (either forearm or back). Each was preceded by a 24-h occlusive pretreatment of the site with 2.5% sodium lauryl sulfate (SLS). Following a 10-day nontreatment period, panelists were challenged at a different site with a 48-h occlusive patch; the site had been pretreated for 1 h with 5% to 10% SLS. Challenge sites were examined at the time of patch removal and 24 h thereafter. No reactions were observed.

Broeckx et al. (1987) reported results of a cosmetic intolerance assay which patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction to Benzoic Acid was noted in 34 (0.7% incidence). A reaction was noted in one of the 155 patients with cosmetic allergy.

Bioresearch Inc. (1992b) tested a liquid/powder foundation containing 0.2% Benzoic Acid in a modified Draize Repeated Insult Patch Test using 75 panelists. Nine 24-h occlusive patches were applied to the back during a 3-week induction period. Following a 2-week nontreatment period, panelists were challenged at a previously unexposed site. Sites were evaluated at 24 and 48 h after patch removal. No reactions were noted during induction or at challenge.

Phototoxicity/Photosensitization

Benzoic Acid

Larmi et al. (1988) and Larmi (1989a, 1989b) reported that clinical studies have demonstrated that ultraviolet (UV) light can produce a dose-dependent inhibition of Benzoic Acid-induced nonimmunologic immediate contact reactions.

Bioresearch Inc. (1991) tested a matte eye shadow and base formulation each containing 0.1% Benzoic Acid under the conditions of the Draize-Shelanski Repeat Insult Patch Test using 77 panelists. The test materials were applied in 48-h occlusive patches to one of three sites on the back. Every third patch was applied to the same site. (This protocol allowed for the observation of delayed reactions.) Sites on the back were irradiated for one minute with UV light (365 nm, at a distance of 12 inches) following removal of induction patches 1, 4, 7, and 10. At the same time, the materials were applied in 48-h open patches to the volar aspect of the right forearm. The protocol was followed for a total of 10 applications within a $3^1/_2$-week period.

Following a 2-week nontreatment period, closed and open challenge patches were applied to previously unexposed sites.

Sites on the back were irradiated after removal of the challenge patch. No reactions were noted during induction or at challenge and no reactions were noted in response to irradiation (Bioresearch Inc. 1991).

Bioresearch Inc. (1992c) applied a liquid/powder foundation containing 0.2% Benzoic Acid at two sites to the back of 10 panelists with Fitzpatrick skin types I, II and III. Sites were not covered. One site on each panelist was irradiated with UV light; the exposure was initiated 30 to 60 minutes after test material application. Sites were irradiated with 0.5 of the previously determined minimal erythema dose (MED) of UVA and UVB light (290 to 400 nm from a Model 12S ultraviolet solar simulator), followed by a total of 14 Joules/cm^2 of UVA (290 to 320 nm). A control site that had not been dermally treated was also irradiated. Panelists were instructed to avoid natural or artificial sunlight exposure throughout the study. All sites were evaluated at 24, 48 and 72 h after irradiation. No reactions were observed.

Bioresearch Inc. (1992d) also tested a liquid/powder foundation containing 0.2% Benzoic Acid in a photosensitization study using 30 panelists with varying Fitzpatrick skin types. The authors stated that the degree of pigmentation did not interfere with UV light response or skin reaction evaluation. During induction, six 24-h occlusive patches were applied to the back within a 3-week period. At the time of patch removal sites were irradiated with 2.0 MEDs of UVB light and 4 Joules/cm^2 of UVA light. Following an 18-day nontreatment period, panelists were challenged at two previously unexposed sites. Challenge sites were scored after 24 h of exposure and one site was then irradiated with 0.5 MED of UVB and 4 Joules/cm^2 of UVA. Another site, not dermally treated, was also irradiated at challenge and served as the UV light control. Challenge sites were evaluated at 24, 48, and 72 h post irradiation. Panelists were instructed to avoid natural or artificial sunlight exposure throughout the study. No reactions were observed.

Carcinostatic Clinical Trial

Kochi et al. (1980) evaluated the carcinostatic activity of Benzaldehyde in a human clinical trial. β-Cyclodextrin Benzaldehyde (CDBA) (10 mg/kg/day) was administered orally or rectally to 57 patients with inoperable carcinoma in the terminal stages or in serious condition with other tumor types. Patients ranged in age from 4 to 83 years with cancerous diseases in a spectrum of tissue types. The CDBA contained approximately 8.3% Benzaldehyde, and the average dose of Benzaldehyde was approximately 500 mg/day. Patients were observed for periods ranging from 2 weeks to more than 2 years.

No toxic effects were seen in any patients given long-term treatments with CDBA. Nineteen patients responded with complete remission, and 10 responded with partial (<50%) remission. The responses continued during treatment with CDBA, but the optimal doses for different carcinomas had not been established (Kochi et al. 1980).

Case Reports

Benzaldehyde

Dadlez (1928) reported that a young woman died shortly after drinking 50 to 60 cc Benzaldehyde. The woman's age and the time between consumption and death were not specified. At autopsy, the stomach contained a yellowish-white pulp having a strong odor of bitter almond. The mucus membrane was whitish, dry, and flushed. Hyperemia was observed in the small intestine, and there were ecchymotic spots on the pleura and pericardium.

Klecak et al. (1977) reported that Benzaldehyde could cause positive patch tests in eczematous patients hypersensitive to complex allergens, such as balsam of Peru and turpentine, which are known to contain Benzaldehyde.

Becker et al. (1994) reported that, out of a sample of 50 human subjects who tested positive for sensitivity to a fragrance mix containing Benzaldehyde, one subject had an immediate positive reaction to Benzaldehyde alone. None of the fragrance mix-sensitive patients had a delayed reaction to Benzaldehyde alone.

Seite-Bellezza et al. (1994) reported a case in which a 19-year-old pastry maker presented with chronic urticaria localized on the hands and forearms. In his work he regularly was in contact with several pastry ingredients, including chocolate, caramel, vanilla, and cinnamon. His history of outbreaks showed a positive correlation between his work and the urticaria. The lesions disappeared within 1 to 2 h of avoiding pastry contact. Patch tests were performed with the European standard series, removed at 20 and 40 min. Balsam of Peru produced an immediate erythemato-oedematous reaction. Benzaldehyde at 5% in petrolatum produced the same response, as did 1% cinnamic aldehyde. Wearing gloves and avoiding contact with pastry resolved the urticaria problem.

Bruze and Zimerson (1997) reported that 10 Swedish patients with hand dermatitis and contact allergy to at least one of six methylol phenols (MPs) were patch-tested for cross reactivity to 19 substances including Benzaldehyde. Patients who showed positive reactions to a variety of MP compounds showed no sensitivity reaction to Benzaldehyde (81% solution) or to Benzoic Acid.

SUMMARY

Benzaldehyde is an aromatic aldehyde used in cosmetics as a denaturant, a flavoring agent, and as a fragrance. In 2001, Benzaldehyde was reportedly used in seven cosmetic products. Its highest reported concentration of use was 0.5% in perfumes, and the next highest use concentration was two orders of magnitude less, at 0.004% in skin care preparations.

Benzaldehyde is used as a GRAS food additive, for its cherry or almond flavor. It occurs naturally in many plant species, including cherry and peach fruits and carnation flowers.

Benzaldehyde can be acquired naturally from the oil of bitter almonds. However, currently most commercially available Benzaldehyde is produced by the oxidation of toluene with a cobalt catalyst.

Benzaldehyde is absorbed through skin and by the lungs. Benzaldehyde and/or its metabolites distribute to all well-perfused organs, but it does not bioaccumulate in any specific tissue type. It is metabolized to benzoic acid, which is then conjugated with glycine or glucuronic acid. Metabolites of Benzaldehyde are excreted in the urine.

In acute toxicity studies, the oral LD_{50} of Benzaldehyde in rats and mice ranged from 800 to 1600 mg/kg. One study found an oral LD_{50} in rats as high as 2850 mg/kg. The oral LD_{50} in guinea pigs was 1000 mg/kg. The intraperitoneal LD_{50} in white rats was 3265 mg/kg. In an inhalation study in mice, 333 to 394 ppm Benzaldehyde reduced respiration rates by 50%.

In 2-weeks of daily (excluding weekends) oral doses of Benzaldehyde in rats and mice, the no observed adverse effect level (NOAEL) was 400. In 13-weeks of daily oral doses of Benzaldehyde, the NOAEL was 400 mg/kg in rats and 600 mg/kg in mice. Rats fed up to 10,000 ppm Benzaldehyde in feed for 16 weeks showed no signs of toxicity. Repeated inhalation of volatilized Benzaldehyde produced ocular and nasal irritation at 500 ppm and death in rabbits at 750 ppm.

Undiluted Benzaldehyde was irritating to rabbit eyes, causing edema, erythema, and pain. Benzaldehyde was determined not to be a contact sensitizer using the Magnusson-Kligman method and the open epicutaneous test. However, it was reported positive for allergenicity in guinea pigs in the Draize test, the maximization test, and a test with Freund's complete adjuvant. Benzoic acid, the primary metabolite of Benzaldehyde, was not phototoxic in an in vitro test using human erythrocytes. Although some clinical cases have been reported, the general incidence of allergenicity to Benzaldehyde appears to be rare. Benzoic Acid did not produce irritation or sensitization reactions in human clinical studies. Benzoic Acid also failed to produce reactions in phototoxicity and photosensitization tests.

Animal studies of Benzaldehyde, Benzoic Acid, and Sodium Benzoate have found that these compounds do not present significant reproductive or developmental toxicity at doses that are nontoxic to the mother. In a behavioral study, blood levels of 0.12 ng/ml Benzaldehyde produced a 44% reduction in motor activity in mice.

Benzaldehyde scored negative for mutagenicity in Ames assays at concentrations up to 3333 μg/plate. Thirteen percent of Chinese hamster cells exposed to 50 nM Benzaldehyde for 24 h showed chromosomal abnormalities. In a mouse lymphoma forward mutation assay, 400 μg/ml Benzaldehyde produced increases in the mutant fraction, but this dose was also very close to the lethal dose, 640 μg/ml.

The results of a 2-year carcinogenicity study in rats and mice by the NTP demonstrated no evidence of carcinogenicity of Benzaldehyde in rats, and some evidence of carcinogenicity of Benzaldehyde in mice. Many other studies have suggested that Benzaldehyde may have carcinostatic or antitumor properties, including one human clinical trial.

DISCUSSION

The CIR Expert Panel noted that Benzaldehyde is a generally recognized as safe (GRAS) food additive in the United States and has been legally accepted as a flavoring substance by the European Commission. The Panel expressed concern regarding the limited irritation and sensitization data available for Benzaldehyde. Although one evaluation of sensitization screening methodologies reported sensitization reactions in animals to Benzaldehyde, other animal tests indicated no sensitization.

To further consider this question, the CIR Expert Panel examined the relevance of the available data on Benzoic Acid from an earlier safety assessment. Available data indicate that Benzyl Alcohol is metabolized in viable skin to Benzoic Acid and hippuric acid, catalyzed by alcohol dehydrogenase. This conversion of Benzyl Alcohol presumably involves Benzaldehyde as an intermediate and the well understood conversion of Benzaldehyde to Benzoic Acid, followed by conjugation with glycine to form hippuric acid. Because much of exposure of the skin to Benzaldehyde actually will result in exposure to Benzoic Acid, the CIR Expert Panel concluded that the irritation and sensitization data in the earlier safety assessment of Benzoic Acid were relevant. Those dermal irritation and sensitization data demonstrated no adverse reactions to Benzoic Acid up to a concentration of 5%, 10 times higher than the maximum use concentration of Benzaldehyde. In addition, based on the professional experience of the Expert Panel, Benzaldehyde does not appear to be an irritant or sensitizer in human skin. Similarly, UV absorption and phototoxicity data for Benzoic Acid demonstrated little UV absorption and no phototoxicity, suggesting that Benzaldehyde also would not be phototoxic.

The highest use concentration of 0.5% is for the perfumes product category. One study of the fate of inhalated Benzaldehyde indicated rapid absorption and distribution to tissue. After peak concentrations in tissues, however, removal from all tissues was rapid and linear over time suggesting little accumulation of Benzaldehyde in tissue.

Benzaldehyde was evaluated by the National Toxicology Program, which found no evidence of carcinogenicity in rats, and some evidence of carcinogenicity in mice. Several studies have suggested that Benzaldehyde can have carcinostatic or antitumor properties. Overall, at the low concentrations used in cosmetics, up to 0.5% in perfumes, and orders of magnitude less in other products, the CIR Expert Panel did not consider Benzaldehyde a carcinogenic risk to humans.

CONCLUSION

Based on the data presented in this safety assessment, the CIR Expert Panel concluded that Benzaldehyde is safe as used in cosmetic products.

REFERENCES

Abramovici, A., and P. Rachmuth-Roizman. 1983. Molecular structure-teratogenicity relationships of some fragrance additives. *Toxicology* 29:143–156.

Andersen, F.A., ed..1998. Final report of the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int. J. Toxicol.* 20(3):23–50.

Barry, B. W., S. M. Harrison, and P. H. Dugard. 1985. Vapour and liquid diffusion of model penetrants through human skin; correlation with thermodynamic activity. *J. Pharm. Pharmacol.* 37:226–235.

Becker, K., E. Temesvari, and I. Nemeth. 1994. Patch testing with fragrance mix and its constituents in a Hungarian population. *Contact Dermatitis* 30:185–186.

Becker, T. W., G. Krieger, and I. White. 1996. DNA single and double strand breaks induced by aliphatic and aromatic aldehydes in combination with copper(II). *Free Rad. Res*. 24(5):325–332.

Beltran-Garcia, M. J., M. Estarron-Espinosa, and T. Ogura. 1997. Volatile compounds secreted by the oyster mushroom (*Pleurotus ostreatus*) and their antibacterial activities. *J. Agric. Food Chem*. 45:4049–4052.

Bioresearch Inc. 1991.Draize-Shelanski repeat insult patch test conducted with eye shadow containing 0.1% benzoic acid. Two studies testing a matte formula #3073-16 and base formula 3073-17 on the same panelists. Project No. 90-7183H. Unpublished data submitted by CTFA. (46 pages.)

Bioresearch Inc. 1992a. Irritation screening study of face make-up containing 0.2% benzoic acid. Project No. 92-7569H. Unpublished data submitted by CTFA. (12 pages.)[2]

Bioresearch Inc. 1992b. Modified Draize repeated insult patch study of face make-up containing 0.2% benzoic acid. Project No. 92 7569H. Unpublished data submitted by CTFA. (11 pages.)[2]

Bioresearch Inc. 1992c. Human phototoxicity test of face make-up containing 0.2% benzoic acid. Project No. 92-7569H. Unpublished data submitted by CTFA. (8 pages.)[2]

Bioresearch Inc. 1992d. Human photoallergy test of face make-up containing 0.2% benzoic acid. Project No. 92-7569H. Unpublished data submitted by CTFA. (9 pages.)[2]

Blum, M. S., F. Padovani, A. Curley, and R. E. Hawk. 1969. Benzaldehyde: Defensive secretion of a harvester ant. *Comp. Biochem. Physiol* 29:461–465.

Boehnlein, J., A. Sakr, J. L. Lichtin, and R. L. Bronaugh. 1994. Characterization of esterase and alcohol dehydrogenase activity in skin. Metabolism of retinol (Vitamin A) during percutaneous absorption. *Pharm. Res*. 11:1155–1159.

Bowen, W. R., S. Y. R. Pugh, and N. J. D. Schmockburgk. 1986. Inhibition of horse liver and yeast alcohol dehydrogenase by aromatic and aliphatic aldehydes. *J. Chem. Tech. Biotechnol.* 36:191–196.

Bowles, B. L., and V. K. Juneja. 1998. Inhibition of foodborne bacterial pathogens by naturally occurring food additives. *J. Food Safety* 18:101–112.

Bray, H. G., W. V. Thorpe, and K. White. 1951. Kinetic studies of the metabolism of foreign organic compounds. i. the formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit. *Biochem. J.* 48:88–96.

Broeckx, W., A. Blondeel, A. Dooms-Goossens, and G. Achten. 1987. Cosmetic Intolerance. *Contact Dermatitis* 16:189–194.

Bruze, M., and E. Zimerson. 1997. Cross-reactivity patterns in patients with contact allergy to simple methylol phenols. *Contact Dermatitis* 37:82–86.

Buchbauer, G., L. Jirovetz, W. Jäger, C. Plank, and H. Dietrich. 1993. Fragrance compounds and essential oils with sedative effects upon inhalation. *J. Pharmaceut. Sci.* 82(6):660–664.

Choi, H. S., A. M. Knevel, and C. Chang. 1992. Molecular complexation: β-Cyclodextrin and benzaldehyde inclusion complex. *Pharmaceut. Res.* 9(5):690–693.

Chow, Y. S., H. S. Shin, A. A. Adesina, and P. L. Rogers. 1995. A kinetic model for the deactivation of pyruvate decarboxylase (PDC) by benzaldehyde. *Biotechnol. Lett.* 17(11):1201–1206.

Clark, G. S. 1995. An aroma chemical profile benzaldehyde. *Perfumer and Flavorist* 20:53–60.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2001. Concentration of Use—Benzaldehyde. Unpublished data submitted by CTFA on October 18, 2001. Washington, DC: CTFA.[2]

Dadlez, J. 1928. The toxicity of benzaldehyde and salicylic aldehyde in humans. *Hebd. Séance Acad. Sci. Paris*. 99:1038.

De Greef, J. A., and C. F. Van Sumere. 1966. The effect of phenolic aldehydes, phenolic acids, coumarins and related compounds on the growth of *Saccaromyces cerevisiae*. *Arch. Int. Physiol. Biochem.* 74:512–513.

Dillon, D., R. Combes, and E. Zeiger. 1998. The effectiveness of *Salmonella* strains TA100, TA102, and TA104 for detecting mutagenicity of some aldehydes and peroxides. *Mutagenesis* 13:19–26.

Eastman Kodak. 1991. Letter from Eastman Kodak to USEPA submitting enclosed material safety data sheet, toxicity health and hazard summary and toxicity report on benzaldehyde with attachments. NTIS Report No. OTS0533617.

Eberlein-König, B. T., T. Bergner, S. Diemer, and B. Przybilla. 1993. Evaluation of phototoxic properties of some food additives: Sulfites exhibit prominent phototoxicity. *Acta Dermato-Venereal*. 73:362–364.

Education and Research Foundation, Inc. 1992. 45 Day usage study in humans -acnegenicity and irritancy of liquid/powder foundation containing 0.2% Benzoic Acid. Unpublished data submitted by CTFA (250 pages.)[2]

Environmental Protection Agency (EPA). 1992. Letter submitting twenty one-page animal skin sensitization summary reports on cinnamic aldehyde, phenylacetaldehyde, benzadlehyde, and glutaraldehyde (sanitized). NTIS Report No. OTS0535611.

European Commission. 1999. Commission decision of 23 February 1999 adopting a register of flavourings used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and the Council of 28 October 1996.

Feron, V. J., H. P. Til, F. de Vrijer, R. A., Woutersen, F. R. Cassee, and P. J. van Bladeren. 1991. Aldehydes: Occurrence, carcinogenic potential, mechanism of action and risk assessment. *Mutat. Res.* 259:363–385.

Florin, I., L. Rutberg, M. Curvall, C. R. Enzell. 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* 18:219–232.

Food and Drug Administration (FDA). 1984. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

FDA. 2001. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Food and Drug Research Labs., Inc. 1972. Teratologic evaluation of FDA 71-37 (sodium benzoate). NTIS Report No. PB-221-777.

Furman, G. M., D. M. Silverman, and R. A. Schatz. J. 1998. Inhibition of rat lung mixed-function oxidase activity following repeated low-level toluene inhalation: Possible role of toluene metabolites. *J. Toxicol. Environ. Health* 54:633–645.

Grier, R. L., D. F. Merkley, J. A. Roth, and M. A. Mouw. 1982. Clinical trial of benzaldehyde in pets with solid tumors: Preliminary study. *Am. J. Vet. Res.* 43:2270–2271.

Guidicelli, Y., R. Nordmann, and J. Nordmann. 1973. Action of aldehydes on lipolysis and glucose metabolism in rat adipose tissue. *Life Sci.* 12:35–47.

Hagan, E. C., W. H. Hansen, O. G. Fitzhugh, P. M. Jenner, W. I. Jones, J. M. Taylor, E. L. Long, A. A., and J. B. Brouwer. 1967. Food flavourings and compounds of related structure. II. subacute and chronic toxicity. *Food Cosmet. Toxicol.* 5:141–157.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger. 1983. *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag. Suppl.* 1:3–142.

Inel, Y., and R. Iseri. 1997. The octanol-water partition coefficient of benzene derivatives based on three dimensional structure directed molecular properties. *Chemosphere* 35:993–1002.

James, T. N., and E. S. Bear. 1968. Cardiac effects of some simple aliphatic aldehydes. *J. Pharmacol. Exp. Therapeut*. 163:300–308.

Jenner, P. M., E. C. Hagan, J. M. Taylor, E. L. Cook, and O. G. Fitzhugh. 1964. Food flavourings and compounds of related structure. i. acute oral toxicity. *Food Chem Toxicol* 2:327–343.

[2]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington DC 20036,USA.

Kasamaki, A., H. Takahashi, N. Tsumura, J. Niwa, T. Fujita, and S. Urasawa. 1982. Genotoxicity of flavoring agents. *Mutat. Res* 105:387–392.

Kimmel, C. A., J. G. Wilson, and H. J. Schumacher. (1971) Metabolism and identification of the causative agent in aspirin teratogenesis in rats. *Teratology* 4:15–24.

Klecak, G., H. Geleick, and J. R. Frey. 1977. Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. *J. Soc. Cosmet. Chem.* 28:53–64.

Kleeberg, J. 1959. Pharmacological and clinical studies on almonds. Inhibition of peptic activity by benzaldehyde. *Arch. Int. Pharmacodyn.* CXX:152–159.

Kligman, A. M. 1977. Maximization test: 2% Benzoic Acid in petrolatum. Report to RIFM. 24 May. Unpublished data from: The Research Institute for Fragrance Materials, Inc., Two University Plaza, Suite 406, Hackensack, NJ 07601.

Kluwe, W. M., C. A. Montgomery, H. D. Giles, and J. D. Prejan. 1983. Encephalopathy in rats and nephropathy in rats and mice after subchronic oral exposure to benzaldehyde. *Food Chem. Toxicol.* 21:245–250.

Kochi, M., S. Takeuchi, T. Mizutani, K. Mochizuki, Y. Matsumoto, and Y. Saito. 1980. Antitumor activity of benzaldehyde. *Cancer Treat. Rep*. 64:21–23.

Krings, U., and R. G. Berger. 1998. Biotechnological production of flavours and fragrances. *Appl. Microbiol. Biotechnol.* 49:1–8.

Kutzman, R.S., G. J. Meyer, and A. P. Wolf. 1980. Biodistribution and excretion of [^{11}C]Benzaldehyde by the rat after two-minute inhalation exposures. *Xenobiotica* 10:281–288.

Laham, S., B. Broxup, M. Robinet, M. Potvin, and K. Schrader. 1991. Subacute inhalation toxicity of benzaldehyde in the Sprague-Dawley rat. *Am. Ind. Hyg. Assoc. J.* 52:503–510.

Laham, S., and M. Potvin. 1987. Biological conversion of benzaldehyde to benzylmercapturic acid in the Sprague-Dawley rat. *Drug Chem. Toxicol*. 10:209–225.

Laham, S., M. Potvin, and M. Robinet. 1988. Metabolism of benzaldehyde in New Zealand White rabbits. *Chemosphere* 17:517–524.

Larmi E. 1989a. PUVA treatment inhibits non-immunologic immediate contact reactions to benzoic acid and methyl nicotinate. *Int. J. Dermatol.* 28:609–611.

Larmi E. 1989b. Systemic effect of ultraviolet irradiation on non-immunologic immediate contact reactions to benzoic acid and methyl nicotinate. *Acta Dermato Venereol.* 69:296–301.

Larmi, E., A. Lahti, and M. Hannuksela. 1988. Ultraviolet light inhibits non-immunologic immediate contact reactions to benzoic acid. *Arch. Dermatol. Res.* 280:420–423.

Leyden, J. J., and A. M. Kligman. 1977. Contact sensitization to benzoyl peroxide. *Contact Dermatitis* 3:273–275.

MacEwen, E. G. 1986. Anti-tumor evaluation of benzaldehyde in the dog and cat.*Am. J. Vet. Res.* 47:451–452.

Macht, D. I. 1922. A pharmacological examination of benzaldehyde and mandelic acid. *Arch. Int. Pharmacodyn. Ther* 27:163–174.

Mattia, C. J., C. P. LeBel, and S. C. Bondy. 1991. Effects of toluene and its metabolites on cerebral reactive oxygen species generation. *Biochem. Pharm*. 42(4):879–882.

McGregor, D. B., A. G. Brown, S. Howgate, D. McBride, C. Riach, and W. J. Caspary. 1991. Responses for the L5178Y mouse lymphoma cell forward mutation assay. v: 27 coded chemicals. *Environ. Mol. Mutagen.* 17:196–219.

Miyakawa, T., J. L. Zundel, and K. Sakaguchi. 1979. Selective inhibitory effect of benzaldehyde on the growth of simian virus 40-transformed cells. *Biochem. Biophys. Res. Commun.* 87:1024–1030.

Ministry of Health, Labor and Welfare. 2001a. Unofficial translation of MHW Ordinance No. 331, Attached Table 1 [Negative List]. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 22, 1-chome, Kasumgaseki, Chiyoda-ku, Tokyo 100-8045, Japan.[2]

Ministry of Health, Labor and Welfare. 2001b. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted List]. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 22, 1-chome, Kasumgaseki, Chiyoda-ku, Tokyo 100-8045, Japan.[2]

Nambata, T., N. Terada, T. Mizutani, and S. Takeuchi. 1980. Effect of benzaldehyde on mouse embryo cell *in vitro*. *Gann*. 71:572–573.

Nambata, T., N. Terada, T. Mizutani, and S. Takeuchi. 1981. Effect of benzaldehyde on proliferation of hela cells. *Gann*. 72:289–292.

National Research Council. 1981. *Formaldehyde and Other Aldehydes*. Washington, DC: National Academy Press.

National Toxicology Program. 1990. Toxicology and carcinogenesis studies of benzaldehyde (CAS no. 100-52-7) in F344/N rats and B6C3F1 mice (gavage studies). NTIS No. PB90253782. Research Triangle Park, NC: NTP.

Nguyen, D. K., A. Bruchet, and P. Arpino. 1994. High resolution capillary GC-MS analysis of low molecular weight organic compounds in municipal wastewater. *J. High Res. Chromatogr.* 17:153–159.

Nishimura, M., M. Umeda, A. Kusanagi, R. Urakabe, K. Yukimatsu, and S. Otawara. 1981. Cytomorphological study of cultured mammalian cells treated with benzaldehyde, catechol, and their derivatives with special reference to inhibition of DNA synthesis. *Gann* 72:272–279.

Opdyke, D. L. J. 1976. Fragrance raw materials monographs: Benzaldehyde. *Food Cosmet. Toxicol.* 14:693–698.

Opdyke, D. L. J. 1979. Monographs on fragrance raw materials. Benzoic acid. *Food Cosmet Toxicol* 17:715–722.

Pellizzari, E. D., T. D. Hartwell, B. S. H. Harris III, R. D. Waddell, D. A. Whitaker, and M. D. Erickson. 1982. Purgeable organic compounds in mother's milk. *Bull. Environ. Contam. Toxicol.* 28:322–328.

Pepe, R.C., J. A. Wenninger, and G. N. McEwen, Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed., vol. 1, 132. Washington, DC: CTFA..

Pettersen, E. O., O. Nome, Ø. W. Rønning, and R. Oftebro. 1983a. Effects of benzaldehyde on survival and cell-cycle kinetics of human cells cultivated *in vitro*. *Eur. J. Cancer Clin. Oncol.* 19:507–514.

Pettersen, E. O., Ø. W. Rønning, O. Nome, and R. Oftebro. 1983b. Effects of benzaldehyde on protein metabolism of human cells cultivated *in vitro*. *Eur. J. Cancer Clin. Oncol*. 19:935–940.

Pettersson, B., M. Curvall, and C. R. Enzell. 1980. Effects of tobacco smoke compounds on the in noradrenalin induced oxidative metabolism in isolated brown fat cells. *Toxicology* 18:1–15.

Polish Academy of Sciences. 1977. Teratologic examination of benzoic acid in rats. Teratologic examination of benzoic acid in golden hamsters. Project No. 05-611-4. Submitted by the FDA in response to a 1995 FOI request (42 pages.)[2]

Research Institute for Fragrance Materials (RIFM). 2001. Unpublished data by RIFM (69 pages.)[2]

Schade, F., R. L. Legge, and J. E. Thompson. 2001. Fragrance volatiles of developing and senescing carnation flowers. *Phytochemistry* 56:703–710.

Schafer, E. W., and W. A. Bowles, Jr. 1985. Acute oral toxicity and repellency of 933 chemicals to house and deer mice. *Arch. Environ. Contam. Toxicol.* 14:111–129.

Seite-Bellezza, F. el Sayed, and J. Bazex. 1994. Contact urticaria from cinnamic aldehyde and benzaldehyde in a confectioner. *Contact Dermatitis* 31:272–273.

Steinhagen, W. H., and C. S. Barrow. 1984. Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. *Toxicol. Appl. Pharmcol.* 72:495–503.

Sporn, A., I. Dinu, and V. Stanciu. 1967. Investigations on the toxicity of benzaldehyde. (Submitted by FDA in response to an FOI request—3 pages.)[2]

Tabatabaie, T., and R. A. Floyd. 1986. Inactivation of gluathione peroxidase by benzaldehyde. *Toxicol. Appli. Pharmacol*. 141:389–393.

Takeuchi, S., M. Kochi, K. Sakaguchi, K. Nakagawa, and T. Mizutani. 1978. Benzaldehyde as a carcinostatic principle in figs. *Agric. Biol. Chem.* 42:1449–1451.

Tan, H. S. I., M. A. Manning, M. K. Hahn, H. G. H. Tan, and U. R. Kotagal. 1991. Determination of benzyl alcohol and its metabolites in plasma by reverse-phase high-performance liquid chromatography. *J. Chromatogr.* 568:145–155.

Taylor, J. M., P. M. Jenner, and W. I. Jones. 1964. A comparison of the toxicity of some allyl, propenyl, and propyl compounds in the rat. *Toxicol. Appl. Pharmacol* 6:378–387.

Watanabe, K., T. Ohta, and Y. Shirasu. 1988. Antimutagenic effects of benzaldehyde and its derivatives on mutagenesis induced by 4-nitroquinoline 1-oxide in*Escherichia coli*. *Agric. Biol. Chem.* 52:1041–1045.

Wolf, C. R., H. Harmon, and C. M. Schiller. 1982. Interaction of aromatic aldehydes with isolated rat liver mitochondria. *Biochem. Pharmcol.* 31:2025–2029.

World Health Organization. 1996. Benzyl acetate, benzyl alcohol, benzaldehyde, and benzoic acid and its salts. *WHO Food Add. Ser*. 37:31–79.

Zeiger, E. 1993. Mutagenicity of chemicals added to foods. *Mutat. Res* 290:53–61.

Zhang, J., Q. He, and P. J. Lloy. 1994. Characteristics of aldehydes: Concentrations, cources, and exposures for indoor and outdoor residential microenvironments. *Environ. Sci. Technol.* 28:146–152.

Zlatkis, A., W. Bertsch, H. A. Lichtenstein, A. Tishbee, F. Shunbo, H. M. Liebich, A. M. Coscia, and N. Fleischer. 1973. Profile of volatile metabolites by gas chromatography-mass spectrometry. *Anal. Chem*. 45:763–767.

Zundel, J. L., T. Miyakawa, and K. Sakaguchi. 1978. Derivatives of benzaldehyde selectively cytotoxic to SV-40 transformed cells. *Agric. Biol. Chem.* 42:2191–2193.