Amended Safety Assessment of Malic Acid and Sodium Malate as Used in Cosmetics

Status:	Re-Review for Panel Review
Release Date:	May 19, 2017
Panel Meeting Date:	June 12-13, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊
cirinfo@cir-safety.org

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst
Date: May 19, 2017
Subject: Re-Review on Malic Acid and Sodium Malate

Enclosed is the Re-Review of the Safety Assessment of Malic Acid and Sodium Malate as Used in Cosmetics. (It is identified as *maacid062017rep* in the pdf document).

The CIR published the Final Report on the Safety Assessment of Malic Acid and Sodium Malate in 2001. Based on the available animal and clinical data available at that time, the Panel concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters in cosmetic formulations; however, the Panel determined that the data were insufficient to determine the safety of these ingredients for any other functions. The data needs, based on the reported function for Sodium Malate (skin conditioning agent – humectant), were concentration of use data, dermal irritation and sensitization data, and ocular irritation data.

Summary information from the original report has been included in italicized text in the safety assessment, as appropriate. The original report (identified as *maacid062017origrep*) is included for the Panel's review so that the detailed information is easily accessible. Very little relevant new data were identified in the published literature, and the data that were gathered were specific only to Malic Acid. CIR staff noticed during the literature review that the European Chemicals Agency (ECHA) has used safety test data on fumaric acid as read across for evaluating the safety of Malic Acid due to the chemical relationship these 2 chemicals have in the citric acid cycle. Staff also noted that Malic Acid and Sodium Malate are monohydroxy succinic acid ingredients. The Panel published the safety assessments of Fumaric Acid (with related salts and esters) in 2009 and Succinic Acid and Sodium Succinate (as part of the report on dicarboxylic acids) in 2012, and concluded that these ingredients are safe as used in cosmetics. These reports (identified as *maacid062017fumrep* and *maacid062017succrep*) are also included for the Panel.

The frequency of use of Malic Acid has increased since safety was originally reviewed, from 47 reported uses in 1998 to 238 reported uses in 2017. Notably, the number of uses near the eye area and mucous membranes increased from no reported uses to 4 and 19, respectively. The reported maximum concentration of use has increased; the maximum leave-on concentration of use reported was 1% (in multiple formulations) in 1984, and the results of the survey conducted by the Council in 2016 now indicate that the maximum leave-on use concentration is 2.1% (in a hair spray). It is used at up to 50% in products diluted for baths. The concentration of use data supplied by the Council as well as data submitted in 2011 (for the report on dialkyl malates, but the data pertain to Malic Acid) are included in this report package (*maacid062017data1* through *maacid062017data3*).

The frequency of use for Sodium Malate has also increased since the original review, from 1 reported use in 1998 to 5 reported uses in 2017. Current uses of Sodium Malate are reported in coloring hair care products and skin care preparations. No concentration of use for Sodium Malate was reported in the 2001 safety assessment, which was a data need outlined in the Panel's discussion. The Council in 2016 reported that Sodium Malate is used at 0.02% in "other" skin care preparations.

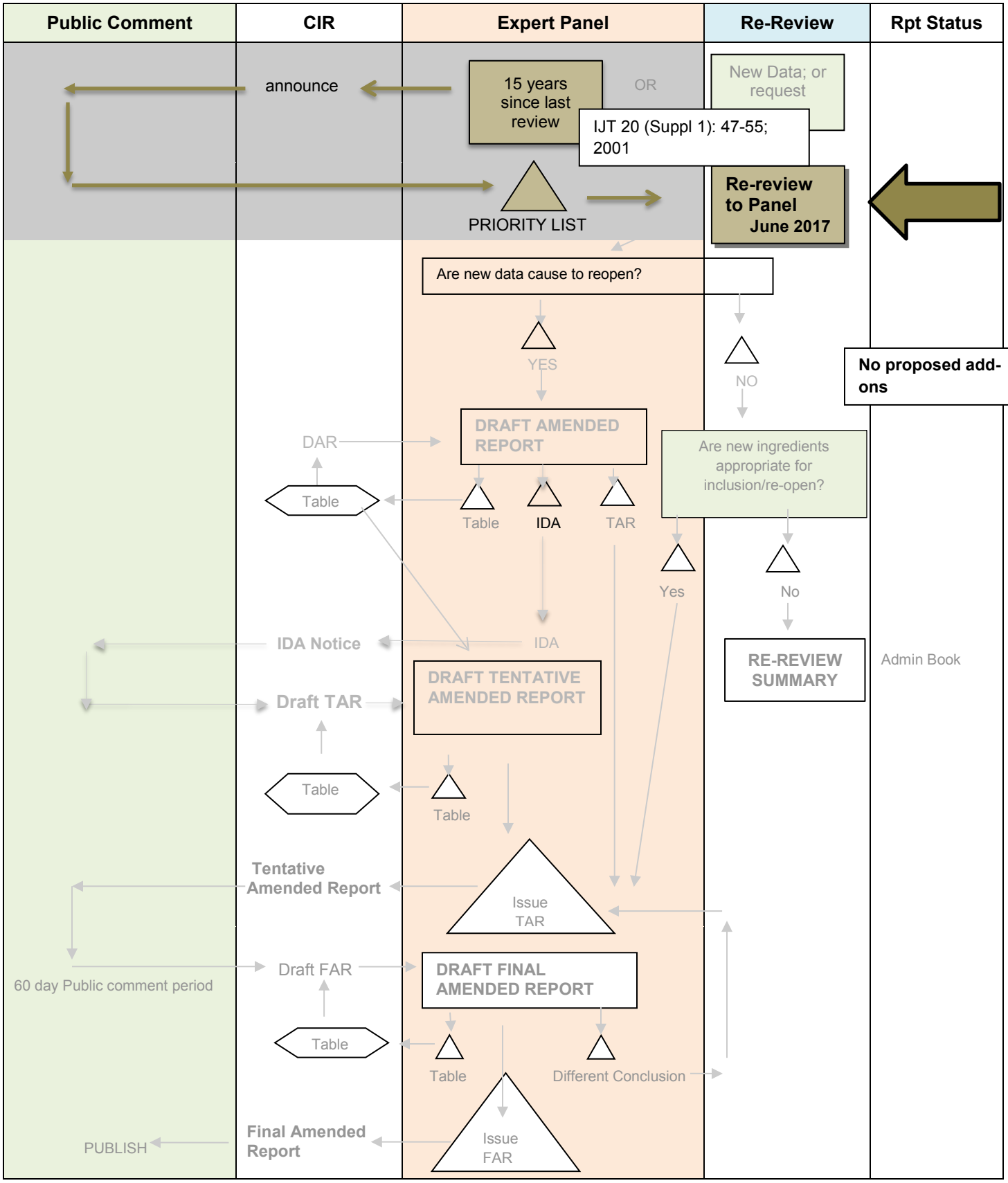
After reviewing the available data in this re-review, the Panel needs to determine whether the published final report on Malic Acid and Sodium Malate should be re-opened to amend the original conclusion or if the original conclusion should be reaffirmed as it stands. If the decision to re-open is made, the Panel should consider whether the available data on fumaric acid and/or succinic acid should be included in the amended safety assessment to be used as read across where there are notable data gaps, specifically with regards to carcinogenicity.

1620 L St NW, Suite 1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Malic Acid and Sodium Malate

MEETING June 2017



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

Malic Acid and Sodium Malate History

2001– The CIR's Final Report on the Safety Assessment of Malic Acid and Sodium Malate in the *IJT* after the report was finalized by the Panel in 1998. Based on the available animal and clinical data available at that time, the Panel concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters in cosmetic formulations; however, the Panel determined that the data were insufficient to determine the safety of these ingredients for any other functions. The data needs, which were based on Sodium Malate's reported function as a skin conditioning agent – humectant, were concentration of use data, dermal irritation and sensitization data, and ocular irritation data.

April/May 2017 – Review of the available published literature since 1998 was conducted in accordance to CIR Procedure regarding re-review of ingredients after ~15 years.

Malic Acid and Sodium Malate Data Profile –June 2017 – Writer, Christina Burnett																
	In-Use	Physical/Chemical Properties	Method of Manufacturing	Composition/Impurities	Acute Toxicity	Repeated Dose Toxicity	Genotoxicity	Reproductive and Developmental Toxicity	Carcinogenicity	Other Relevant Toxicity Studies	Irritation/Sensitization - Nonhuman	Irritation/Sensitization - Human	Ocular/Mucosal	Phototoxicity	Clinical Studies/Case REports	Toxicokinetics
Original Report																
Malic Acid	X	X	X	X	X	X	X	X			X	X	X		X	X
Sodium Malate	1 use, no concentration				X											
Re-Review																
Malic Acid	X						X					X	X		X	X
Sodium Malate	X															

“X” indicates that data were available in the category for that ingredient.

Malic Acid and Sodium Malate RR

(prepared by Christina Burnett)

Ingredient	CAS #	InfoB	SciFin	PubMed		FDA	EU	ECHA		SIDS	ECETOC		NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA
PREVIOUSLY REVIEWED																			
Malic Acid	636-61-3 (D-) 6915-15-7 97-67-6 (L-)	√	√	√		21 CFR 184.1069; 21 CFR 582.60; 21 CFR 582.1069	No	Yes; most data already in report; read across data with fumaric acid		HPV chemical, no report			No						
Sodium Malate	676-46-0	√	√	√		---	No	No		No			No						

Search Strategy

4/26/17 - all previously-reviewed ingredients were searched for the years 1998-2017

PubMed

Malic Acid OR Sodium Malate AND ("1998"[Date - Publication] : "2017"[Date - Publication])) – 2263 hits. Further refinement of search detailed below:

malic acid toxicity –120 hits (including original report)/ 1 useful
sodium malate toxicity – 32 hits (including original report)/ 0 useful
dermal effects of malic acid –2 hits (including original report)/1 useful
dermal effects of sodium malate – 1 hit (original report)
irritation of malic acid – 3 hits (including original report)/1 useful
irritation of sodium malate – 2 hits (including original report)/1 useful
sensitization of malic acid – 5 hits (including original report)/0 useful
sensitization of sodium malate – 1 hit (original report)
carcinogenicity of malic acid – 1 hit/0 useful
carcinogenicity of sodium malate – 0 hits

LINKS

online database (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
wINCI (to cite publicly) - http://webdictionary.personalcarecouncil.org
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed ;
Also search: PubMed Dietary Supplement Subset https://ods.od.nih.gov/Research/PubMed_Dietary_Supplement_Subset.aspx and
https://ods.od.nih.gov/Health_Information/IBIDS.aspx
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)

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

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

Note: ChemPortal can be used to search several of the above databases simultaneously - http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en

SIXTY-FOURTH MEETING OF THE EXPERT PANEL
September 22-23, 1997

Malic Acid and Sodium Malate

Dr. Andersen noted that during Team meetings on the preceding day, it was determined that, based on the available data, the Panel may be able to arrive at a conclusion on the safety of Malic Acid and Sodium Malate during the open session of the Panel meeting.

The Panel determined during the open session that if Malic Acid and Sodium Malate are used as pH adjusters, the available safety test data are sufficient to support the safety of these ingredients in cosmetics, and the Panel would plan to issue a Tentative Report with this conclusion at the December 8-9, 1997 Panel meeting. It was also determined that if these ingredients are used for purposes other than as pH adjusters, then the Panel is informally requesting the following kinds of data in order to complete its safety assessment.

(1) Current concentration of use

(2) Dermal irritation and sensitization data

(3) Ocular toxicity data, if available

(4) Examination of the basis for the absence of GRAS approval for baby foods

The Panel also stated that if Malic Acid and Sodium Malate are used as exfoliants, then additional data similar to that needed to assess the safety of Alpha Hydroxy Acids will be needed, including skin penetration enhancement and UV radiation damage enhancement data.

SIXTY-FIFTH MEETING OF THE EXPERT PANEL
December 8-9, 1997

Malic Acid and Sodium Malate

Dr. Schroeter noted that Malic Acid has two possible uses in cosmetics, as a pH adjuster and as an exfoliant. Dr. Schroeter's Team recommended that Malic Acid be considered safe as used in cosmetics as a pH adjuster, and that the available data are insufficient for evaluating the safety of Malic Acid as an exfoliant in cosmetics. According to the Schroeter Team, the data needed for evaluation of the safety of Malic Acid as an exfoliant in cosmetics are as follows: (1) Concentration of use as an exfoliant; (2) Irritation at exfoliant pH and concentration; (3) Penetration enhancement at exfoliant pH and concentration; and (4) Sun sensitivity enhancement at pH and concentration of the exfoliant.

Dr. Belsito noted that his Team concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters, and that the available data are insufficient for evaluating the safety of these ingredients with respect to other uses in cosmetics. Dr. Belsito agreed with the four data needs mentioned on the preceding page, and requested that ocular toxicity data (if available) be added to the list.

Dr. Belsito asked if the Panel is certain that the only two uses of Malic Acid are as a pH adjuster and an exfoliant.

Dr. Bergfeld said that Malic Acid may be used as a humectant.

Ms. Fise said that she did not recall any decision made by the Expert Panel in which it was concluded that the data were insufficient for evaluating the safety of an ingredient relative to a specific use.

Dr. McEwen favored the conclusion that Malic Acid and Sodium Malate are safe for use as pH adjusters and that the data are insufficient for evaluating safety relative to other uses. He also said that the following statements should be included in the report discussion: The data were sufficient to address the question of pH adjuster. Other uses of this ingredient would require additional information. The type of information would depend on the type of use of the ingredient.

Dr. Bailey asked members of the Panel if they would want to be more specific, and which tests would satisfy the sun sensitivity question. He noted that, with AHAs, there were tests measuring MED, and that these were supplemented with a test measuring sunburn cells, but not MED. Dr. Bailey wanted to know which information is needed in order for the Panel to address sun sensitivity issues for the AHA-type of exfoliant.

Dr. Andersen said that as demonstrated in its safety assessment of AHAs, the Panel has said very clearly that a sunburn cell assay is an assay that will identify increases in sun sensitivity. This assay identified AHAs as ingredients that increase sun sensitivity, and these data were used by the Panel in reaching a final conclusion.

Dr. Bailey said that in the AHA report, there was an MED study as well as a sunburn cell test. Given the strengths and weaknesses of the two tests, Dr. Bailey asked if the Panel needs to answer this important safety question for an ingredient that is widely used.

Dr. Belsito said that if Malic Acid is an exfoliant, then the Panel's concern is going to be an enhancement of sensitivity to sun exposure. He noted that the Panel has not specified any studies, but that if industry is interested in the studies that were found to be sufficient, the AHA report can be consulted.

Dr. Belsito noted that the Panel has reviewed a negative 2-year oral toxicity study and negative reproductive toxicity data. Given these results, he said that his concern about Malic Acid relates to its exfoliant capabilities, the level at which it causes irritation, and the effect that exfoliation has on subsequent sun activity.

Dr. McEwen wanted to know if there is any concern about sensitization potential.

Dr. Belsito said that Malic Acid is not a sensitizer, but could be an irritant.

The Panel voted unanimously in favor of issuing a Tentative Report with the following conclusion: Based on the available data, Malic Acid and Sodium Malate are safe for use in cosmetics as pH adjusters. The available data are insufficient to support the safety of these ingredients if used other than as a pH adjuster.

SIXTY-SEVENTH MEETING OF THE EXPERT PANEL
May 18-19, 1998

Malic Acid and Sodium Malate

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Malic Acid and Sodium Malate are safe for use as pH adjusters in cosmetic formulations. The data were insufficient to determine the safety of these ingredients for any other functions.

The discussion leading up to the above conclusion is stated below:

Specifically, the data are insufficient to determine the safety of Sodium Malate when used as a skin conditioning agent - humectant. The types of data required for the Expert Panel to determine the safety of Sodium Malate as a skin conditioning agent are:

(1) Concentration of use data

(2) Dermal irritation and sensitization data

(3) Ocular irritation data, if available

The data needed to assess the safety of Malic Acid or Sodium Malate for some function other than as a pH adjuster or skin conditioning agent-humectant cannot be specified without knowing the intended function. Were these ingredients to be used as exfoliants, for example, data similar to that included in the report on Glycolic and Lactic Acid (i.e., the Alpha Hydroxy Acid report) (CIR, 1997) would be needed.

Amended Safety Assessment of Malic Acid and Sodium Malate as Used in Cosmetics

Status: Re-Review for Panel Review
Release Date: May 19, 2017
Panel Meeting Date: June 12-13, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊
cirinfo@cir-safety.org

INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) published the Final Report on the Safety Assessment of Malic Acid and Sodium Malate in 2001.[1] Based on the available animal and clinical data available at that time, the Panel concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters in cosmetic formulations; however, the Panel determined that the data were insufficient to determine the safety of these ingredients for any other functions. The data needs, based on the reported function of Sodium Malate (skin conditioning agent – humectant), were concentration of use data, dermal irritation and sensitization data, and ocular irritation data. In accordance with its Procedures, the CIR evaluates the conclusions of previously-issued reports every 15 years; therefore this re-review document has been prepared.

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), Malic Acid is reported to function in cosmetics as a fragrance ingredient and a pH adjuster, while Sodium Malate is reported to function in cosmetics as a skin-conditioning agent – humectant.[2,3] These reported functions are the same as those indicated in the 2001 assessment, although fragrance ingredient is a new function for Malic Acid.

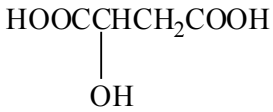
Malic Acid (or malate) is an intermediate in the citric acid cycle (also known as the tricarboxylic acid (TCA) cycle or Krebs cycle) formed during the hydration reaction of fumarate (or fumaric acid) with the enzyme fumarase.[4] Fumarate is formed by the oxidation reaction of succinate (succinic acid) and coenzyme Q (ubiquinone) with succinic dehydrogenase. Because of the chemical relationship for fumaric acid and Malic Acid, the European Chemicals Agency (ECHA) has used safety test data on fumaric acid as read across for evaluating the safety of Malic Acid.[5] The Panel published the safety assessments of Fumaric Acid (with related salts and esters) in 2009 and Succinic Acid and Sodium Succinate (as part of the report on dicarboxylic acids) in 2012 and concluded that these ingredients are safe as used in cosmetics.[6,7]

Excerpts from the summary of the 2001 report on are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information, except for chemical and physical properties, is not included in the tables or the summary section.) Additionally, the Discussion from the original report (available at http://www.cir-safety.org/ingredients) is also included in this document.

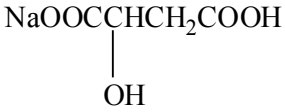
CHEMISTRY

Definition

The *Dictionary* defines Malic Acid as the organic acid that conforms to the formula described below.[2]



Sodium Malate is the sodium salt of Malic Acid (q.v.). It conforms to the formula described below.[3]



Malic Acid and Sodium Malate are monohydroxy succinic acid ingredients. Malic Acid has a stereocenter denoted by D, L, DL, or meso. The *Dictionary* name as defined is ambiguous to these stereochemical details. Stereochemistry is identified where provided in the data.

Physical and Chemical Properties

Physical and chemical properties of Malic Acid were previously reported in the 2001 safety assessment and the pertinent information from that document is described in Table 1. No physical or chemical properties for Sodium Malate were reported previously nor found in the updated literature search.

Methods of Manufacture

DL-Malic acid is made by the catalytic oxidation of benzene to maleic acid, which is converted to malic acid by heating with steam under pressure.[1] L-Malic acid is available through the microbiological fermentation of fumaric acid.

Natural Occurrence

The L-isomer is naturally occurring and common metabolite of plants (most commonly found in fruits, such as unripe apples) and animals.[1]

Impurities

Maleic and fumaric acids are by-products of the manufacture of Malic Acid.[1] Malic Acid is generally purified until the amounts of fumaric and maleic acid are 7.5 and <500 ppm, respectively.

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

The frequency of use of Malic Acid has increased since safety was originally reviewed, from 47 reported uses in 1998[1] to 238 reported uses in 2017[8] (Table 2). Notably, the number of uses near the eye area and mucous membranes increased from no reported uses to 4 and 19, respectively. The reported maximum concentration of use has increased; the maximum leave-on concentration of use reported was 1% (in multiple formulation types) in 1984,[1] and the results of the survey conducted by the Council in 2016 now indicate that the maximum leave-on use concentration is 2.1% (in a hair spray).[9] It is used at up to 50% in products diluted for baths.

The frequency of use for Sodium Malate has also increased since the original review, from 1 reported use in 1998[1] to 5 reported uses in 2017[8]. Current uses of Sodium Malate are reported in coloring hair care products and skin care preparations. No concentration of use for Sodium Malate was reported in the 2001 safety assessment.[1] The Council in 2016 reported that Sodium Malate is used at 0.02% in "other" skin care preparations.[9]

Malic Acid is used in products that are used near the eye at a maximum concentration of 0.000012% (in eyeliners; no maximum use concentration was reported in 1984 for this category) and in those that can come in contact with mucous membranes at maximum concentrations up to 50% (in bath oils, tablets and salts; again, no previously reported concentrations in this category).[1,9] Additionally, Malic Acid is used in body and hand products and pump hair sprays formulations at concentrations up to 2.1%; these product-types could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles <10 μm compared with pump sprays.[10,11] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[12,13]

Malic Acid and Sodium Malate are not restricted from use in any way under the rules governing cosmetic products in the European Union (EU).[14]

Non-Cosmetic

D- and L-Malic Acid are generally recognized as safe (GRAS) as direct food additives for use as flavor enhancers, flavoring agents, adjuvants, and as pH control agents.[1] These stereochemicals are not GRAS for baby foods.

The *Merck Index* reports that Malic Acid is an intermediate in chemical synthesis.[15] It is a chelating and buffering agent. In foods, it is a flavoring agent, a flavor enhancer, and an acidulant.

TOXICOKINETICS STUDIES

Absorption, Distribution, Metabolism, and Excretion

Most of the radioactivity from 2.5 mg/kg U-^{14}C-L-malic acid (specific activity 61μCi/mmol) or 4-^{14}C-DL-malic acid (specific activity 93 μCi/mmol) administered orally or intraperioneally (i.p.) to male rats was excreted as carbon dioxide.[1] Daily oral administration of 4 g/kg Malic Acid resulted in increased glucuronic acid excretion in the urine.

Skin Penetration

The ability for Malic Acid in rinse-off personal care products to penetrate the skin was assessed in an in vitro study.[16] A shampoo with radiolabeled Malic Acid (L-(U)-^{14}C-malic acid; <1%; pH 5.0-7.0) was applied as a single dose to human epidermal membranes mounted in static diffusion cells. The membranes were not occluded. The exposures were 1 min in duration. Epidermal penetration of Malic Acid from the shampoo was considered negligible, with > 99% removed by rinsing. The actual skin dose for Malic Acid was 2.69 μg/cm^2, the total absorbable dose was 0.003% and the total dose delivered was 0.000067 μg/cm^2.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The oral LD_{50} values of Malic Acid for mice, rats, and rabbits ranged from 2.66 to greater than 3.2, 1.60 to 3.5, and 3 to 5 g/kg, respectively.[1] The acute LD_{50} of Malic Acid given intravenously was 2.4 g/kg for rabbits, and the i.p. LD_{50} for mice and rats were 50 to 100 and 100 to 200 mg/kg, respectively.

Chronic Toxicity Studies

In a chronic oral study in rats, Malic Acid at concentrations up to 50,000 ppm (5.0%) for 104 weeks resulted in some changes in body weight gains and feed consumption, but compound-related lesions were not observed.[1] No significant changes or lesions were observed when dogs were fed Malic Acid at concentrations up to 50,000 ppm for 104 weeks.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Oral dosing of Malic Acid did not cause developmental toxicity in mice (at up to 266 mg/kg), rats (at up to 350 mg/kg), or rabbits (at up to 300 mg/kg).[1] In a multigenerational oral DART study, no significant adverse effects were observed in rats that received up to 10,000 ppm Malic Acid.

GENOTOXICITY STUDIES

In Vitro

Malic Acid was not mutagenic in Ames tests or a chromosomal assay.[1] In one study, pyrolyzates of Malic Acid were not mutagenic, but in another study they were. Products formed from treatment of Malic Acid with aqueous solutions of chlorine were mutagenic.

DL-Malic Acid was not mutagenic in an Ames test in *Salmonella typhimurium* strains TA97 and TA102 when tested with and without metabolic activation.[5] The material was tested at up to 10 mg/plate in distilled water.

CARCINOGENICITY STUDIES

No published carcinogenicity studies on Malic Acid or Sodium Malate were discovered and no unpublished data were submitted either currently or in the 2001 safety assessment.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Animal Studies

Malic Acid was moderately irritating to rabbit skin (500 mg for 24 h) and was a strong irritant to guinea pigs (concentration not reported).[1]

Human Studies

In a test determining subjective skin irritation potential, the average irritation scores over a 15-minute period were 39.4, 37.1, and 23.1 for 1 M Malic Acid at pH 3, 5, and 7, respectively.[1]

In a modified human repeat insult patch test (HRIPT) under a semi-occlusive patch, a hair styler formulation with 0.022725% Malic Acid at pH 3.6 was not a significant skin irritant and did not induce allergic contact dermatitis.[17] This study included 101 subjects.

In another HRIPT, a hair shampoo with 0.00375% Malic Acid at pH 3.0 was not a significant skin irritant and did not induce allergic contact dermatitis.[17] The 98 subjects in this study received occlusive patches.

OCULAR IRRITATION STUDIES

In Vitro Studies

The irritation potential of Malic Acid was tested in formulation in chorioallantoic membrane vascular assays (CAMVA) and bovine corneal opacity and permeability tests (BCOP).[17] Malic acid at 2.2725% was tested in a hair styler and a hair shampoo at pH 3.6 and pH 3.0, respectively. The assays predicted that the formulation with Malic Acid at pH 3.6 would be a severe ocular irritant and the formulation with Malic Acid at pH 3.0 would be an ocular irritant.

Animal Studies

Malic Acid (750 µg) caused severe ocular irritation in rabbit eyes.[1]

CLINICAL STUDIES

In predictive testing using patients with atopic dermatitis, 18 of 34 patients reacted to a diet high in Malic Acid and citric acid, and 6 reacted to a diet high in Malic Acid.[1] In assessing the effect of Malic Acid on cell renewal, an 18%, 10%, and 5% increase was observed at pH 3, 5, and 7, respectively. Malic Acid (200 mg) was not toxic in a clinical efficacy and safety test.

The occupational cumulative irritation potential of Malic Acid with other fruit acids was tested in 20 healthy volunteers.[18] The volunteers were exposed twice daily for 4 days to 2% Malic Acid (pH 2 and pH 4), citric acid, or lactic acid, either alone or in tandem with 0.5% sodium lauryl sulfate (SLS). Positive and negative controls were SLS and distilled water, respectively. Approximately 50 µl of the test materials were applied to each test area on the paravertebral mid back by

occlusive patches (Finn Chambers on Scanpor, 12 mm diameter). The patches were removed after 30 min, rinsed with ~10 ml of tap water, and dried with tissue paper without rubbing. Irritant cutaneous reactions were quantified by visual scoring, transepidermal water loss, and skin color reflectance. The twice daily application of either Malic Acid (pH 2 or pH 4) or citric acid alone did not induce significant irritant reactions and were comparable to the negative control. Combined exposures to one of the acids and SLS caused marked barrier disruption, but the effect was less than that observed from combined exposure to SLS and water, which indicated a protective effect by the fruit acids. The authors of the study concluded that Malic Acid, citric acid, and lactic acid did not significantly contribute to the occurrence of irritant contact dermatitis or increase susceptibility to SLS-induced irritation.

SUMMARY

The frequency of use of Malic Acid has increased since safety was originally reviewed, from 47 reported uses in 1998 to 238 reported uses in 2017. Notably, the number of uses near the eye area and mucous membranes increased from no reported uses to 4 and 19, respectively. The reported maximum concentration of use has increased; the maximum leave-on concentration of use reported was 1% (in multiple formulation types) in 1984, and the results of the survey conducted by the Council in 2016 now indicate that the maximum leave-on use concentration is 2.1% (in a hair spray). It is used at up to 50% in products diluted for baths.

The frequency of use for Sodium Malate has also increased since the original review, from 1 reported use in 1998 to 5 reported uses in 2017. Current uses of Sodium Malate are reported in coloring hair care products and skin care preparations. No concentration of use for Sodium Malate was reported in the 2001 safety assessment. The Council in 2016 reported that Sodium Malate is used at 0.02% in "other" skin care preparations.

Malic Acid is an intermediate in chemical synthesis. It is a chelating and buffering agent. In foods, it is a flavoring agent, a flavor enhancer, and an acidulant.

In an in vitro study, epidermal penetration of <1% radiolabeled Malic Acid (pH 5.0-7.0) in a shampoo was considered negligible, with > 99% removed by rinsing. The actual skin dose for Malic Acid was 2.69 $\mu g/cm^2$, the total absorbable dose was 0.003% and the total dose delivered was 0.000067 $\mu g/cm^2$.

DL-Malic Acid at up to 10 mg/plate was not mutagenic in an Ames test.

In modified HRIPTs, formulations that contained up to 0.00375% Malic Acid were not significant skin irritants and did not induce allergic contact dermatitis.

Malic Acid in formulations at 2.2725% was predicted to be an ocular irritant was tested in vitro.

Malic Acid (2%, pH 2 and pH 4) did not significantly contribute to the occurrence of irritant contact dermatitis or increase susceptibility to SLS-induced irritation

No published carcinogenicity studies on Malic Acid or Sodium Malate were discovered and no unpublished data were submitted.

DISCUSSION FROM THE FINAL SAFETY ASSESSMENT OF MALIC ACID AND SODIUM MALATE

The Expert Panel considered separately the ways in which Malic Acid and Sodium Malate are used. As a pH adjuster, Malic Acid historically has been used at concentrations less than 1%. The available data demonstrate that what toxicity has been demonstrated for Malic Acid and Sodium Malate is related to concentration. Accordingly, the Expert Panel concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters (even though Sodium Malate is not currently used for that purpose).

The data included in this report, however, were insufficient to determine the safety of these ingredients when used in cosmetics as other than pH adjusters. Specifically, the data are insufficient to determine the safety of Sodium Malate when used as a skin conditioning agent – humectant. The types of data required for the Expert Panel to determine the safety of Sodium Malate as a skin conditioning agent are:

1. *concentration of use data;*
2. *dermal irritation and sensitization data; and*
3. *ocular irritation data, if available.*

The data needed to assess the safety of Malic Acid or Sodium Malate for some function other than as a skin conditioning agent – humectant cannot be specified without knowing the intended function. Were these ingredients to be used as exfoliants, for example, data similar to that included in the report on Glycolic and Lactic Acid (i.e. the Alpha Hydroxy Acid report)[19] would be needed.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Physical and chemical properties of Malic Acid.[1]

Physical Form	White or colorless crystals
Molecular Weight (Da)	134.09
Density	1.601 (DL-form); 1.595 (D- or L- form; 20º/4ºC)
Melting Point ºC	126-132 (DL-form): 101 (D-form); 100 (L-form)
Boiling Point ºC	150 (DL-form; decomposes); 140 (D- or L- form; decomposes)
Solubility	Soluble in water; slightly soluble in alcohol and ether

Table 2. Current and historical frequency and concentration of use of Malic Acid and Sodium Malate according to duration and exposure.

	Malic Acid			
	# of Uses		*Max Conc of Use (%)*	
	2017[8]	**1998[1]**	**2016[9]**	**1984[1] [#]**
Totals*	**238**	**47**	**0.000012-50**	**< 0.1-1**
Leave-On	*110*	*31*	*0.000012-2.1*	*< 0.1-1*
Rinse-Off	*126*	*16*	*0.00013-4*	*<0.1-1*
Diluted for (Bath) Use	*2*	*NR*	*0.006-50*	*NR*
Eye Area	4	NR	0.000012	NR
Incidental Ingestion	4	NR	0.0006-0.55	NR
Incidental Inhalation-Spray	3; 26[a]; 22[b]	2; 3[a]; 3[b]	0.0011-2.1; 0.00013-1.9[a]	NR
Incidental Inhalation-Powder	22[b]	3[b]	0.0004-1[c]	NR
Dermal Contact	106	7	0.000012-50	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	100	18	0.00013-4	0.1-1
Hair-Coloring	13	NR	0.00015-0.05	< 0.1-1
Nail	15	22	0.3	< 0.1-1
Mucous Membrane	19	NR	0.0006-50	NR
Baby Products	8	1	NR	NR
	Sodium Malate			
	# of Uses		*Max Conc of Use (%)*	
	2017[8]	**1998[1]**	**2016[9]**	**1984[1] [#]**
Totals*	**5**	**1**	**0.02**	**NR**
Leave-On	*2*	*1*	*0.02*	*NR*
Rinse-Off	*3*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	1[a]	1[b]	NR	NR
Incidental Inhalation-Powder	NR	1[b]	NR	NR
Dermal Contact	3	1	0.02	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	2	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR

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

[#] At the time of the original safety assessment, concentration of use data were not reported by the FDA; however, the FDA provided historic data

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

[b] 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

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

NR – no reported use

REFERENCES

1. Fiume MZ, Bergfeld WF, Belsito DV, Klaassen CD, Schroeter AL, Shank RC, Slaga TJ, Snyder PW, and Andersen FA. Final Report on the Safety Assessment of Malic Acid and Sodium Malate. *Int J Toxicol*. 2001;20(Suppl 1):47-55.

2. Web-Based Ingredient Dictionary (wINCI): Malic Acid. http://webdictionary.personalcarecouncil.org/jsp/IngredientDetail.jsp?monoid=1538. Washington, DC. Last Updated 2017. Date Accessed 4-27-2017.

3. Web-Based Ingredient Dictionary (wINCI): Sodium Malate. http://webdictionary.personalcarecouncil.org/jsp/IngredientDetail.jsp?monoid=9633. Washington, DC. Last Updated 2017. Date Accessed 4-27-2017.

4. Horton HR, Moran LA, Ochs RS, Rawn JD, and Scrimgeour KG. The Citric Acid Cycle. Chapter: 13. In: *Principles of Biochemistry*. 2nd ed. Prentice-Hall, Inc.; 1996:355-380.

5. European Chemicals Agency. Malic Acid. http://echa.europa.eu/. Last Updated 2017. Date Accessed 4-27-2017.

6. Robinson VC, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DA, Marks JG, Shank RC, Slaga TJ, Snyder PW, and Andersen FA. Safety Assessment of Fumaric Acid and Related Salts and Esters as Used in Cosmetics. Washington, DC, Cosmetic Ingredient Review. 2009. http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/FR464.pdf.

7. Fiume MZ, Heldreth B, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DA, Marks JG, Shank RC, Slaga TJ, Snyder PW, and Andersen FA. Final Report of the Cosmetic Ingredient Review Expert Panel on the Safety Assessment of Dicarboxylic Acids, Salts, and Esters. *Int J Toxicol*. 2012;31(Suppl 1):5S-76S. http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/pr528.pdf.

8. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2017. Washington, DC: FDA.

9. Personal Care Products Council. 2-8-2016. Concentration of Use by FDA Product Category: Malic Acid and Sodium Malate. Unpublished data submitted by Personal Care Products Council.

10. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27.

11. Rothe H. Special Aspects of Cosmetic Spray Evalulation. 9-26-2011. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, D.C.

12. Bremmer HJ, Prud'homme de Lodder LCH, and Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006. Report No. RIVM 320104001/2006. pp. 1-77.

13. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.

14. European Union. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. 2009. Date accessed 5-17-2016. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

15. O'Neil MJ (ed.). The Merck Index. 15th *ed*. Royal Society of Chemistry, 2013.

16. Okuda M, Donahue DA, Kaufman LE, Avalos J, Simion FA, Story DC, Skaguchi H, Fautz R, and Fuchs A. Negligible penetration of incidental amounts of alpha-hydroxy acid from rinse-off personal care products in human skin using an in vitro static diffusion cell model. *Toxicol In Vitro*. 2011;25:2041-2047.

17. Personal Care Products Council. 9-19-2011. Summaries of Studies of Products Containing Malic Acid. Unpublished data submitted by the Personal Care Products Council. 3 pages.

18. Schliemann-Willers S, Fuchs S, Kleesz P, Grieshaber R, and Elsner P. Fruit acids do not enhance sodium lauryl sulphate-induced cumulative irritant contact dermatitis *In vivo*. *Acta Derm Venereol*. 2005;85:206-210.

19. Fiume MZ, Bergfeld WF, Belsito DV, Carlton WW, Klaassen CD, Schroeter AL, Shank RC, Slaga TJ, and Andersen FA. Final report on the safety of Glycolic Acid, Ammonium, Calcium, Potassium, and Sodium Glycolates, Methyl, Ethyl, Propyl, and Butyl Glycolates, and Lactic Acid, Ammonium, Calcium, Potassium, Sodium, and TEA-Lactates, Methyl, Ethyl,

Isopropyl, and Butyl Lactate, and Lauryl, Myristyl, and Cetyl Lactates. *Int J Toxicol*. 1998;17(Suppl 1):1-241. http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/pr34.pdf.

Final Report on the Safety Assessment of Malic Acid and Sodium Malate[1]

Malic Acid functions in cosmetic formulations as a pH adjuster, and Sodium Malate functions as a skin conditioning agent-humectant. Malic Acid is reportedly used in almost 50 cosmetic formulations across a range of product types at low concentrations, whereas Sodium Malate is used in only one. As a pH adjuster, Malic Acid is used at low concentrations. One commercial method of preparing Malic Acid is hydration of fumaric acid or maleic acid, and then purified to limit the amount of the starting material present. Because Malic Acid is a component of the Kreb's cycle, another method is fermentation. Malic Acid was relatively nontoxic in acute toxicity studies using animals. In a chronic oral study, feeding Malic Acid to rats resulted only in weight gain changes and changes in feed consumption. Malic Acid did not cause reproductive toxicity in mice, rats, or rabbits. Malic Acid was a moderate to strong skin irritatant in animal tests, and was a strong ocular irritant. Malic Acid was not mutagenic across a range of genotoxicity tests. Malic Acid was irritating in clinical tests, with less irritation seen as pH of the applied material increased. Patients patch tested with Malic Acid, placed on a diet that avoided foods containing Malic or citric acid, and then challenged with a diet high in Malic and citric acid had both immediate urticarial and delayed contact dermatitis reactions. These data were considered sufficient to determine that Malic Acid and Sodium Malate would be safe at the low concentrations at which these ingredients would be used to adjust pH (even though Sodium Malate is not currently used for that purpose). The data, however, were insufficient to determine the safety of these ingredients when used in cosmetics as other than pH adjusters and specifically, the data are insufficient to determine the safety of Sodium Malate when used as a skin conditioning agent-humectant. The types of data required for the Expert Panel to determine the safety of Sodium Malate as a skin-conditioning agent are: concentration of use data; dermal irritation and sensitization data; and ocular irritation data, if available. The data needed to assess the safety of Malic Acid or Sodium Malate for some function other than as a skin-conditioning agent cannot be specified without knowing the intended function. Were these ingredients to be used as exfoliants, for example, data similar to that included in the Cosmetic Ingredient Review safety assessment of Glycolic Acid would be needed. Until these data are available, it is concluded that the available data are insufficient to support the safety of these ingredients in cosmetic formulations for functions other than use as a pH adjuster.

Received 15 June 2000; accepted 2 November 2000.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Monice Zondlo Fiume, former Scientific Analyst/Report Management Coordinator, prepared this report. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 20(Suppl. 1):47–55, 2001

1071-7544/01 $12.00 + .00

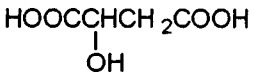
INTRODUCTION

The safety of Malic Acid and Sodium Malate as used in cosmetic formulations is reviewed in this report. Malic Acid functions in cosmetics as a pH adjuster and Sodium Malate functions as a skin conditioning agent-humectant (Wenninger, Canterbery, and McEwen 2000).

CHEMISTRY

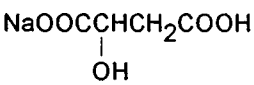
Definition and Structure

Malic Acid (CAS No. 97-67-6, Wenninger, Canterbery, and McEwen 2000; 6915-15-7, Lewis 1993a; 1993b) is the organic acid that conforms to the formula (Wenninger, Canterbery, and McEwen 2000):



Malic Acid is also known as (±)-Malic Acid (US Pharmacopeial Convention, Inc. 1995); D,L-Malic Acid (Food and Agriculture Organization of the United Nations/World Health Organization [FAO/WHO] 1994; Food and Drug Research Labs, Inc. [FDRL] 1973a); DL-Malic Acid (Wenninger, Canterbery, and McEwen 2000); Hydroxysuccinic Acid; Hydroxybutanedioic Acid (Wenninger, Canterbery, and McEwen 2000; FAO/WHO 1994; FDRL 1973a); (±)-Hydroxysuccinic Acid; Hydroxybutanedioic Acid, (±)- (US Pharmacopeial Convention, Inc. 1995); Butanedioic Acid, Hydroxy- (Wenninger, Canterbery, and McEwen 2000); Succinic Acid, Hydroxy-; alpha-Hydroxysuccinic Acid; Deoxytetraric Acid (Registry of Toxic Effects of Chemical Substances [RTECS] 1997); 1-Hydroxy-1,2-Ethanedicarboxylic Acid; Methyl Tartronic Acid (FDRL 1973a); Oxyethylenesuccinic Acid (Grant 1972); Pomalous Acid (FAO/WHO 1994); and Apple Acid (Lewis 1993b).

Sodium Malate (CAS No. not available) is the sodium salt of Malic Acid (q.v.) that conforms to the formula (Wenninger, Canterbery, and McEwen 2000):



Sodium Malate is also known as Malic Acid, Monosodium Salt (Wenninger, Canterbery, and McEwen 2000).

TABLE 1
Physical and chemical properties of Malic Acid

Property	Description	Reference
Physical characteristics	White crystals	Nikitakis and McEwen 1990
	White or nearly white crystalline powder or granules with a strongly acidic taste	National Academy of Sciences (NAS) 1996
	Colorless crystals with a sour taste	Lewis 1993a
	White or colorless crystals with an acid taste	Lewis 1993b
	White odorless triclinic crystals with a smoothly tart taste	Furia 1972
Molecular weight	134.09 Da	NAS 1996; Budavari 1989
Grades	Technical, active, and inactive	Lewis 1993a
Melting point		
DL-form	126–132°C	Nikitakis and McEwen 1990
	128°C	Lewis 1993a; 1993b
	131–132°C	Budavari 1989
D-(+)-form	101°C	Budavari 1989
L-(−)-form	100°C	Budavari 1989
Boiling point		
DL-form	150°C (decomposes)	Lewis 1993a
D- or L-form	140°C (decomposes)	Lewis 1993a
Density		
DL-form	1.601	Lewis 1993a
D- or L-form	1.595 (20°/4°C)	Lewis 1993a
Solubility	Soluble in water; slightly soluble in alcohol and ether	Nikitakis and McEwen 1990
	Very soluble in water and alcohol; slightly soluble in ether	Lewis 1993a; 1993b
	Soluble in water, methanol, ethanol, acetone, diethyl ether, and dioxane; practically insoluble in benzene	Budavari 1989
Optical rotation		
L-(−)-form	$[\alpha]_D - 2.3°$ (8.5 g in 100 ml water)	Budavari 1989
Ionization constants		
K_{A1}; K_{A2}	3.9×10^{-4}; 1.4×10^{-5}	Miltenberger 1989
K_1; K_2	4×10^{-4}; 9×10^{-6}	Furia 1972
Reactivity	Combustible	Lewis 1993a

Physical and Chemical Properties

The physical and chemical properties of Malic Acid are described in Table 1.

Manufacture and Production

DL-Malic Acid is made by the catalytic oxidation of benzene to maleic acid, which is converted to Malic Acid by heating with steam under pressure (Lewis 1993b). Malic Acid can be prepared by fermentation from sugars (Anonymous 1975). L-Malic Acid is available through the microbiological fermentation of fumaric acid (Miltenberger 1989). The L-form of Malic Acid is the naturally occurring isomer and is found in unripe apples and other fruits (Lewis 1993b).

Analytical Methods

Malic Acid has been detected and quantitated in biological fluids using gas chromatography (GC), enzymatic methods, and fluorometry; in general foods and in fruits and fruit derivatives using fluorometry, GC, gas-liquid chromatography, thin-layer chromatography (TLC), paper chromatography, polarimetry, manometry, and ion exchange plus ultraviolet (UV); and in synthetic mixtures of food acids using TLC, thin-layer electrophoresis plus chromatography, and fluorometry (FDRL 1973a). Liquid chromatography (Agarwal 1988; Eisele 1996), GC (Agarwal 1988), and ligand-exchange photometric ion chromatography (Yamamoto, Matsunaga, and Mizukami 1991) have been used to determine Malic Acid in apple juice. A spectrophotometric method with use of a malic enzyme was used to determine Malic Acid in other liquids (Suye, Yoshihara, and Inuta 1992).

Impurities

Maleic and fumaric acids are by-products of the manufacture of Malic Acid (Miltenberger 1989). Malic Acid is generally purified until the amounts of fumaric and maleic acid are 7.5 and <500 ppm, respectively.

USE

Cosmetic

Malic Acid functions as a pH adjuster and Sodium Malate functions as a skin conditioning agent—humectant in cosmetic formulations (Wenninger, Canterbery, and McEwen 2000). The product formulation data submitted to the FDA in 1998 stated that Malic Acid was contained in 47 cosmetic product formulations and that Sodium Malate was contained in one cosmetic formulation (FDA 1998). Concentration of use values are no longer reported to the FDA by the cosmetics industry (FDA 1992) and no current concentration of use data were provided by industry; however, historical product formulation data submitted to the FDA in 1984 stated that Malic Acid was used at concentrations of $\leq$1% (FDA 1984). Such low concentrations are expected to correspond to use as a pH adjuster. Sodium Malate was not reported to be used in 1984. This information is summarized in Table 2.

International

Malic Acid is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (*CLS*) as DL-Malic Acid (Rempe and Santucci 1997). DL-Malic Acid, which conforms to the specifications of the *Japanese Cosmetic Ingredients Codex* or *Japanese Standards of Food Additives*, has precedent for use without restriction in all *CLS* categories except eyeliner preparations, for which there is no precedent for use. Malic Acid does not appear in Annex II (list of substances which must not form part of the composition of cosmetic products) or Annex III (list of substances which cosmetic products must not contain except subject to restrictions and conditions) of the Cosmetics Directive of the European Union (European Economic Community 1995).

Noncosmetic

DL- and L-Malic Acid, when meeting Food Chemicals Codex specifications, are generally recognized as safe (GRAS) as direct food additive for use as a flavor enhancers, flavoring agents, and adjuvants, and as pH control agent, but are not GRAS for use in baby foods (FDA 1997). Using good manufacturing practices, the following maximum concentrations are allowed in foods as served: hard candy, 6.9%; processed fruits and fruit juices, 3.5%; nonalcoholic beverages, 3.4%; soft candy, 3.0%; chewing gum, 3.0%; jams and jellies, 2.6%; gelatins, puddings, and fillings, 0.8%; and all other food categories, 0.7%. Malic Acid can be used as an acidifying ingredient in milk and cream, and can be used in French dressing, mayonnaise, and salad dressing. There is no set limit on the human acceptable daily intake (ADI) of L-Malic Acid, and the ADI for D-Malic Acid is limited only by good manufacturing practice (FAO/WHO 1967; 1969). Neither D- or DL-Malic Acid should be added to food of young infants.

TABLE 2
Product formulation and concentration of use data

Product category (number of formulations reported to FDA) (FDA 1984)	Number of formulations containing ingredient (FDA 1998)	Historical concentration of use (FDA 1984)
Malic Acid		
Other baby products (29)	1	
Hair conditioners (636)	8	
Hair sprays (aerosol fixatives) (261)	2	
Shampoos (noncoloring) (860)	7	
Tonics, dressings, and other hair-grooming aids (549)	1	
Wave sets (55)	—	0.1–1.0%
Hair rinses (coloring) (33)	—	<0.1–1.0%
Basecoats and undercoats (48)	9	<0.1%
Nail polish and enamel (80)	9	<0.1–1.0%
Other manicuring preparations (61)	4	<0.1–1.0%
Face and neck preparations (excluding shaving preparations) (263)	2	
Body and hand preparations (excluding shaving preparations) (796)	1	
Moisturizing preparations (769)	1	
Night preparations (188)	1	
Paste masks (mud packs) (255)	1	
1998 total for Malic Acid	47	
Sodium Malate		
Body and hand preparations (excluding shaving preparations) (796)	1	
1998 total for Sodium Malate	1	

Malic Acid is used in foods as an acidifier and flavoring agent (National Academy of Sciences [NAS] 1996). It can also be used as a discoloration inhibitor and a synergist with antioxidants (Furia 1972). It is used in the manufacture of various esters and salts, in wine manufacturing, and as a chelating agent (Lewis 1993a). Malic Acid is also used in medicine and in the preparation of esters and salts (Patty 1981-2).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Male albino Wistar Alderly Park SPF rats were given 2.5 mg/kg ^{14}C-L-Malic Acid (diluted with L-Malic Acid to a specific activity of 61 μCi/mmol) or 4-^{14}C-DL-Malic Acid (specific activity 93 μCi/mmol) in an aqueous solution by gavage or by intraperitoneal (IP) injection (Daniel 1969). (The number of animals per group was not specified.) Urine, feces, and expired carbon dioxide were collected. Most of the radioactivity was excreted as carbon dioxide; after 24 hours, 91.6% and 83.4% of orally and intraperitoneally administered DL-Malic Acid, respectively, and 88.0% and 86.6% of orally and intraperitoneally administered L-Malic Acid, respectively, was found in expired air. The amount of radioactivity recovered after oral and IP administration of DL-Malic Acid was 3.1% and 8.8% in the urine and 0.6% and 0.3% in the feces, respectively, and the amount recovered after oral and IP administration of L-Malic Acid was 3.2% and 3.1% in the urine and 1.4% and 1.4% in the feces, respectively. After 24 hours, the total amount of radioactivity recovered was 95.3% and 92.5% after oral and IP administration of DL-Malic Acid, respectively, and 92.6% and 91.1% after oral and IP administration of L-Malic Acid, respectively.

Daily oral administration of 4 g/kg Malic Acid resulted in increased glucuronic acid excretion in the urine (Martin and Stenzel 1944).

Biochemistry

Malic Acid is an intermediate in the tricarboxylic acid (Kreb's) cycle (Taylor 1988). It is formed from fumaric acid and is oxidized to oxaloacetic acid (Patty 1981-2). Malic Acid plays an essential role in carbohydrate metabolism (Liebrand 1992).

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The oral LD_{50} values of Malic Acid for albino CD-1 outbred mice, albino Wistar rats, and Dutch-Belted rabbits were approximately 2.66 (FDRL 1973b), 3.5 (FDRL 1973c), and 3 g/kg (FDRL 1973d) respectively. Each study used 50 animals, consisting of five groups of 5 males and 5 females, and Malic Acid was administered as a 25% aqueous solution. Mortality was observed for 14 days. Signs of toxicity included ataxia, prostration, convulsions, and death.

In a review of studies done in the 1920s, FAO/WHO (1967) stated that the oral lethal dose of L-Malic Acid for rabbits was 5 g/kg, and for Sodium Malate in dogs was 1 g/kg. In a more recent review, the oral LD_{50} of Malic Acid for rabbits was 5 g/kg (Sax 1979). In a review of industrial chemicals, Patty (1981–2) stated that the oral LD_{50} values of Malic Acid for mice and rats were reported to be 1.6 to 3.2 and >3.2 g/kg, respectively. The signs of acute poisoning in rats and mice were weakness, retraction of the abdomen, respiratory distress, and cyanosis.

Parenteral

The acute toxicity of intravenously administered 0.25 N Malic Acid aqueous solution to four rabbits was 2.4 g/kg (FDRL 1973a).

The IP administration to rats of 1 g/kg L-Malic Acid was not lethal, but the same dose of D-Malic Acid killed rats within 20 to 25 minutes (Brookdale Dental Center of New York University 1973). The vehicle was not reported. A mixture of 1 g/kg D-Malic Acid and 1 g/kg L-malic Acid was lethal, and death occurred sooner than it did with D-Malic Acid alone. The IP administration of 2 g/kg DL-Malic Acid was not lethal to rats.

In a review of hazardous substances, Patty (1981–2) reported that the IP LD_{50} of Malic Acid for mice and rats as 50 to 100 and 100 to 200 mg/kg, respectively.

Chronic Toxicity

Oral

Groups of 30 male and 30 female Charles River rats were fed 500, 5000, or 50,000 ppm (0.05%, 0.5%, and 5.0%, respectively) Malic Acid for 104 weeks, and a control group of 60 male and 60 female rats was given untreated feed (TRW/Hazleton Laboratories 1971a). Animals were observed daily for mortality. Clinical observations were made and body weights and feed consumption were determined weekly for the first 26 weeks, biweekly for the second 26 weeks, and monthly thereafter. Clinical pathology studies were performed on five males and five females per group prior to study initiation and at 13, 26, 52, and 104 weeks. After 26 and 52 weeks, 5 male and 5 female test animals per group and 10 male and 10 female control animals were killed; the remaining animals were killed at study termination.

Physical appearance, behavior, and survival were similar for test and control animals. Body weight gains were significantly decreased for males and females of the high-dose group during weeks 0 to 52. Feed consumption was statistically significantly decreased for males of the high-dose group during this period. For females of the high-dose group, feed consumption was significantly decreased during weeks 0 to 26 and decreased, but not to a significant degree, during weeks 27 to 52, as compared to controls. These differences were less distinct during the second year; terminal body weights of the high-dose group were similar to controls for male animals and decreased, but not significantly, for female animals. Significant changes were not observed in hematological, blood, or urine parameters. Significant lesions were not found at gross and microscopic examination. For males

of the high-dose group, relative thyroid weights were significantly decreased at week 26, relative testes weights were significantly increased and liver weights were significantly decreased at week 52, and spleen weights were significantly increased and relative kidney weights were significantly decreased at study termination as compared to control animals. For females of the high-dose group, heart and body weights were significantly decreased at week 26, body weights were significantly decreased at week 52, and thyroid gland weights were significantly decreased at study termination. These differences were considered incidental.

Groups of four male and female beagle dogs were fed 500, 5000, or 50,000 ppm Malic Acid for 104 weeks, and a control group was given untreated feed (TRW/Hazleton Laboratories 1971b). Clinical observations were made daily. Body weights and feed consumption were determined weekly for the first 26 weeks, biweekly for the second 26 weeks, and monthly thereafter. Clinical pathology studies were performed prior to study initiation and at 4, 13, 26, 52, 78, and 104 weeks. One male and one female from each group was killed after 52 weeks, and the remaining animals were killed at study termination.

Body weight gains were normal for all animals. Significant changes were not observed in hematological, blood, or urine parameters. Significant lesions were not observed at necropsy or at microscopic examination, and dose-related differences in absolute and relative organ weights were not found.

Dermal Irritation

In a review of industrial chemicals, Patty (1981–2) stated that Malic Acid was moderately irritating to rabbit skin (500 mg/24 h) and was a strong irritant to guinea pigs.

Ocular Irritation

In a review of industrial chemicals, Patty (1981–2) stated that application of Malic Acid, 750 μg, to the conjunctival sac of rabbits caused severe ocular irritation.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of 25 albino CD-1 outbred mice were mated and dosed orally with 2.66, 12.4, 57.3, or 266 mg/kg Malic Acid on days 6 through 15 of gestation (FDRL 1974a). A negative-control group was given vehicle (water) and a positive-control group was given 150 mg/kg aspirin. All animals were observed daily. Body weights were determined on days 0, 6, 11, 15, and 17 of gestation. On day 17 of gestation, the number of implantation sites, resorption sites, and live and dead neonates were determined. The body weights of live pups were recorded, and all neonates were examined grossly.

At gross examination, 19, 22, 21, and 21 animals of the 2.66, 12.4, 57.3, and 266 mg/kg dose groups, respectively, were gravid. All animals except one of the 12.4 mg/kg test group survived until study termination. The researchers concluded that "the administration of up to 266 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in sham-treated controls."

A study following a similar procedure was conducted using groups of 25 to 29 Wistar albino rats dosed orally with 3.5, 16.2, 75.4, or 350 mg/kg Malic Acid (FDRL 1974b). The number of implantation sites, resorption sites, and live and dead neonates were determined on day 20 of gestation. At gross examination, 20/25, 21/29, 22/25, and 26/28 animals of the 3.5, 16.2, 75.4, and 350 mg/kg dose groups, respectively, were gravid. All animals except three of the 350-mg/kg test group (two were gravid) survived until study termination. The researchers concluded that "the administration of up to 350 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in sham-treated controls."

A study was also conducted using groups of 15 to 23 Dutch-belted rabbits that were inseminated artificially and dosed orally with 3, 14, 65, or 300 mg/kg Malic Acid on days 6 to 18 of gestation (FDRL 1974c). A negative-control group was given water and a positive-control group was dosed with 6-aminonicotinamide. All animals were observed daily and body weights were determined on days 0, 6, 12, 18, and 29 of gestation. On day 29 of gestation, the number of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were determined.

At gross examination, 12/15, 10/20, 13/15, and 13/23 animals of the 3, 14, 65, and 300 mg/kg dose groups, respectively, were gravid. All animals of the negative and positive control groups and the 3 and 14 mg/kg dose groups, 12/15 of the 65-mg/kg dose group (two were gravid), and 15/23 of the 300-mg/kg dose group (four were gravid) survived until study termination. The researchers concluded that "the administration of up to 300 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in sham-treated controls."

Groups of 10 male and 20 female weanling albino rats were fed 1000 or 10,000 ppm Malic Acid for 9 weeks prior to mating for the F_{1A} litter through weaning of the F_{1B} litter, and a control group was fed untreated feed (Hazleton Laboratories, Inc. 1970). The F_{1A} litters were culled to a maximum of eight pups, reproductive indices were monitored, and after 21 days, approximately one third of the pups were necropsied. One week after weaning of the last F_{1A} litter, the P_1 parents were remated to produce the F_{1B} litter, which was also culled and monitored. After 21 days, 10 male and 20 female weanlings from each group were selected for the P_2 generation. Approximately one third of the remaining pups were necropsied. The P_2 generation was

fed the appropriate diet and mated when the animals reached approximately 100 days of age to produce the F_{2A} generation, and the same procedures were followed as above. One half of the F_{2B} litters were delivered naturally and held until weaning, whereas the other half were delivered by caesarean section on day 19 of gestation.

Prior to mating of the P_1 generation, body weight gains of males of the test groups were slightly decreased compared to control animals; female body weights were comparable. Feed consumption and survival were similar for test and control animals. Appearance and behavior were similar for P_1 test and control rats. For all litters, the various indices, litter sizes, and pup body weights were comparable among test and control animals. In the F_{1A} litters, all of the necropsied pups in three of the low-dose litters had rough surfaces on the spleen. In the F_{2A} litters, the number of pups that were weak or had labored respiration during lactation was increased in the high-dose group. Abnormal findings were not reported at necropsy. None of the P_1 animals died during the F_{1A} or F_{1B} phase. The P_2 test and control animals were similar throughout the study; wheezing was observed in all groups during the F_{2B} phase. In the F_{2A} litters, renal discoloration (two animals), dark renal medullas (four animals), rough surfaces on the spleen (four animals), and white foci on the spleen (three animals) were found in low-dose weanling animals. Renal discoloration (three animals), dark red corticomedullary zones (three animals), dark renal medullas (three animals), rough surfaces on the spleen (two animals), and a firm, enlarged, irregularly-shaped cecum with a hole penetrating it (one animal) were found in high-dose weanling animals at necropsy. In the F_{2B} litters, weakness and labored respiration were reported for a few low-dose pups, and the renal pelvis of one high-dose pup was dilated at necropsy. The animals of the F_{2B} generation delivered by caesarean section had no "meaningful differences" between test and control animals in the number and placement of implantation and resorption sites or in the number, weight, or length of live neonates, and none of the neonates died. The skeletal development of the F_{2B} neonates was similar between test and control animals. Slight differences in developmental indices were "considered to be within the range of normal variations in fetal development. No trends toward lesser or greater skeletal development were observed."

GENOTOXICITY

The mutagenic potential of 0.001% Malic Acid was determined in a plate test using *Salmonella typhimurium* strains TA1535, TA1537, and TA1538 without and with metabolic activation (Litton Bionetics, Inc. 1974). Negative and positive controls were used, and duplicate testing was done. Malic Acid was not mutagenic.

An Ames test was performed to determine the mutagenic potential of Malic Acid in phosphate buffer, $\leq$10.0 mg/plate, using *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98 with metabolic activation (Ishidate et al. 1984). Testing was done in duplicate. Malic Acid was not mutagenic.

The mutagenic potential of 1100 to 2000 μg/plate Malic Acid in distilled water was determined in a plate test using *S. typhimurium* TA97, TA98, TA100, and TA104 with and without metabolic activation (Al-Ani and Al-Lami 1988). Distilled water was used as a negative control and 2-aminoanthracene was used as a positive control. Testing was done in triplicate. Malic Acid was not mutagenic.

The mutagenic potential of Malic Acid was examined in a suspension test using *S. typhimurium* strains TA1535, TA1537, and TA1538 and *Saccharomyces cerevisiae* strain D4 without and with metabolic activation (Litton Bionetics, Inc. 1974). Malic Acid was tested at concentrations of 0.0005% and 0.001% using *S. typhimurium* and 0.05% and 0.1% using *S. cerevisiae*. Negative and positive controls were used. Malic Acid was not mutagenic for either *S. typhimurium* or *S. cerevisiae*.

A chromosomal aberration test was performed without metabolic activation using a Chinese hamster fibroblast cell line to determine the mutagenic potential of $\leq$1.0 mg/ml Malic Acid in physiological saline (Ishidate et al. 1984). The incidence of polyploid cells and cells with structural chromosomal aberrations was 0% and 1%, respectively, after 48 hours. Malic Acid was not mutagenic.

The effect of pyrolysis on the mutagenic potential of Malic Acid was first determined using *S. typhimurium* TA98 and TA100 with and without metabolic activation (Yoshida and Okamoto 1982). In this study, pyrolyzates of Malic Acid were not mutagenic. In a study by Kuroda, Yoshida, and Mizusaki (1985), pyrolyzates of Malic Acid were mutagenic when tested using *S. typhimurium* TA97 with and without metabolic activation. The pyrolyzates of Malic Acid were fractionated into neutral, acidic, phenolic, and basic fractions, and the mutagenicity of each fraction was determined using TA97 and TA98. Most of the mutagenicity was found in the neutral fraction, with TA97 being more sensitive, and weak activity was found in the acidic and phenolic fractions. No activity was found in the basic fractions with either strain.

Malic Acid was treated with aqueous solutions of chlorine, pH 2.5, 4, and 7 (Chang et al. 1988). Diethyl ether extraction followed by gas chromatography/mass spectrometry (GC/MS) was performed and the results indicated that "large amounts" of trichloroacetaldehyde were present in the treated Malic Acid. Methyl esters of dichloro- and trichloroacetic acid were detected by GC/MS analysis when comparably treated Malic Acid was reacted with diazomethane. The products that are formed are considered mutagenic.

CARCINOGENICITY

Published data on the carcinogenic potential of Malic Acid and Sodium Malate were not found.

CLINICAL ASSESSMENT OF SAFETY

Irritation

The subjective skin irritation potential of Malic Acid was evaluated by applying 2 mg/cm^2 of 1 M Malic Acid in vehicle

(15% ethanol [SD 40], 5% ethoxydiglycol, and 5% butylene glycol) to the nasal fold area of at least 10 subjects (Smith 1996). Irritation was graded on a scale of 0 to 4 every minute for 15 minutes. The irritation scores, as an average of the summation of each individual irritation score over the 15-minute test period, were 39.4, 37.1, and 23.1 for pH 3, 5, and 7, respectively.

Sensitization

Predictive Testing

Thirty-four patients with atopic dermatitis were tested to determine their sensitivity to foods containing Malic (and citric) Acid (Walsh 1979). The patients were first patch tested with Malic (and citric) Acid applied as a 10% aqueous solution under occlusive patches for 48 hours. For 2 weeks, the patients followed a diet that avoided processed foods in which Malic (and citric) Acid were used, and then challenged themselves with a diet high in Malic (and citric) Acid the during the third week. Eighteen patients reacted to both Malic and citric Acid and 6 patients reacted to only Malic Acid. Both immediate reactions (seasonal allergic rhinitis and urticaria) and delayed reactions (contact dermatitis) were present. Patch-test results were reliable in predicting results of the challenge with diet.

Skin Effects

The effect of Malic Acid on cell renewal was assessed using the dansyl chloride method (Smith 1996). Two mg/cm^2 of 1 M Malic Acid in a simple liquid vehicle (15% ethanol [SD 40], 5% ethoxydiglycol, and 5% butylene glycol) was applied to the volar forearm which was stained with dansyl chloride twice daily until all the stain was removed. An 18%, 10%, and 5% increase in cell renewal was observed at pH 3, 5, and 7, respectively.

Medical/Therapeutic

The data from clinical use of Malic Acid are included here to provide a complete record of reported dermal effects. Information included in this section represents the opinions of researchers; such information is only included in order to provide the full scope of information available. Inclusion is not an endorsement of validity.

Fourteen patients, 11 males and 3 females, with various forms of ichthyosiform dermatoses were used to evaluate the therapeutic potential of more than 60 chemicals, including Malic Acid (Van Scott and Yu 1974). Malic Acid was dissolved in either water or ethanol and incorporated into a hydrophilic ointment of plain petrolatum. The ointment, containing 5% Malic Acid (pH not specified), was applied twice daily to the appropriate test site for 2 weeks. Daily to weekly observations were made. Malic Acid provided 3+ (disappearance of scales from lesions) or 4+ (restoration to normal looking skin) improvement in all patients except one with epidermolytic hyperkeratosis.

An efficacy and safety test of a tablet containing 200 mg Malic Acid (and 50 mg magnesium) was conducted using patients with primary fibromyalgia syndrome (Russell et al. 1995). In the first part of the test, 24 patients were given three tablets twice daily (bid) for 4 weeks. In the second part, 16 patients started with three tablets bid and increased the dosage every 3 to 5 days as necessary; at month 6, the average dose was 8.8 tablets per day. (For a 50-kg person, ingestion of six tablets would be equivalent to 24 mg of malate/kg of body weight.) In the first part of the study, one test patient reported diarrhea, one reported nausea, and one reported dyspepsia. (In the placebo group, two patients reported diarrhea and one reported dyspepsia.) In the second part of the study, five test patients reported diarrhea, one reported nausea, one reported dyspepsia, one reported panic attacks, and one reported dizziness.

SUMMARY

Malic Acid, an intermediate in the Kreb's cycle, is an organic acid that functions as a pH adjuster and Sodium Malate is an organic salt that functions as a chemical additive. In 1998, it was reported to the Food and Drug Administration (FDA) that Malic Acid was used in 47 cosmetic formulations and that Sodium Malate was used in 1 formulation. In 1984, Malic Acid was reported to be used at concentrations of $\leq$1%; Sodium Malate was not reported to be used in 1984.

Malic Acid is generally purified until the amounts of the by-products fumaric and maleic acid are 7.5 and <500 ppm, respectively. Malic Acid is a direct food additive.

Upon oral and IP administration of radioactive Malic Acid to rats, most of the radioactivity was excreted as carbon dioxide.

The oral LD_{50} values of Malic Acid for mice, rats, and rabbits ranged from 2.66 to >3.2, 1.60 to 3.5, and 3 to 5 g/kg, respectively. The acute LD_{50} of Malic Acid given intravenously was 2.4 g/kg for rabbits, and the IP LD_{50} values for mice and rats were 50 to 100 and 100 to 200 mg/kg, respectively. In a chronic oral study, feeding Malic Acid to rats resulted in some changes in body weight gains and feed consumption, but compound-related lesions were not observed. No significant changes or lesions were observed when dogs were fed Malic Acid in a chronic study. Malic Acid did not cause reproductive toxicity in mice, rats, or rabbits.

Malic Acid was moderately irritating to rabbit skin and was a strong irritant to guinea pigs. Malic Acid caused severe ocular irritation in rabbit eyes.

Malic Acid was not mutagenic in plate tests, an Ames test, a suspension test, or a chromosomal aberration assay. In one study, pyrolyzates of Malic Acid were not mutagenic, but in another study they were. Products formed from treatment of Malic Acid with aqueous solutions of chlorine were mutagenic.

In a test determining the subjective skin irritation potential, the average irritation scores over a 15-minute period were 39.4, 37.1, and 23.1 for Malic Acid at pH 3, 5, and 7, respectively. In predictive testing using patients with atopic dermatitis, 18 of 34 patients reacted to a diet high in Malic and citric acids, and 6 reacted to a diet high in Malic Acid. In assessing the effect of

Malic Acid on cell renewal, an 18%, 10%, and 5% increase was observed at pH 3, 5, and 7, respectively.

Malic Acid was not toxic in a clinical efficacy and safety test.

DISCUSSION

The Expert Panel considered separately the ways in which Malic Acid and Sodium Malate are used. As a pH adjuster, Malic Acid historically has been used at concentrations less than 1%. The available data demonstrate that what toxicity has been demonstrated for Malic Acid and Sodium Malate is related to concentration. Accordingly, the Expert Panel concluded that Malic Acid and Sodium Malate are safe for use as pH adjusters (even though Sodium Malate is not currently used for that purpose).

The data included in this report, however, were insufficient to determine the safety of these ingredients when used in cosmetics as other than pH adjusters. Specifically, the data are insufficient to determine the safety of Sodium Malate when used as a skin conditioning agent—humectant. The types of data required for the Expert Panel to determine the safety of Sodium Malate as a skin conditioning agent are:

1. concentration of use data;
2. dermal irritation and sensitization data; and
3. ocular irritation data, if available.

The data needed to assess the safety of Malic Acid or Sodium Malate for some function other than as a skin conditioning agent—humectant cannot be specified without knowing the intended function. Were these ingredients to be used as exfoliants, for example, data similar to that included in the report on Glycolic and Lactic Acid (i.e., the Alpha Hydroxy Acid report) (Andersen 1998) would be needed.

CONCLUSION

On the basis of the animal and clinical data included in this report, the Cosmetic Ingredient Review (CIR) Expert Panel concludes that Malic Acid and Sodium Malate are safe for use as pH adjusters in cosmetic formulations. The Expert Panel determined that the data are insufficient to determine the safety of these ingredients for any other functions.

REFERENCES

Agarwal, V. K. 1988. Simultaneous determination of D- and L-Malic Acids in apple juice by gas chromatography. *J. Assoc. Off. Anal. Chem.* 71:466–468.

Al-Ani, F. Y., and S. K. Al-Lami. 1988. Absence of mutagenic activity of acidity regulators in the Ames *Salmonella*/microsome test. *Mutat. Res.* 206:467–470.

Andersen, F. A., ed. 1998. Final report on the safety of Glycolic Acid, Ammonium, Calcium, Potassium, and Sodium Glycolates, Methyl, Ethyl, Propyl, and Butyl Glycolates, and Lactic Acid, Ammonium, Calcium, Potassium, Sodium, and TEA-Lactates, Methyl, Ethyl, Isopropyl, and Butyl Lactate, and Lauryl, Myristyl, and Cetyl Lactates. *Int. J. Toxicol.* 17:1–241.

Brookdale Dental Center of New York University. 1973. Letter from Dr. JR Stern to Dr. B La Du summarizing the results of testing acute testing with forms of Malic Acid. (Submitted by FDA in response to an FOI request; 1 page.)[2]

Budavari, S., ed. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals.* 11th ed., 896. Rahwah, NJ: Merck & Co.

Chang, T-L, R. P. Streicher, H. Zimmer, and J. W. Munch. 1988. The interaction of aqueous solutions of chlorine with Malic Acid, tartaric acid, and various fruit juices. A source of mutagens. *Anal. Lett.* 21:2049–2067.

Daniel, J. W. 1969. The metabolism of L- and DL-Malic Acid by rats. *Food Cosmet. Toxicol.* 7:103–106.

Eisele, T. A. 1996. Determination of D-Malic Acid in apple juice by liquid chromatography: Collaborative study. *J. A. O. A. C. Intl.* 79:50–54.

European Economic Community (EEC). 1995. EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII. Brussels: EEC.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 1967. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. (Submitted by FDA in response to an FOI request; 3 pages.)[2]

FAO/WHO. 1969. 13th Report—Specifications for the identity and purity of food additives, and their toxicological evaluation, etc. May 27–June 4. (Submitted by FDA in response to an FOI request; 3 pages.)[2]

FAO/WHO. 1994. *Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, M-6. USA: International Life Sciences Institute.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database.* Washington, DC: FDA.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw composition statements. *Fed. Register* 57:3128–3130.

FDA. 1997. Direct food substances generally recognized as safe and requirements for specific standardized milk and cream. *Code of Federal Regulations*, Title 21. §131.111; §169.115; §169.140; §169.150; §184.1069.

FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database.* Washington, DC: FDA.

Food and Drug Research Labs, Inc. (FDRL). 1973a. Scientific literature reviews on generally recognized as safe (GRAS) food ingredients—Malic Acid. Prepared for the FDA. June. NTIS Report No. PB-223 865.

FDRL. 1973b. Oral LD_{50} for mice of FDA 71-90 (Pomalus®; Malic Acid). Laboratory No. 1762r; contract No. FDA 71-260. Study dated Oct 16. (Submitted by FDA in response to an FOI request; 2 pages.)[2]

FDRL. 1973c. Oral LD_{50} for rats of FDA 71-90 (Pomalus®; Malic Acid). Laboratory No. 1763r; Contract No. FDA 71-260. Study dated Oct 16. (Submitted by FDA in response to an FOI request; 2 pages.)[2]

FDRL. 1973d. Oral LD_{50} for rabbits of FDA 71-90 (Pomalus®; Malic Acid). Laboratory No. 1764r; Contract No. FDA 71-260. Study dated Nov 29. (Submitted by FDA in response to an FOI request; 2 pages.)[2]

FDRL. 1974a. Teratologic evaluation of FDA 71-90 (Pomalus®; Malic Acid) in mice. Laboratory No. 1765r; Contract No. FDA 71-260. Study dated Jan 14. (Submitted by FDA in response to an FOI request; 10 pages.)[2]

FDRL. 1974b. Teratologic evaluation of FDA 71-90 (Pomalus®; Malic Acid) in rats. Laboratory No. 1766r; Contract No. FDA 71-260. Study dated Jan 14. (Submitted by FDA in response to an FOI request; 17 pages.)[2]

FDRL. 1974c. Teratologic evaluation of FDA 71-90 (Pomalus®; Malic Acid) in rabbits. Laboratory No. 1767r; Contract No. FDA 71-260. Study dated May 17. (Submitted by FDA in response to an FOI request; 14 pages.)[2]

Furia, T. E., ed. 1972. *Handbook of food additives*, 2nd ed., 239–241. Cleveland: CRC Press.

Furia, T. E., ed. 1975. *Fenaroli's handbook of flavor ingredients*, 2nd ed., 326. Boca Raton, FL: CRC Press.

Grant, J., ed. 1972. *Hackh's chemical dictionary*, 4th ed., 404. New York: McGraw-Hill.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

Hazleton Laboratories, Inc. 1970. Final report: Two-generation reproduction study using rats of X-5120. Project No. 165-128. Study dated Nov 11. (Submitted by FDA in response to an FOI request; 25 pages.)[2]

Ishidate, M. Jr., T. Sofuni, K. Yoshikawa, et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.* 22:623–636.

Kuroda, M., D. Yoshida, and S. Mizusaki. 1985. Mutagenicity of pyrolyzates of natural substances toward *Salmonella typhimurium* TA97. *Agric. Biol. Chem.* 49:1893–1895.

Lewis, R. J., Sr. 1993a. *Hawley's condensed chemical dictionary*, 12th ed., 725. New York: Van Nostrand Reinhold.

Lewis, R. J., Sr. 1993b. *Hazardous chemicals desk reference*, 3rd ed., 791. New York: Van Nostrand Reinhold Company.

Liebrand, J. T. 1992. *Encyclopedia of food science and technology*, vol 1, 1–6. New York: Wiley and Sons.

Litton Bionetics, Inc. 1974. Mutagenic evaluation of compound FDA 71-90. Report dated Nov 25 submitted to the FDA. NTIS Report No. PB-245440.

Martin, G. J., and W. Stenzel. 1944. The influence of various chemicals and vitamin deficiencies on the excretion of glucuronic acid in the rat. *Arch. Biochem.* 3:325–331.

Miltenberger, K. 1989. *Ullmann's encyclopedia of industry chemistry*, 5th ed., vol. A13, 515–517. New York: VCH Publishing.

National Academy of Sciences (NAS). 1996. *Food Chemicals Codex*, 4th ed., 237–238. Washington, DC: National Academy Press.

Nikitakis, J. M., and G. N. McEwen, Jr., eds. 1990. *CTFA Compendium of cosmetic ingredient composition—descriptions I*. Washington, DC: CTFA.

Patty. (1981–2). *Patty's industrial hygiene and toxicology*, 3rd ed., 4937, 4941–4942. New York: Wiley and Sons.

Registry of Toxic Effects of Chemical Substances (RTECS). 1997. Malic Acid entry. RTECS database. Bethesda, MD: National Library of Medicine.

Rempe, J. M., and L. G. Santucci. 1997. *CTFA list of Japanese cosmetic ingredients*, 3rd ed., 62. Washington, DC: CTFA.

Russell, I. J., J. E. Michalek, J. D. Flechas, and G. E. Abraham. 1995. Treatment of fibromyalgia syndrome with Super Malic®. A randomized, double blind, placebo controlled, crossover pilot study. *J. Rheumatol.* 22:953–958.

Sax, N. I. 1979. *Dangerous properties of industrial materials*, 5th ed., 785. New York: Van Nostrand Reinhold Company.

Smith, W. P. 1996. Comparative effectiveness of α-hydroxy acids on skin properties. *Intl. J. Cosmet. Sci.* 18:75–83.

Suye, S.-I., N. Yoshihara, and S. Inuta. 1992. Spectrophotometric determination of L-Malic Acid with a malic enzyme. *Biosci. Biotech. Biochem.* 56:1488–1489.

Taylor, E. J., ed. 1988. *Dorland's illustrated medical dictionary*, 27th ed., 975. Philadelphia, PA: WB Saunders.

TRW/Hazleton Laboratories. 1971a. Final report: 24-month dietary administration to rats of compound X-5120. Project No. 165-126. Study dated Oct 19. (Submitted by FDA in response to an FOI request; 215 pages.)[2]

TRW/Hazleton Laboratories. 1971b. Final report: 104-week dietary administration to dogs of X-5120. Project No. 165-129. Study dated Oct 15. (Submitted by FDA in response to an FOI request; 150 pages.)[2]

US Pharmacopeial Convention, Inc. 1995. *The United States pharmacopeia*, vol 23, and *The national formulary*, vol 18, 2262–2263. Taunton, MA: Rand McNally.

Van Scott, E. J., and R. J. Yu. 1974. Control of keratinization with α-hydroxy acids and related compounds. I. Topical treatment of ichthyotic disorders. *Arch. Dermatol.* 110:586–590.

Walsh, W. E. 1979. Atopic dermatitis associated with citric and Malic Acid intolerance. *Minn. Med.* 62:637–639.

Wenninger, J. A., R. C., Canterbery, and G. N. McEwen, Jr., eds. 2000. *International cosmetic ingredient dictionary and handbook*, 8th ed., vol 1–2. Washington, DC: CTFA.

Yamamoto, A., A. Matsunaga, and E. Mizukami. 1991. Ligand-exchange ion chromatographic determination of Malic Acid enantiomers in apple juice with photometric detection. *J. Chromatogr.* 585:315–317.

Yoshida, D., and H. Okamoto. 1982. Mutagenicity of the pyrolysis products of ammonium salts. *Agric. Biol. Chem.* 46:1087–1088.

Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of Fumaric Acid And Related Salts and Esters as Used in Cosmetics

March 23, 2009

The 2009 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Valerie C. Robinson, Scientific Analyst/Writer.

1101 17th Street, NW, Suite 412
Washington, DC 20036

Final Report of the Safety Assessment of Fumaric Acid and Related Salts and Esters as Used in Cosmetics

Abstract: Fumaric Acid, Dibehenyl Fumarate, Di-C12-15 Alkyl Fumarate, Diethylhexyl Fumarate, Diisostearyl Fumarate, Disodium Fumarate, Ferrous Fumarate, Sodium Fumarate, and Sodium Stearyl Fumarate are used in cosmetics. Salts of dimethyl fumarate are used as antipsoriatic pharmaceuticals, but not in cosmetics. Fumarate metabolism occurs in the citric acid cycle to produce water and carbon dioxide. Most animal studies demonstrate no significant single or repeated dose toxicity, including genotoxicity and carcinogenicity assays. One repeated dose animal study did report gonadotropic and estrogenic activity, and progressive testicular atrophy, but a comparable study reported that spermatogenesis and testicular structure were unaffected. In pre-clinical studies, dimethyl fumarate, at doses approaching maternal toxicity levels, was not a developmental toxicant; embryo-fetal toxicity was only observed at maternally toxic doses. These ingredients are not irritants or sensitizers. The CIR Expert Panel considered that the available data were adequate to support the safety of these ingredients as used in cosmetics.

INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel has considered the information in this report to assess the safety of Fumaric Acid, Dibehenyl Fumarate, Di-C12-15 Alkyl Fumarate, Diethylhexyl Fumarate, Diisostearyl Fumarate, Disodium Fumarate, Ferrous Fumarate, Sodium Fumarate, and Sodium Stearyl Fumarate as used in cosmetics as binders, bulking agents, buffering agents, emollient skin conditioning agents, nonaqueous viscosity increasing agents, pH adjusters, and slip modifiers.

Fumaric Acid is a trans dicarboxylic acid. The corresponding cis dicarboxylic acid is Maleic Acid. Previously, the CIR Expert Panel had reviewed the safety of Maleic Acid (Andersen 2007) with the conclusion of safe for use in cosmetic formulations as a pH adjustor in the practices of use as described in that safety assessment; i.e., up to 0.0004%. These two isomers are not considered readily interconverted and have different chemical and physical properties.

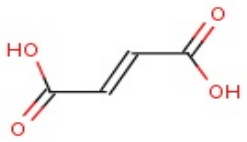
CHEMISTRY

Definition and Structure

Fumaric Acid

As listed in the *International Cosmetic Ingredient Dictionary and Handbook* (G ıric Acid (CAS Nos.110-17-8 c acid shown in **Figure 1a**.

(a)



(b)

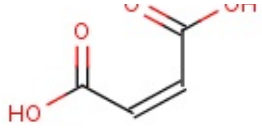


Figure 1. (a) Fumaric Acid and (b) Maleic Acid (ChemIDplus Lite 2008).

The difference in structure of the cis dicarboxylic acid form of Maleic Acid may be seen in the structure shown in Figure 1b.

Technical names for Fumaric Acid include: Allomaqleic Acid, Boletic Acid, 2-Butenedioic Acid; trans-1,2-Ethylenedicarboxylic Acid, and Lichenic Acid. Trade name mixtures include: Lipoderma - Shield BG, Lipoderma - Shield PG, and Unicontrozon C-49 (Gottschalck and Bailey 2008).

According to Hansson and Thorneby-Andersson (2003), Fumaric Acid is an endogenous compound formed mainly in the citric acid cycle. Fumaric Acid is also a fruit acid, ubiquitous in plants.

Dibehenyl Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Dibehenyl Fumarate (CAS No. not listed) is the diester of behenyl alcohol and Fumaric Acid with the chemical structure shown in **Figure 2.**

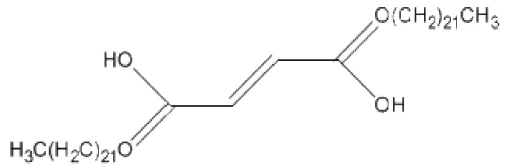


Figure 2. Dibehenyl Fumarate (Gottschalck and Bailey 2008).

A synonym for Dibehenyl Fumarate is 1,4-Bis-Docosanyl Butenedioate. A trade name is listed as Marrix 222 (Gottschalck and Bailey 2008).

Di-C12-15 Alkyl Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Di-C12-15 Alkyl Fumarate (CAS No. not listed) is the diester of C12-15 Alcohols (q.v.) and Fumaric Acid. It has the chemical structure shown in **Figure 3.**

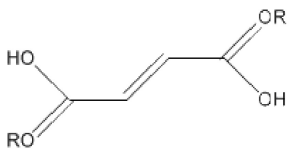


Figure 3. Di-C12-15 Alkyl Fumarate, where R represents the C12-15 alkyl group (Gottschalck and Bailey 2008).

A trade name for Di-C12-15 Alkyl Fumarate is Marrix S.F. (Gottschalck and Bailey 2008).

Diethylhexyl Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Diethylhexyl Fumarate (CAS Nos. 141-02-6; 128111-61-5) is the diester of 2-ethylhexanol and Fumaric Acid. It has the chemical structure shown in Figure 4.

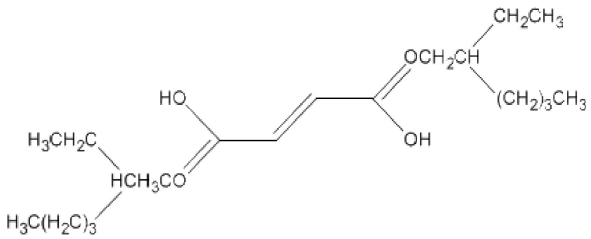


Figure 4. Diethylhexyl Fumarate (Gottschalck and Bailey 2008).

Synonyms for Diethylhexyl Fumarate include: Bis(2-Ethylhexyl) 2-Butenedioate; 2-Butenedioic Acid, Bis(2-Ethylhexyl) Ester; and Dioctyl Fumarate. A trade name for this chemical is Bernel Ester 284 (Gottschalck and Bailey 2008).

Diisostearyl Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Diisostearyl Fumarate (CAS Nos. 112385-09-8; 113431-53-1) is the diester of isostearyl alcohol and Fumaric Acid (q.v.). It has the chemical structure shown in Figure 5.

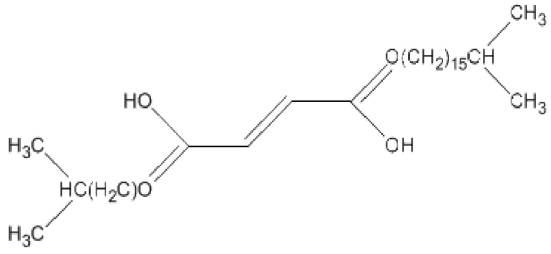


Figure 5. Diisostearyl Fumarate (Gottschalck and Bailey 2008).

Synonyms for Diisostearyl Fumarate include 2-Butenedioic Acid, Diisooctodecyl Ester and Diisooctadecyl 2-Butendioate. A trade name is Schercemol DISF Ester (Gottschalck and Bailey 2008).

Disodium Fumarate

As listed in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Disodium Fumarate (CAS No. 17013-01-3) is the disodium salt of Fumaric Acid (q.v.). It has the chemical structure shown in Figure 6.

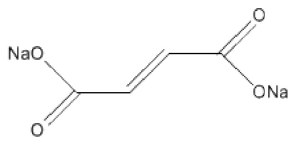


Figure 6. Disodium Fumarate (Gottschalck and Bailey 2008).

Another name for Disodium Fumarate is Fumaric Acid, Disodium Salt. A trade name mixture is listed as Extrapone Apple 2/033317 (Gottschalck and Bailey 2008).

Ferrous Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Ferrous Fumarate (CAS No. 40770-80-8) is the salt in the chemical structure shown in Figure 7.

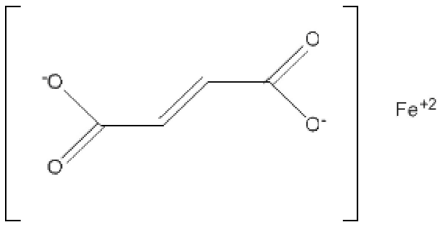


Figure 7. Ferrous Fumarate (Gottschalck and Bailey 2008).

A synonym for Ferrous Fumarate is 2-Butenedioic Acid (2E1-, Iron (2^+) Salt (1:1).

Sodium Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Sodium Fumarate (CAS Nos. 5873-57-4; 7704-73-6) is the mono-sodium salt of Fumaric Acid (q.v.). It has the chemical structure shown in Figure 8.

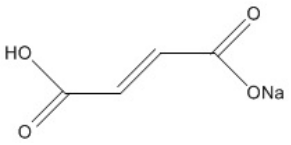


Figure 8. Sodium Fumarate (Gottschalck and Bailey 2008).

Synonyms for Sodium Fumarate include: 2-Butenedioic Acid, Monosodium Salt; Fumaric Acid, Monosodium Salt; and Monosodium Fumarate (Gottschalck and Bailey 2008).

Sodium Stearyl Fumarate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008), Sodium Stearyl Fumarate (CAS No. 1120-04-3) is the organic compound shown in the structure shown in Figure 9.

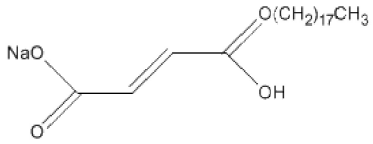


Figure 9. Sodium Stearyl Fumarate (Gottschalck and Bailey 2008).

A trade name for Sodium Stearyl Fumarate is Covafluid FS (Gottschalck and Bailey 2008).

Physical and Chemical Properties

Table 1 presents physical and chemical properties of Fumaric Acid, Disodium Fumarate, Diisostearyl Fumarate, Sodium Stearyl Fumarate, and Ferrous Fumarate; and chemical class information on all ingredients in this safety assessment.

Reactivity

Hansson and Thorneby-Andersson (2003) reported that maleic acid and Fumaric Acid have several chemical reactions in common, particularly those based on their electrophilic properties. Their electrophilic character is displayed in their reactions with

Table 1. Physical and Chemical Properties of Fumaric Acid, its salts and esters.

Property	Description	Reference
	Fumaric Acid	
Chemical Class	Carboxylic Acids	Gottschalck and Bailey (2008)
Appearance	White crystals or clear, crystalline powder, solid	Bartek Ingredients, Inc. (2007); and Food Chemicals Codex (2008)
Odor/Taste	None/fruit-like	Bartek Ingredients, Inc. (2007)
pH (1:30 aqueous solution)	2.0 - 2.5	Food Chemicals Codex (2008)
Molecular Weight:	116.07	Bartek Ingredients, Inc. (2007)
Acid Equivalent Weight	58.04	Bartek Ingredients, Inc. (2007)
Specific Gravity (20°C/4°C)	1.635	Bartek Ingredients, Inc. (2007)
Melting Point (°C)	286	Bartek Ingredients, Inc. (2007)
Flash Point (°C)	282	Bartek Ingredients, Inc. (2007)
Solubility	Soluble in alcohol, slightly soluble in water and ether, very slightly soluble in chloroform.	Food Chemicals Codex (2008)
log P_{ow}	0.33	European Chemicals Bureau (2000)
	Disodium Fumarate	
Chemical Class	Organic Salts	Gottschalck and Bailey (2008)
Molecular Weight:	160	Bimax Ingredients, Inc. (2007)
Appearance	White powder	Bimax Ingredients, Inc. (2007)
pH (10% in water):	6.8	Bimax Ingredients, Inc. (2007)
	Sodium Fumarate	
Chemical Class	Organic Salts	Gottschalck and Bailey (2008)
	Dibehenyl Fumarate	
Chemical Class	Esters	Gottschalck and Bailey (2008)
	Di-C12-15 Alkyl Fumarate	
Chemical Class	Esters	Gottschalck and Bailey (2008)
	Diethylhexyl Fumarate	
Chemical Class	Esters	Gottschalck and Bailey (2008)
	Diisostearyl Fumarate	
Chemical Class	Esters	Gottschalck and Bailey (2008)
Appearance @ 25°C	Clear to slightly hazy viscous liquid	Lubrizol (2007)
Color, Gardner	1 max	Lubrizol (2007)
Odor	Slight, characteristic	Lubrizol (2007)
Specific Gravity @ 25°C	0.890 - 0.910	Lubrizol (2007)
Refractive Index @ 25°C	1.461 - 1.464	Lubrizol (2007)
Acid Value, mg KOH/g	2.0 max	Lubrizol (2007)
Saponification Value, mg KOH/g	160 - 180	Lubrizol (2007)
IR (neat)	Conforms to reference	Lubrizol (2007)
Solubility	Solubile in most hydrophobic solvents such as esters, vegetable oils, mineral oils, alcohols, aliphatic, aromatic and chlorinated hydrocarbons; partly soluble in glycols; dispersible in triols and polyols; and insoluble in water.	Lubrizol (2007)
	Sodium Stearyl Fumarate	
Molecular weight	390.54	Science Lab (2007a)
Melting Point	decomposes	Science Lab (2007a)
Appearance	Fine, white powder	Food Chemicals Codex (2008)
Solubility	Slightly soluble in methanol, but practically insoluble in water	Food Chemicals Codex (2008)
	Ferrous Fumarate	
Appearance	red-orange to red-brown powder; may contain soft lumps that produce a yellow streak when crushed	Food Chemicals Codex (2008)
Solubility	Soluble in water and alcohol	Food Chemicals Codex (2008)
Molecular weight	169.9	Science Lab (2007b)
Melting Point	decomposes	Science Lab (2007b)

thiols, such as cysteine and glutathione. The reaction of endogenous Fumaric Acid with glutathione and with cysteine give S-(1,2-dicarboxylethyl)glutathione and S-(1,2-dicarboxylethyl)cysteine, respectively, as products.

Method of Manufacture

According to Gottschalck and Bailey (2008), Disodium Fumarate, Sodium Fumarate, Di-C12-15 Alkyl Fumarate, and Diethylhexyl Fumarate are derived solely from a synthetic source. Dibehenyl Fumarate is derived from plant and synthetic sources, and Diisostearyl Fumarate is derived from animal and synthetic sources.

The *Merck Index* stated that Fumaric Acid is prepared industrially from glucose by the action of fungi (i.e., *Rhizopus nigricans*) and that laboratory preparation of Fumaric Acid is performed by the oxidation of furfural with sodium chlorate in the presence of vanadium pentoxide (O'Neill 2006).

Natural Occurrence

Haviv et al. (1999) reported that fumarates are derived from succinates by succinate dehydrogenase, an enzyme that is unique because it is an integral part of the inner mitochondrial membrane, and directly linked to the electron transport chain. Human skin naturally produces Fumaric Acid when exposed to sunlight.

The Merck Index (O'Neil et al. 2006) stated that Fumaric Acid naturally occurs in many plants, including *Fumaria officinalis* L.

Analytical Methods

Kim and Karasek (1981) compared the negative ions observed by plasma chromatography (PC) and atmospheric pressure ionization mass spectrometry (APIMS) for maleic acid, Fumaric Acid, and the isomeric phthalic acids. All 3 isomers of phthalic acid (phthalic acid, isophthalic acid, and terephthalic acid) showed the identical species of (M-18)- and (M + O)- in APIMS, whereas phthalic acid and isophthalic acid showed a single ionic species with different ion mobilities in PC. Maleic acid and Fumaric Acid showed the same patterns of negative production ions in either PC or APIMS. The authors concluded that if the ion survival time from a compound is longer than the time of PC detection, then the ion can be observed by both techniques; if the ion survival time is $\leq 10^{-5}$ sec then the ion can be observed only by APIMS.

Impurities

Lonza (2006) provided the specifications for Fumaric Acid (in the trade name mixture, Unicontrozon C-49) shown in **Table 2**.

UV Absorption

European Chemicals Bureau (2000) stated that Fumaric Acid does not absorb UV light above 290 nm in methanol, acidic methanol or basic methanol solution.

Table 2. Specifications for Fumaric Acid in the trade name mixture, Unicontrozon C-49 as given by Lonza (2006).

Characteristics	Guaranteed
Purity (%)	99.7 min.
Moisture (%)	0.25 max.
Color in alcohol (sol. 5%) (Hazen)	10 max.
Ash (ppm)	50 max.
Iron (ppm)	5 max.
Arsenic (ppm)	1 max.
Heavy Metals (as Pb) (ppm)	10 max.
Lead (ppm)	1 max.
Mercury (ppm)	1 max.
Maleic Acid (%)	0.1 max.
Granulometric Analysis	
Granular form	
on 30 mesh sieve (%)	0.5 max.
on 140 mesh sieve (%)	90 min.
Granular FF form	
on 30 mesh sieve (%)	2.5 max.
on 140 mesh sieve (%)	60 min.
Powder form	
through 80 mesh sieve (%)	100 min.
through 100 mesh sieve (%)	95 min.
Microbiology Data[1]	
Bacteria (CFU/g)	<10
Molds and Yeasts (CFU/g)	<10
Total Coliforms (CFU/g)	<10
Fecal Coliforms (CFU.g)	<10
Salmonella (CFU/g)	Absent

[1] This is a statistical control realized in a external laboratory and did not appear on the Certificate of Quality.

USE

Cosmetic

The functions of Fumaric Acid and its salts in cosmetics as given in the International Cosmetic Ingredient Dictionary and Handbook are shown in **Table 3**.

Industry provided reports of ingredient usage to the Food and Drug Administration (FDA) through the Voluntary Cosmetic Registration Program (VCRP) and the Cosmetic, Toiletry and Fragrance Association (CTFA - now the Personal Care Products Council) conducted a survey of current use concentrations. These data are given in **Table 4**.

As provided to the VCRP, both Fumaric Acid an Di-C12-15 Alkyl Fumarate have 4 uses and Diisostearyl Fumarate has 1 use in cosmetics (FDA 2006). Based on industry survey data, Fumaric Acid, Di-C12-15 Alkyl Fumarate, Diisostearyl Fumarate, and Ferrous Fumarate are used at concentrations of 0.0008% - 5%, 0.4% - 5%, 1% - 20%, and 0.0003%, respectively (CTFA 2007).

No current uses or use concentrations were available for Disodium Fumarate, Sodium Fumarate, Dibehenyl Fumarate, Diethylhexyl Fumarate, or Sodium Stearyl Fumarate.

According to Lubrizol (2007), Diisostearyl Fumarate is a high shine emollient with good conditioning properties. In lip care products, it helps to disperse pigments and is used to decrease feathering and bleeding. It provides a rich skin feel in creams and lotions. With its high contact angle, Diisostearyl Fumarate is ideal for applications requiring target delivery.

Certain uses of these ingredients as given in **Table 4** suggested that the product type could be aerosolized or sprayed.

Jensen and O'Brien (1993) reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.

The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, $\mathbf{d_a}$, defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of 4.25 ± 1.5 µm for respirable particles that could result in lung exposure (Jensen and O'Brien, 1993).

Bower (1999), reported diameters of anhydrous hair spray particles of 60 - 80 µm and pump hair sprays with particle diameters of ≥80 µm. Johnsen (2004) reported that the mean particle diameter is around 38 µm in a typical aerosol spray. In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 - 110 µm range.

Non-Cosmetic

According to Davidson and Juneja (1990), Fumaric Acid is used to prevent the occurrence of malolactic fermentation in wines and as an antimicrobial agent in wines.

According to Hansson and Thorneby-Andersson (2003), Fumaric Acid is used as an additive to food for acidification purposes. It is also used in the plastics industry in the form of its dicarboxylic esters, especially in the production of polyesters. Esters of Fumaric Acid are also used as pharmacological tools in the depletion of glutathione.

FDA (2007), established by regulation (21CFR172.350) that Fumaric Acid and its calcium, ferrous, magnesium, potassium, and sodium salts may be safely used as food additives: (a) if the additives meet the following specifications: (1) Fumaric Acid contains a minimum of 99.5 percent by weight of Fumaric Acid, calculated on the anhydrous basis, and (2) the calcium, magnesium, potassium, and sodium salts contain a minimum of 99 percent by weight of the respective salt, calculated on the anhydrous basis, and Ferrous Fumarate contains a minimum of 31.3 percent total iron and not more than 2 percent ferric iron; (b) with the exception of Ferrous Fumarate, Fumaric Acid and the named salts are used singly or in combination in food at a level not in excess of the amount reasonably required to accomplish the intended effect; and (c) Ferrous Fumarate is used as a source of iron in foods for special dietary use, when the use is consistent with good nutrition practice.

FDA (2007) also established by regulation (21CFR172.826) that Sodium Stearyl Fumarate may be safely used as a food additive if: (a) it contains not less than 99 percent Sodium Stearyl Fumarate calculated on the anhydrous basis, and not more than 0.25 percent sodium stearyl maleate; and (b) The additive is used or intended for use: (1) as a dough conditioner in yeast-leavened bakery products in an amount not to exceed 0.5 percent by weight of the flour used, (2) as a conditioning agent in dehydrated potatoes in an amount not to exceed 1 percent by weight thereof, (3) as a stabilizing agent in non-yeast-leavened bakery products in an amount not to exceed 1 percent by weight of the flour used, (4) as a conditioning agent in processed cereals for cooking in an amount not to exceed 1 percent by weight of the dry cereal, except for foods for which standards of identity preclude such use, or (5) as a conditioning agent in starch-thickened or flour-thickened foods in an amount not to exceed 0.2 percent by weight of the food.

European Chemicals Bureau (2000) described Fumaric Acid as being used in paints, lacquers, varnishes, paper, pulp and wood fixing agents, food and foodstuff additives, intermediates, pH-regulating agents, and stabilizers.

Table 3. Functions of Fumaric Acid and its Salts and Esters in Cosmetics (Gottschalck and Bailey 2008).

Ingredient	Function
Fumaric Acid	Fragrance Ingredient; pH Adjuster
Disodium Fumarate	Buffering Agent; pH Adjuster
Sodium Fumarate	Buffering Agent; pH Adjuster
Dibehenyl Fumarate	Viscosity Increasing Agent - Nonaqueous
Di-C12-15 Alkyl Fumarate	Skin-Conditioning Agent - Emollient
Diethylhexyl Fumarate	Skin-Conditioning Agent - Emollient
Diisostearyl Fumarate	Skin-Conditioning Agent - Emollient
Sodium Stearyl Fumarate	Binder; Bulking Agent; Slip Modifier
Ferrous Fumarate	Not Reported

Table 4. Current uses and concentrations of Fumaric Acid and its salts and esters in cosmetics.

Product Category	2005 uses (FDA 2006)	2007 concentrations (CTFA 2007)
Fumaric Acid		
Bath Preparations		
Oils, tablets and salts	-	5%
Capsules	-	2%
Other bath preparations	-	0.08%
Non-coloring Hair Preparations		
Hair conditioners	-	0.2%
Skin Care Preparations		
Face and neck skin care preparations	-	0.2%
Body and hand skin care preparations	1	0.008%
Foot powders and sprays	1	
Moisturizers	-	0.02%
Night skin care preparations	-	0.2%
Paste masks (mud packs)	2	0.2%
Other skin care preparations	-	0.0008%
Total uses/ranges for Fumaric Acid	**4**	**0.0008% - 5%**
Ferrous Fumarate		
Personal Hygiene Products		
Other personal cleanliness products[1]	-	0.0003%
Skin Care Preparations		
Body and hand creams, lotions and powders	-	0.0003%
Total uses/ranges for Ferrous Fumarate	**-**	**0.0003%**
Di-C12-15 Alkyl Fumarate		
Baby Products		
Lotions, oils, powders and creams	-	5%
Non-coloring Hair Preparations		
Hair conditioners	-	0.4%
Makeup Preparations		
Face powders	-	1%
Foundations	-	2%
Lipsticks	1	5%
Skin Care Preparations		
Face and neck skin care preparations	-	1% -2%
Body and hand skin care preparations	1	1%
Foot powders and sprays	-	
Moisturizers	2	4%
Night skin care preparations	-	3%
Total uses/ranges for Di-C12-15 Alkyl Fumarate	**4**	**0.4% - 5%**
Diisostearyl Fumarate		
Eye Makeup Preparations		
Other eye makeup preparations	1	-
Non-coloring Hair Preparations		
Hair conditioners	-	1%
Hair sprays/aerosol fixatives	-	1%
Shampoos	-	1%
Other non-coloring hair preparations	-	1%
Makeup Preparations		
Blushers	-	3%
Lipsticks	-	20%
Total uses/ranges for Diisostearyl Fumarate	**1**	**1% - 20%**

GENERAL BIOLOGY

Citric Acid Cycle

According to Haviv et al. (1999), succinate and fumarate are readily oxidized by the kidneys. Succinate, fumarate and malate enhance cellular respiration catalytically, rather than stoichiochemically. Hydration of fumarate occurs via fumarase, which catalyzes a stereospecific trans addition of H^+ and OH^- to form L-malate, the only isomer that occurs naturally. Fumarate is an intermediate in the citric acid cycle used by cells to produce energy in the form of ATP from food. Fumarate is formed in the citric acid cycle via the oxidation of adenylsuccinate by the enzyme succinate dehydrogenase. Fumarate is then converted by the enzyme fumarase to malate.

Absorption, Distribution, Metabolism, Excretion

Fumaric Acid is a normal constituent of tissues as an intermediate in the tricarboxylic acid cycle. Distribution of Fumaric Acid in rat tissue has been studied by partition chromatography and it was found that blood contained 3 mg/l, brain tissue 150 mg/kg, kidney tissue 95 mg/kg, liver 78 mg/kg and muscle 23 mg/kg (Marshall et al. 1949).

According to Mrowietz et al. (1999), Fumaric Acid is poorly absorbed after oral intake. However, Fumaric Acid esters are almost completely absorbed in the small intestine. Dimethylfumarate (DMF) is rapidly hydrolyzed by esterases to monoethylfumarate (MEF), which is regarded as the active metabolite. MEF is further metabolized in the citrate cycle into water and carbon dioxide. The authors noted that there is no evidence for a cytochrome P450-dependent metabolism of Fumaric Acid esters. Excretion of metabolites is mainly through breathing, with only small amounts being excreted via urine and feces. DMF has a half-life of about 12 min, and MEF has a half-life of 36 h. Peak concentrations of monomethylfumarate are seen between 5 h and 6 h. DMF and free Fumaric Acid do not bind to serum proteins. Monomethylfumarate shows a protein binding of about 50%. The oral absorption of the esters refers to smaller esters (methyl and ethyl) than those used in cosmetics.

According to Hansson and Thorneby-Andersson (2003), the Fumaric Acid concentration in normal human plasma is about 2 µM, with the total body content in a adult human ranging from 8 to 80 g.

Membrane Effects

Butterfield et al. (1986) studied of the effect of various dicarboxylic acid compounds on the physical state of membrane proteins in human erythrocytes. Fumaric Acid, produced highly significant alteration in the physical state of membrane proteins.

Enzyme Effects

Spencer et al. (1990) reported that dimethyl fumarate and dimethyl maleate are potent inducers of cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase activity in Hepa 1c1c7 murine hepatoma cells in culture, whereas Fumaric Acid and maleic acids are much less potent. Dimethyl fumarate in the diet (0.2 - 0.5%) of female CD-1 mice and female Sprague-Dawley rats elevated cytosolic glutathione transferases and quinine reductase activities in a variety of organs. The widespread induction of such detoxification enzymes by dimethyl fumarate suggested to these authors the potential value of this compound as a protective agent against chemical carcinogenesis and other forms of electrophile toxicity. The authors concluded that this study supports the finding that the concentrations of dimethyl fumarate required to obtain substantial enzyme induction were well-tolerated by rodents.

Hepatoprotective Effects

Rao and Mishra (1997) assessed the hepatoprotective activity of Fumaric Acid in the aqueous extract of the whole plants of *Sida cordifolia* Linn. (*Malvaceae*), commonly known as Bala.

Table 5 describes the effect of Fumaric Acid from this source on the viability of isolated rat hepatocytes exposed to galactosamine and thioacetamide. Fumaric Acid was found to be non-hepatotoxic at the maximum dose of 1000 µg/ml in vitro and hepatoprotective of thioacetamide at all concentrations and of galactosamine at the two highest concentration levels.

The authors also stated that Fumaric Acid from this source was non-hepatotoxic at 20 mg/kg p.o. in vivo. The compound had significant protection against thioacetamide induced hepatic cytotoxicity at all the tested concentration levels. It also had significant protection against galactosamine induced hepatic cytotoxicity at 100 and 1000 µg/ml, but it did not show any protection at 10 µg/ml (Rao and Mishra 1997).

Antiproliferative Effects

Hagedorn et al. (1975) reported on the effect of Fumaric Acid monoethylester (MEF) on DNA-synthesis. The incorporation of ^{14}C-thymidine into the DNA of cultured human lymphocytes was depressed by added MEF depending on the dosage of MEF. Decreasing incorporation was due to a lower number of DNA synthesizing cells. No selective inhibition of proliferation during one of the cell cycle phases was observed.

The effect of Fumaric Acid was examined on DNA synthesis in hepatocytes or hepatoma cells from rats treated with toxic agents (Kuroda et al. 1986).

Table 5. Effect of Fumaric Acid on viability of isolated rat hepatocytes exposed to galactosamine and thioacetamide (Rao and Mishra 1997).

Group		% Viability, Mean ± SEM (% Protection)			
		Galactosamine		Thioacetamide	
		% Viable Cells	Oxygen Uptake (µl/hr/mg protein)	% Viable Cells	Oxygen Uptake (µl/hr/mg protein)
Control		98.05 ± 0.56	4.13 ± 0.13	98.05 ± 0.56	4.13 ± 0.13
Toxicant		50.01 ± 0.11	1.98 ± 0.02	24.73 ± 1.14	0.98 ± 0.01
Fumaric Acid	10 µg/ml	32.50 ± 0.94	NU[a]	70.59 ± 1.31[b]	NU[a]
	100 µg/ml	98.10 ± 1.03[b]	NU[a]	92.96 ± 0.54[b]	NU[a]
	1000 µg/ml	99.10 ± 1.12[b]	4.28 ± 0.05[b]	90.01 ± 1.15[b]	3.91 ± 0.07[b]

[a] NU = Not undertaken
[b] Significant reduction compared to toxicant ($P < 0.01$).

Male Donryu rats were injected with mitomycin C or aflatoxin B1, singly or in combination with Fumaric Acid. After a specified period, hepatocytes were isolated from the liver by the collagenase perfusion method and placed in culture, and their activities for DNA synthesis were measured. Mitomycin C (0.5 mg/kg) reduced the semiconservative DNA synthesis, but simultaneous dosing of Fumaric Acid (40 mg/kg) enhanced the recovery. DNA synthesis in hepatoma cells was also reduced with mitomycin C but, in contrast to that of the hepatocytes, was little influenced by the simultaneous dosing of Fumaric Acid. The i.p. injection of Fumaric Acid also reduced the toxicity of aflatoxin B1 (0.25 mg/kg, ip), preventing the reduction of DNA synthesis as well as the occurrence of nuclear degenerative changes in the aflatoxin B1-exposed hepatocytes.

Sebok (1993), as part of an effort to study antipsoriatic effects, examined the antiproliferative and cytotoxic profile of Fumaric Acid in keratinocyte cultures. Hyperproliferative HaCaT keratinocytes in monolayer cultures were exposed to Fumaric Acid at concentrations between 0.4 µM and 960 µM for 48 h. Cell proliferation was studied by [^{3}H]thymidine incorporation. In addition ^{14}C-labelled amino acid uptake and total protein content were measured. Direct cytotoxicity was determined by the release of cytoplasmic lactate dehydrogenase (LDH) into the culture medium. The corresponding 50% inhibition concentration (IC_{50}) was calculated for DNA/protein synthesis: > 960 µM (Fumaric Acid), respectively. The total protein content was less sensitive. The authors concluded that there was no association between the cytotoxic and antiproliferative potential of Fumaric Acid.

Vandermeeren et al. (1997) reported that Western blots of normal human dermal fibroblast cytoplasmic extracts showed that dimethylfumarate had minor effects on the I kappa B alpha, beta and epsilon proteins: their cytokine-induced degradation and synthesis was only slowed down, an effect most prominently observed for I kappa B beta. No inhibitory effect of dimethylfumarate was observed on cytokine-induced RelA/p65 or c-Rel accumulation in nuclear extracts of cytokine-treated normal human dermal fibroblast cells. In contrast, cytokine-induced nuclear factor kappa B1/p50 nuclear accumulation was specifically inhibited by dimethylfumarate. This inhibitory effect was sufficient to inhibit nuclear factor kappa B1-RelA binding to nuclear factor kappa B consensus oligonucleotides in DNA binding assays. Likewise, cytokine-induced activation of a pNF kappa B:luciferase reporter construct in transiently transfected normal human dermal fibroblasts was inhibited by dimethylfumarate. The authors stated that the observations supported a mechanistic model for the oral antipsoriatic dimethylfumarate in which lowering of nuclear factor kappa B1 leads to changes in the nuclear factor kappa B1-RelA nuclear balance and inhibition of cytokine-induced adhesion molecule expression in normal human dermal fibroblasts.

ANIMAL TOXICOLOGY

Acute Toxicity

Table 6 summarizes acute animal toxicity studies involving Fumaric Acid, Sodium Fumarate, Disodium Fumarate, Diisostearyl Fumarate, and Di-C12-15 Alkyl Fumarate. Overall, these ingredients exhibited little acute toxicity. In some instances, the number of animals used were not provided by the study authors.

Short-term Oral Toxicity

Disodium Fumarate

Fourteen rabbits were fed 320-2080 mg/kg bw of Disodium Fumarate daily for 28 days without any deaths. An additional 6 rabbits received 2880-3680 mg/kg bw for 17 days with 3 deaths. Two rabbits were fed a daily diet containing 640 mg/kg bw Disodium Fumarate for 36 days without consistent adverse effect on body weight, hematology, non-protein nitrogen or creatinine levels, or histopathological findings (Locke et al. 1942).

Short-term Parenteral Toxicity

Sodium Fumarate

Each of 5 rabbits received i.v. injections of 50-500 mg/kg Sodium Fumarate every 2nd or 3rd day for 10-32 days without any injurious effect on blood levels of non-protein nitrogen or creatinine, phensulfolphthalein excretion, or kidney and liver histology (Bodansky et al. 1942).

Six rabbits received twice weekly i.p. injections of 60 mg/kg bw of Sodium Fumarate over 17-29 weeks. Swelling and congestion of the thyroids and atrophy of testes, with low hyaluronidase content, were found (Arai and Suchiro 1953).

Subchronic Oral Toxicity

Sodium Fumarate

In a study by Packman et al. (1963), 4 groups of 15 rabbits were fed diets containing 0 or 6.9% Sodium Fumarate (equivalent to 5% Fumaric Acid) for 150 days. There were no significant differences from controls in body weight gain, feed consumption, mortality rate, blood counts, blood sugar, non-protein nitrogen level and urine. Organ weights were not significantly different between the groups and histologic examination showed no adverse findings attributable to the diet. In particular, spermatogenesis and testicular structure were unaffected.

Chronic Oral Toxicity

Fumaric Acid

Levey et al. (1946) reported a study in which rats (14/group) were maintained on daily diets containing 0.1% or 1.0% Fumaric Acid for 2 years. The control group received 0.2% acetic acid. After 6 months, 7 of the animals were necropsied and examined grossly and histologically. The remaining 7 animals were continued on the experiment for the remainder of the 2-year period. Because of respiratory infections, survival in all groups (including control) was reduced significantly during the second half of the study: 0/7 control, 1/7 0.1% Fumaric Acid treated, and 2/7 1.0% Fumaric Acid treated animals survived until the end of the study. This reduction was not attributed to the test material. No clinical or pathological effects attributed to dosing were observed in rats fed 0.1% or 1.0% Fumaric Acid.

Eight groups of 14 weanling rats were kept on diets containing 0, 0.1 or 1.0% Fumaric Acid or 1.38% Sodium Fumarate for one year (half the groups) or 2 years. No adverse effect was noted on rate of weight gain, hemoglobin, blood picture, calcium balance as shown by bone histology, or on the histology of liver, kidney, spleen and stomach (Levey et al. 1946).

Fitzhugh and Nelson (1947) reported that 5 groups of 12 male and 12 female rats were fed diets containing 0, 0.1, 0.5, 0.8 or 1.2% Fumaric Acid for 2 years without toxic effects on growth or feed consumption. A further 4 groups of 12 male rats were kept for 2 years on diets containing 0, 0.5, 1.0 or 1.5% Fumaric Acid. At the 1.5% level was there a very slight increase in mortality rate and some testicular atrophy. Gross and microscopic examination of major organs revealed no abnormalities and tumor incidence was not significantly different between the groups.

Arai et al. (1955) reported a study in which 9 male rabbits received 60 mg/kg Sodium Fumarate every second day by i.p. injection, for 150 days. By the end of the test period, serum gonadotropic activity and estrogenic activity were detected. There was progressive testicular atrophy in all animals, resulting in disappearance of seminiferous epithelium and survival of Sertoli cells only. Chromophobe cells were increased in the pituitary.

Table 6. Acute animal toxicity studies with Fumaric Acid, Sodium Fumarate, Disodium Fumarate, Diisostearyl Fumarate, and Di-C12-15 Alkyl Fumarate.

Animal	Route	LD_{50} (mg/kg b.w.)/Results	Reference
		Fumaric Acid	
Rat	Oral	10,000	Ullmann's Encyclopedia of Industrial Chemistry (1996)
Rat	Oral	Female: 9,300; range: 6,300 - 13,800 Male: 10,700; range: 7,200 -15,800	Vernot et al. (1977)
Rat	Oral	10,700	Lewis (1991)
Rabbit	Oral	5,000	National Institute for Occupational Safety and Health (NIOSH 1986)
Rabbit	Dermal	> 20,000	Vernot (1977)
Mouse	i.p.	100	NIOSH (1986)
Mouse	i.p.	200	Smith et al. (1963)
Rat	i.p.	< 587 [a]	Levey et al. (1946)
		Sodium Fumarate	
Rat	Oral	~8,000	Levey et al. (1946)
		Disodium Fumarate	
Rabbit	Oral	~3,600	Locke et al. (1942)
Not specified	Not specified	~4,800	Weiss et al. (1923)
		Diisostearyl Fumarate	
Ten (5 males, 5 females) albino rats, 200 - 288 g	Oral	>5000	Consumer Product Testing (1993)
		Di-C12-15 Alkyl Fumarate	
3 male and 3 female HanBri:WIST (SPF) rats	oral gavage	>2000	RCC (2001)
5 male and 5 female rabbits	Acute dermal toxicity limit test	>2000	LebercoCelsis Testing (1996)

[a] necropsy showed hemorrhagic spots on the intestine near the site of injection; the surface of the liver appeared to be seared; and there was engorgement of the intestine and liver.

Fumaric Acid was fed to 4 groups of 6 young dogs at 0, 1, 3 and 5% of the diet for 2 years without adverse effect on body weight gain, development, hematology, blood sugar and urea levels, hemoglobin or urine analysis. Organ weights and gross and histopathological examination of all principal organs and tissues revealed no effects attributable to the treatment (Harrisson and Abbott 1962).

Ocular Irritation

Table 7 lists ocular irritation studies regarding the use of Fumaric Acid, Di-C12-15 Alkyl Fumarate, and Diisostearyl Fumarate in rabbits. Overall, Fumaric Acid was irritating and the esters were not significant ocular irritants. In some instances, the number of animals used were not provided by the study authors.

Table 7. Ocular Irritation Studies with Fumaric Acid, Di-C12-15 Alkyl Fumarate,and Diisostearyl Fumarate.

Animal	Method	Result	Reference
	Fumaric Acid		
Rabbit	100 mg/ 24h	Moderately irritating	NIOSH (1986); Sax-Lewis (1991)
Rabbit	No details provided	Irritating	European Chemicals Bureau (2000)
	Di-C12-15 Alkyl Fumarate		
6 New Zealand White Rabbits	0.1 mL placed on the everted lower lid of one eye; the upper and lower lids were gently held together for 1 second; lesions evaluated at 24, 48 and 72 h.	Non-irritating	AMA Laboratories (1991)
	Diisostearyl Fumarate		
6 New Zealand White Rabbits	0.1 mL intraocular 1 eye; eyes unwashed for 24 h; lesions evaluated at 24, 48, and 72 h.	Mild ocular irritant	Consumer Product Testing (1993)

Dermal Irritation

Table 8 lists dermal irritation studies regarding the use of Fumaric Acid, Di-C12-15 Alkyl Fumarate, and Diisostearyl Fumarate in rabbits. Overall these ingredients were not significant dermal irritants. In some instances, the number of animals used were not provided by the study authors.

Dermal Sensitization

Di-C12-15 Alkyl Fumarate

RCC (2002) reported on the contact hypersensitivity in 15 male albino guinea pigs (10 test, 5 control) in a maximization test using Di-C12-15 Alkyl Fumarate. The intradermal induction of sensitization in the test group was performed in the nuchal region with a 50% dilution of the test material in corn oil and in an emulsion of FCA/physiological saline. The induction of sensitization was conducted for 48 h under occlusion with the test item at 75% in corn oil 1 week after the intradermal induction and following pre-treatment of the test areas with 10% Sodium Lauryl Sulfate (SLS) approximately 24 h prior to application of the test item.

The animals of the control group were intradermally induced with corn oil under occlusion following pre-treatment with 10% SLS. Two weeks after epidermal induction, the control and test animals were challenged by epidermal application of the test item at 75% and 15% in corn oil and corn oil alone under occlusive dressing. Cutaneous reactions were evaluated at 24 h and 48 h after removal of the dressing. No toxic symptoms were evident in the guinea pigs of the control or test group. No deaths occurred. None of the control or test animals had skin reactions after the challenge treatment with Di-C12-15 Alkyl Fumarate at 75% and 15% (w/w/) in corn oil. Based on the findings, Di-C12-15 Alkyl Fumarate was determined to be a non-sensitizer under the conditions of this study.

Diisostearyl Malate

According to Research and Development (1988), Diisostearyl Malate was tested for contact allergy in the guinea pig (no details were provided on number of animals). The induction concentration was 0.05% in propylene glycol and FCA. The challenge concentration was 0.50% (100% in petrolatum). There were no reactions to the test material. Diisostearyl Malate was determined to be a non-sensitizer.

Other Fumarates

Dimethylfumarate (DMF) and monoethylfumarate (MEF) are Fumaric Acid derivatives used in psoriasis treatment, primarily in Europe, and have been studied to identify potential adverse effects. DMF and MEF are not cosmetic ingredients.

In order to determine the irritating and sensitizing properties of DMF and MEF, De Haan et al. (1994) used a cytotoxicity, flank irritation, ear swelling and guinea pig maximization test. Twenty guinea pigs were used for immunization: 10 with DMF and 10 with MEF. Each guinea pig received 1 ml of a compound dissolved in 6 ml phosphate buffer saline and mixed with 6 ml Freund's complete adjuvant (FCA): in the nucha (0.4 ml), in front and hind legs (each 0.1 ml), and in both ears (0.1ml).

Another 10 guinea pigs were injected with FCA and served as the control animals. The results of the cytotoxicity test demonstrated that DMF was the most toxic derivative. DMF induced contact-urticarial reactions in contrast to MEF. Challenge experiments 21 days after immunization (open epicutaneous) with Fumaric Acid (400 mM), MEF (100 mM) and DMF (20 mM) in MEF- and DMF-sensitized guinea pigs demonstrated that both MEF and DMF are moderate contact sensitizers. Readings were done after 20 min, 24, 48, and 72 h. In DMF-sensitized animals cross-reactions with MEF were found. As DMF and MEF have cytotoxic, contact-urticarial and/or sensitizing properties, topical application should be avoided. Fumaric Acid was not found to be a sensitizer. No further study details were provided.

REPRODUCTIVE and DEVELOPMENTAL TOXICITY

Fumaric Acid

Levey et al. (1946) reported a study in which 12 guinea pigs (male and female) were used to determine whether Fumaric Acid might have an effect on reproduction and lactation. The animals received 1% Fumaric Acid in the diet (~400 mg/kg b.w./day). The exposure period was not reported. There were no detectable toxic effects on growth, reproduction or lactation of the Fumaric Acid-treated guinea pigs.

Table 8. Dermal Irritation Studies with Fumaric Acid, Di-C12-15 Alkyl Fumarate, and Diisostearyl Fumarate.

Animal	Method	Result	Reference
	Fumaric Acid		
Rabbit	500 mg/ 24 h	Slightly irritating	NIOSH (1986); Sax-Lewis (1991)
Rabbit	no details provided	Non-irritating	European Chemicals Bureau (2000)
	Di-C12-15 Alkyl Fumarate		
6 New Zealand Albino Rabbits	Trunks of the rabbits were clipped free of hair; patches were placed over intact and abraded skin; 0.5 g test material was place under each patch; the trunk animal was wrapped to retard evaporation and maintain test patch position; skin lesions were evaluated at 24 and 72 h	Non-primary irritant	AMA Laboratories, Inc. (1991)
	Diisostearyl Fumarate		
6 New Zealand White Rabbits	single dermal application of 0.5 mL of the test material on 2 occluded test sites (1 abraded, 1 non-abraded); observed at 24 and 72 h	Primary Irritation Index: 1.35 Non-primary irritant	Consumer Product Testing (1993)

Other Fumarates

According to BiogenIdec (2008), the drugs, Fumaderm® and Fumaderm® initial, are approved for use in Germany in the treatment of plaque psoriasis. These drugs contain 120 mg of dimethyl fumarate (aka DMF), 87 mg of ethyl hydrogen fumarate (calcium salt), 5 mg ethyl hydrogen fumarate (magnesium salt), and 3 mg ethyl hydrogen fumarate (zinc salt), in the case of Fumaderm®; or 30 mg of dimethyl fumarate, 67 mg of ethyl hydrogen fumarate (calcium salt), 5 mg ethyl hydrogen fumarate (magnesium salt), and 3 mg ethyl hydrogen fumarate (zinc salt) in the case of Fumaderm® initial.

While the studies provided to support approval of these pharmaceuticals were not available, a summary of preclinical data is provided in the package insert. This summary stated that studies on rats and rabbits exposed to doses approaching levels causing maternal toxicity, yielded no evidence of any teratogenic effect. Embryo-fetal toxicity (growth retardation, mortality) was only observed at doses known to cause maternal toxicity. In one reproduction study on rats, there was no evidence to indicate any effect on fertility (BiogenIdec 2008).

GENOTOXICITY

Fumaric Acid

Table 9 describes in vitro genotoxicity studies of Fumaric Acid. Fumaric Acid was not mutagenic in several Ames tests and in CHO cells in culture, but was mutagenic in one assay using L5178Y cells in culture.

Diisostearyl Fumarate

SafePharm Laboratories (2008) reported on a reverse mutation assay (Ames Test) using *S. typhimurium* and *Escherichia coli*. *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain WP2uvrA were treated with Diisostearyl Fumarate at 5 dose levels, in triplicate, both with and without the addition of metabolic activation. In experiment 1, the dose range was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate. The experiment was repeated on a separate day using the same dose range as Experiment 1, fresh cultures of the bacterial strains and fresh material formulations.

The vehicle (acetone) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Therefore, the sensitivity of the assay and the efficacy of the S9-mix were validated. The test material caused no visible reduction in the growth of the bacteria at any dose level. The test material was therefore tested up to the maximum recommended dose level of 5000 µg/plate. A precipitate (oily in appearance) was observed at and above 1500 µg/plate; this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation. Diisostearyl Fumarate was considered to be non-mutagenic under the test conditions (SafePharm Laboratories 2008).

Di-C12-15 Alkyl Fumarate

According to Sitek Research Laboratories (1995), Di-C12-15 Alkyl Fumarate was tested for its potential to cause mutation at the histidine operon of *S. typhimurium* strains TA98, TA100, TA1537, and TA1538 and at the tryptophan operon of *E. coli* strain WP2uvrA. The test article, dissolved in acetone, was tested for toxicity to strains TA100 and WP2uvrA in a range finding test at test article concentrations ranging from 5.0 to 5000 µg/plate. The tester strains were exposed to the test article in the absence of exogenous activation and in the presence of Aroclor 1254-induced rat liver S-9 plus cofactors.

Based on the results of the range finding test, the first mutation assay was performed with the 5 *S. typhimurium* tester strains and *E. coli* strain WP2uvrA using concentrations of 100, 250, 500, 750 and 1000 µg/plate in the presence and absence of S-9 activation. The second mutation assay was performed with the preincubation method to confirm the results of the first assay, using the same concentrations. The results of both mutation assays indicated that the test article did not induce any positive increase in the number of revertant colonies for any of the tester strains in the presence or absence of Aroclor 1254-induced rat liver S-9. Under the conditions of this study, Di-C12-15 Alkyl Fumarate is negative in the *S. typhimurium/E. coli* plate incorporation/preincubation mutation assay.

Table 9. Fumaric Acid Genotoxicity Studies.

System	Concentration	Result	Reference
S. typhimurium - TA100	10000, 1000, 100, and 10 µg/plate	not mutagenic	Rapson (1980)
S. typhimurium - TA92, TA94, TA98, TA100, TA1535, and TA1537 with and without metabolic activation	Up to 10 µg/plate	not mutagenic	Ishidate et al. (1984)
S. typhimurium - TA97, TA98, TA100, TA1535 with and without metabolic activation	33, 100, 333, 1000, and 2000 µg/plate	not mutagenic	Ishidate et al. (1984)
S. typhimurium - TA98, TA100, TA1535, TA1537, TA1538 with and without metabolic activation	10 - 5000 µg/plate	not mutagenic	European Chemicals Bureau (2000)
Chinese hamster lung fibroblast (CHL) with and without metabolic activation	0.125, 0.25, and 0.5 µg/mL	incidence of polyploidy or structural aberrations of treated cells did not differ from the negative controls.	Ishidate et al. (1984)
L5178Y cells (TK+/-) with and without metabolic activation.	2856 - 8000 µg/mL	mutagenic with and without metabolic activation	Saffioti and Shubik (1963)

CARCINOGENICITY

According to Levey et al. (1946), Fumaric Acid was not carcinogenic in male and female Osborne-Mendel rats. The rats received Fumaric Acid in the diet daily at 0.1, 0.5, 0.8, 1.0, 1.2, and 1.5% (~750 mg/kg/day). The exposure period was not reported. In the highest dose group, there was a low level of survival (2 out of 12) at the end of the experiment, while in the lower dose groups, mortality did not differ significantly from the controls (details not given). The gross and microscopic findings showed no difference between the control and treated animals. Tumors showed no difference in incidence among the animal groups.

According to Saffioti & Shubik (1963), Fumaric Acid was found to induce moderate focal hyperplasia of the epidermis, but not tumors in female Swiss mice. The animals were initially treated once a week to the clipped dorsal skin with 7, 12-dimethylbenz(a)anthracene (1.5% in mineral oil), then with Fumaric Acid twice a week (1% in acetone). The entire treatment period was 76 weeks with Fumaric Acid alone.

Antitumor Activity

Kuroda and Akao (1981) studied the antitumor and anti-intoxication activities of Fumaric Acid in cultured cells. The Ehrlich, MH-134, and L1210 cell lines were grown in the peritoneal cavity of ICR/JCL, C2H/He, and DBA/2 male mice, respectively. Fumaric Acid was isolated as the active component of Capsella bursa-pastoris herb for inhibiting the solid growth of Ehrlich tumors in mice, and was found to significantly reduce the growth and viability of Ehrlich, MH134, and L1210 mouse tumor cells in culture at concentrations of 0.3 ~ 1.2 mg/mL. Also, at these concentrations, Fumaric Acid in the culture medium had no deleterious effect on monolayer development of mouse and chick embryo cells, but exhibited activity to enhance the recovery of cells from the toxic effects of mitomycin C, aflatoxin B_1, N-methyl-N'-nitro-N-nitrosoguanidine, and potassium 1-methyl-7-[2-(5-nitro-2-furyl) vinyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (NFN).

The inhibitory effect of Fumaric Acid on carcinogenesis by NFN was examined histologically with male ICR/JCL mice (Kuroda et al. 1982). NFN was fed to 62 mice at a dose level of 0.012% in the diet for 14 weeks. These mice were then divided into 2 groups. One group was given a basal diet, and the other group was given a diet containing 1% Fumaric Acid in the subsequent 39 weeks. In the group of 30 mice fed NFN alone, squamous cell carcinomas were found in the stomachs of 7 mice, multiple papillomas in the stomachs of 13 mice, and multiple and large papillary adenocarcinomas in the lungs of 27 animals. The administration of Fumaric Acid suppressed the NFN-induced stomach and lung carcinogenesis. In the group of 32 mice fed NFN and Fumaric Acid, no stomach tumors developed except 1 early-stage squamous cell carcinoma. In the lungs, only a small focus of mild atypical hyperplasia and a few early-stage adenocarcinomas were noted in 7 and 11 animals in the group of 32 mice, respectively.

In a study by Kuroda et al. (1983), Fumaric Acid was examined for its effect on hepatocarcinogenesis in rats fed 3-methyl-4'-(dimethylamino)azobenzene (3-Me-DAB). Male Donryu rats received approximately 0.5 g 3-Me-DAB in a diet containing 0.06% 3-Me-DAB for 50 days; they then received a diet containing 1% Fumaric Acid and drinking water containing 0.025% Fumaric Acid for 51 weeks. The administration of Fumaric Acid effectively suppressed the development of hepatocellular carcinoma, hyperplastic nodules, and hyperplastic areas in the livers of rats fed 3-Me-DAB.

Pereira et al. (1994) examined the use of azoxymethane (AOM)-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. Foci of aberrant and/or hexosaminidase-negative crypts in rat colon are putative precancerous lesions that have been proposed as biomarkers for short-term bioassays for chemical carcinogens and chemopreventive agents. The ability of a substance to reduce the yield of AOM-induced foci in the colon of male Fischer 344 rats was evaluated as a screening assay for chemopreventive agents. Twenty-eight test agents were administered continuously in the diet from the start of the experiments until the animals were killed 35 days later. Calcium salts of carbonate, chloride and glucarate decreased the yield of AOM-induced foci while the acidic salts of lactate and phosphate did not inhibit the formation of foci. Dimethyl fumarate, Fumaric Acid, genistein, piroxicam, simethicone, sodium suramin and sulindac reduced the yield of AOM-induced foci of aberrant crypts, with genistein being the most potent.

Kuroda et al. (1987) examined the inhibitory effect of Fumaric Acid on hepatocarcinogenesis in mice fed thioacetamide (TAA). A group of male ICR mice was fed TAA at a level of 0.035% in the diet for 40 weeks and then fed a basal diet for 48 weeks. Hepatic tumors developed in 11 of the 24 animals of this group and they were diagnosed as hepatocellular carcinomas. However, cirrhotic lesions and the enlargement of hepatocyte nucleoli were not as marked in mice as in previous findings in rats fed TAA. The effect of Fumaric Acid on carcinogenesis was examined in a group of mice fed this compound at a level of 1% in a basal diet after ingestion of TAA. The inhibitory effect of Fumaric Acid on TAA carcinogenesis was so marked that no hepatic carcinomas were found in any of the 15 animals fed Fumaric Acid in combination with TAA.

According to Kuroda and Akao (1989), Fumaric Acid suppressed the carcinogenesis in the liver of rats fed 3'-Me-DAB, and a study was performed to examine the effect of Fumaric Acid on DNA synthesis and subcellular structures of hepatocytes under the anticarcinogenic regimen. Male Donryu strain rats were given 3'-Me-DAB by being fed a diet containing 0.06% 3'-Me-DAB for 50 d. They then received a diet containing 1% Fumaric Acid and drinking water containing 0.025% Fumaric Acid for 53 to 69 weeks. Hepatocytes were isolated from the liver by the collagenase perfusion method and placed in culture, and their activity for DNA synthesis was measured in terms of the incorporation of [^{3}H]dThd into DNA. An enhanced DNA synthesis of hepatocytes was noted in the rats given Fumaric Acid, indicating that Fumaric Acid enhanced the proliferation of hepatocytes to counteract the carcinogenic effect of 3'-Me-DAB. An electron microscopic examination indicated that the distribution of subcellular organella was almost normal in the Fumaric Acid-treated hepatocytes.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation/Sensitization

Fumaric Acid

De Haan et al. (1994) reported that topical therapy for psoriasis with Fumaric Acid and its derivatives in the treatment of psoriasis can produce perilesional skin irritation, macular papular rashes and urticarial reactions.

Dermtest (1997) studied the primary skin irritation and allergic hypersensitivity potential in humans patch tested with 20% aqueous solution of the trade name mixture Unicotrozon C-49 containing 5% Fumaric Acid and 17.5% Fumaris Officinalis Extract that also contains some Fumaric Acid. This trade name mixture also included water, citrus medica limonum (lemon) fruit extract and propylene glycol. Fifty subjects (male and female) were used in the study. No evidence of primary irritation or

allergic hypersensitivity was seen in any of the subjects. No positive reactions were found in any of the subjects after 24, 48, and 72 h. The authors of this study concluded that under the test conditions, 20% aqueous Unicontrozon C-49 was not an irritant or sensitizer.

Di-C12-15 Alkyl Fumarate

Stephens & Associates (1997) conducted a 14-day cumulative irritation study using 5% Di-C12-15 Alkyl Fumarate. Twenty-seven subjects completed the study. Subjects were patched with test and control materials daily for 14 days. Subjects wore the patches for approximately 24 h and removed them approximately 2 h before grading of the test sites. There was no experimental irritation demonstrated with the test material.

Stephens & Associates (1998) evaluated the safety, effectiveness, comedogenicity, and acnegenicity of 5% Di-C12-15 Alkyl Fumarate, a topically applied cosmetic product designed to improve skin moisturization in women. Thirty-nine female subjects (50% Japanese, 50% Caucasian) completed the 8-week controlled usage study. Results indicated that the test material was non-acnegenic and non-comedogenic.

In another study by Stephens & Associates (1998), a human repeat insult patch test using 5% Di-C12-15 Alkyl Fumarate was performed. Ninety-eight subjects completed the study. During the induction phase, patches containing the test material were applied 9 times at approximately 48 to 72 h intervals. Reactions at the application sites were graded approximately 48 to 72 h after each application. Twelve to twenty-four (12-24) days after application of the last induction patches, challenge patches were applied to original and alternate sites, and reactions were graded at approximately 48 and 96 h post-application. No edema, vesicles, bullae, spreading, or weeping were observed during the study. The test material did not induce allergic contact dermatitis in any of the subjects.

Clinical Research Laboratories, Inc. (2004) determined the dermal irritation and sensitization potential of a leave-on product containing 1% Di-C12-15 Alkyl Fumarate. A total of 112 subjects, male and female, between the ages of 18 - 70, enrolled in the study, of which 108 subjects completed the study - 4 subjects discontinued participation for reasons unrelated to the testing. Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material was applied under a semi-occlusive patch to the upper back and was allowed to remain in direct skin contact for a 24 h period.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the induction period. The sites were graded for dermal irritation and sensitization 24 h after removal of the patches by the subjects on Tuesday and Thursday and 48 h after the removal of the patches on Saturday. The sites were graded according to the following scoring system: 0, no visible skin reaction; ±, barely perceptible erythema (minimal); 1+, mild erythema (diffuse); 2+, well-defined erythema; 3+, erythema and edema; and 4+, erythema and edema with vesiculation.

After a 2-week rest period, challenge patches were applied to previously untreated areas on the back. After 24 h, the patches were removed and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 h and 72 h. Based on the conditions of this study, the test material containing 1% Di-C12-15 Alkyl Fumarate did not demonstrate a potential for eliciting dermal irritation or sensitization (Clinical Research Laboratories 2004).

Clinical Research Laboratories, Inc. (2005), determined the dermal irritation and sensitization potential of a leave-on product containing 1% Di-C12-15 Alkyl Fumarate. A total of 112 subjects, male and female, between the ages of 18 - 70, were enrolled in the study, of which 104 subjects completed the study - 8 subjects discontinued participation for reasons unrelated to the testing. Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material was applied under a semi-occlusive patch to the upper back (between the scalpulae) and was allowed to remain in direct skin contact for a 24 h period. Patches were applied to the same site according to the protocol described previously for a total of 9 applications during the induction period. Based on the conditions of this study, the test material did not demonstrate a potential for eliciting dermal irritation or sensitization (Clinical Research Laboratories 2005).

Diisostearyl Fumarate

KGL, Inc. (2006) evaluated the contact-sensitization potential of a lip gloss containing 20% Diisostearyl Fumarate to human skin by means of maximization assay. A total of 26 healthy adults (23 females, 3 males) participated in the study; of which 25 completed the study. Approximately 0.05 mL of aqueous SLS (0.25%) was applied to a designated site under a 15 mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24-h. After 24-h, the SLS patch was removed and 0.05 mL of the test material (SPF-15 lip gloss) was applied to the same site before the site was again covered with occlusive tape (induction patch). The induction patch was left in place for 48-h (or for 72-h when placed over the weekend) following which it was removed and the site again examined for irritation. If no irritation was present, a 0.25% aqueous SLS patch was reapplied to the same site for 24-h, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence was continued for a total of 5 induction exposures. If irritation developed at any time-point during the induction phase, the 24-h SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-h rest period during which no patch was applied.

After a 10-day rest period which followed the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed. Pre-treatment with SLS was performed prior to challenge. Approximately 0.05 mL of a 5.0% aqueous solution was applied to a fresh skin site under a 15 mm disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for 1-h. It was then removed and the test material was applied to the same site. The challenge patch was left in place for 48-h. After that period, the patch was removed and the site graded 15-30 min later and again 24-h later for any reaction. No adverse or unexpected reactions were seen in any of the subjects during the induction phase and no instances of contact allergy were recorded during the challenge phase at either 48 or 72-h (KGL, Inc. 2006).

KGL, Inc. (2007) evaluated the contact-sensitization potential of a lip gloss containing 17.41% Diisostearyl Fumarate to human skin by means of maximization assay. A total of 26 healthy adults (20 - 61 years old) participated in the study; 25 completed the study. Approximately 0.05 mL of aqueous SLS (0.25%) was applied to a designated site under a 15 mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24-h. After 24-h, the SLS patch was removed and 0.05 mL of the test material (SPF-15 lip gloss) was applied to the same site before the site was again covered with occlusive tape (induction patch). The induction patch was left in place for 48-h (or for 72-h when placed over the weekend) following which it was removed and the site again examined for irritation. If no

irritation was present, a 0.25% aqueous SLS patch was reapplied to the same site for 24-h, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence was continued for a total of 5 induction exposures. If irritation developed at any time-point during the induction phase, the 24-h SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-h rest period during which no patch was applied.

After a 10-day rest period which followed the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed. Pre-treatment with SLS was performed prior to challenge. Approximately 0.05 mL of a 5.0% aqueous solution was applied to a fresh skin site under a 15 mm disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for 1-h. It was then removed and the test material was applied to the same site. The challenge patch was left in place for 48-h. After that period, the patch was removed and the site graded 15-30 min later and again 24-h later for any reaction. Under the conditions of this test, the lipgloss containing 17.4% Diisostearyl Fumarate did not possess a detectable contact-sensitizing potential and is not likely to cause contact sensitivity reactions under normal use conditions (KGL, Inc. 2007).

Dimethyl Fumarate

Rantanen (2008) reported on an epidemic of severe contact dermatitis cases related to newly acquired Chinese sofas and chairs. Five patients were studied. Furniture samples were analyzed by gas chromatography-mass spectrometry. Compounds were identified using a mass spectrum library and measured semiquantitatively. Patch tests were performed with commercial standard allergens, furniture upholstery and chemicals found in the analysis. Patch tests with commercial allergens did not solve the problem. Up to 470 µg/kg of DMF was found in chairs (kg refers to the upholstery). The patients showed strong positive patch test reactions to upholstery fabric samples and to DMF, down to a level of 1 ppm in the most severe case. It was concluded by the author that the cause of the Chinese sofa/chair dermatitis epidemic is likely to be contact allergy to DMF, a novel potent contact sensitizer. As noted earlier, DMF is not a cosmetic ingredient.

Psoriasis Treatment

Fumaric Acid and its derivatives have been studied as anti-psoriatic agents, primarily the salts of the dimethyl and monoethyl forms which are not used as cosmetic ingredients.

The drugs, Fumaderm® and Fumaderm® initial, are approved for use in Germany in the treatment of plaque psoriasis (BiogenIdec 2008). Raab (1984) expressed the view that MEF is anti-psoriatic, but is too toxic for clinical use, while Fumaric Acid itself may produce secondary changes that have beneficial effects on psoriatic lesions, but is not itself an anti-psoriatic. Raschka and Koch (1999) reported that Fumaric Acid preparations can be used as long-term and effective treatment of psoriasis, but that gastrointestinal, dermatological and hematological side-effects, and transient renal damage may be present during treatment with Fumaric Acid.

Table 10 briefly summarizes published studies related to Fumaric Acid and its derivatives in the treatment of psoriasis.

Table 10. Psoriasis Treatment using Fumaric Acid and its derivatives.

Study Description	Reference
Fumaric Acid compound therapy (FACT) consists of the oral intake of dimethylfumaric acid ester (DMFAE) and several salts of monoethylfumaric acid ester (MEFAE) in combination with topical Fumaric Acid therapy (1% to 3% MEFAE in an ointment or Fumaric Acid in bathing oils) and a diet. An open pilot study was conducted using 36 patients in which FACT therapy was effective. Thereafter, several controlled studies with MEFAE sodium in 2 different dosages versus placebo, and DMFAE versus placebo, were done. The results indicated that MEFAE sodium in dosages up to 240 mg daily was ineffective, whereas daily dosages of 720 mg resulted in a significant decrease in scaling and itching but did not affect extension of the eruption. DMFAE, 240 mg daily, produced a significant amelioration and prevented extension. Side effects of Fumaric Acid treatment were nausea, diarrhea, general malaise, and severe stomach ache. Mild disturbances of liver and kidney function during treatment were observed with the 720 mg dosage of MEFAE and with the 240 mg dosage of DMFAE. Moreover, a relative lymphopenia with a selective decrease of suppressor T lymphocytes occurred in about 50% of the patients treated with DMFAE.	Neiboer et al. (1989)
in a 4-month double-blind study, the effects of dimethylfumaric acid esters (DMFAE-EC) and DMFAE (120 mg per tablet) plus salts of monoethylfumaric acid esters (Fumaric Acid combination, FAC-EC) in enteric-coated tablets were compared in 22 and 23 patients, respectively, with psoriasis. In both groups about 50% showed a considerable improvement, i.e. the initial score was more than halved. The therapeutic effects showed no significant differences in both groups with respect to the total psoriasis score or the different parameters. In the FAC-EC (120 mg per tablet) group the effects were obtained more rapidly. Most frequently observed side effects in both groups were flushings, stomach ache and diarrhea. Due to these complaints, patients discontinued therapy. Eosinophilia, leukopenia and lymphopenia were the most frequently observed differences in lab tests. It was concluded that FAC-EC had no significantly better effect than monotherapy with DMFAE-EC. Moreover, enteric coating of the tablets did not prevent stomach complaints.	Nieboer et al. (1990)
196 patients, 18 years of age and older with nummular and plaque-type psoriasis over at least 10% of the body surface, were given one of two reatments: dimethyl Fumaric Acid ester (DMFAE, monotherapy) and Fumaric Acid combination (FAC) therapy. The DMFAE group was treated with capsules filled with 60 mg of semienteric coated granulate of DMFAE. In the first week, the dosage was 60 mg/day. This was increased weekly by 60 mg to a maximum of 240 mg/day. The FAC group was treated with 2 types of enteric-coated tablets: (1) mite tablet, containing 30 mg of DMFAE, 5 mg Mg-, 3 mg Zn-, and 56 mg Ca-MEFAE; or (2) forte tablet, containing 120 mg of DMFAE, 5 mg Mg-, 3 mg Zn-, and 87 mg Ca-MEFAE. Medication started with 1 mite tablet per day. In the 4th week, medication was switched to 1 forte tablet per day and this was increased weekly to a maximum of 4 tablets per day, in 2 divided doses after meals. Topical treatment consisted of the application of a bland cream or ointment or a mild topical corticosteroid. Therapeutic evaluation was done in the periods of 3 to 6 months, 6 to 12 months, 12 to 18 months, and 18 to 24 months. No significant differences could be found between DMFAE monotherapy and FACT therapy when equivalent doses of DMFAE were taken. Several side effects were observed (gastrointestinal complaints, general malaise, mild liver and kidney disturbances (seen in 3 and 1 patient), and leukocytopenia (in 4% of the patients). The symptoms disappeared immediately after discontinuation of treatment. Recurrent psoriasis after discontinuation varied, but in most cases complete healing occurred.	Kolbach and Nieboer (1992)

Table 10 (continued). Psoriasis Treatment using Fumaric Acid and its derivatives.

Study Description	Reference
a randomized double-blind study in 100 patients (male and female, 18-70 years old) with psoriasis, comparing Fumaric Acid derivatives with placebo. Patients were treated with either drug or placebo in tablet form. The drug consisted of a mixture of dimethylfumarate and monoethyl-hydrogenfumarates. The low dose contained 105 mg of ester mixture up to a maximum dose of 1290 mg by week 16. The results indicated statistically significant superiority of the Fumaric Acid derivatives over placebo. Adverse events (flush, gastrointestinal disturbances) were initially relatively frequent, but decreased thereafter. Fumaric Acid derivatives were reported to be effective and safe in the treatment of psoriasis.	Altmeyer et al. (1994)
2041 psoriatic patients over a 9-yr period using Fumaric Acid preparations (Fu-P-mite; 105 mg ester mixture, Fu-P-forte; 215 mg ester mixture). Many of the patients exhibited side effects when first being introduced to the forte tablets and when reaching the dosage of 4 to 6 tablets daily. For this reason, the protocol was altered as follows: mite tablets were increased daily for 6 days, then for 2 to 3 days a dose of 6 mite tablets were given as maintenance, and subsequently every 2 to 3 days two mite tablets were replaced by 1 forte tablet. The forte tablets were increased to consider body weight and side effects up to a maximum of 3 to 4 tablets per day. The maximum dose was obtained for 3 to 6 months. Thereafter, the dose was reduced continuously by 1 forte tablet per month until signs of deterioration disappeared. At this time, dosage was again increased with mite tablets. Many of the patients improved after only 2 to 6 months of treatment. No serious side effects were noted.	Skaria and Schmid (1996)
the safety of Fumaric Acid esters (Fumaderm initial, 215 mg; Fumaderm, 105 mg) was evaluated in the oral long-term therapy of severe psoriasis vulgaris. A total of 83 patients with severe psoriasis were investigated in a 12-month clinical trial. The antipsoriatic effect of Fumaric Acid derivatives was clear, with a mean reduction of 76% in psoriasis area and severity index (PASI). Adverse events were noted in 62% of the patients – mainly gastrointestinal complaints. These were dose-dependent and decreased in frequency throughout the course of the study. No severe adverse effects occurred. The authors concluded that Fumaric Acid derivatives are indicated in cases of severe therapy-resistant psoriasis and can be used even for long-term application.	Altmeyer and Nuchel (1996)
based on the premise that psoriasis is not primarily a skin disorder, but an immunological disturbance under the skin and that the skin manifestations are a result of overstimulation of superficial skin cells (Langerhans cells) due to increased production of interleukin 2, 6 and 8, as well as transforming growth-factor-alpha. Interleukin-10 production is diminished, this study addressed the immunotherapeutic effect of Fumaric Acid in combination with thymus extract and selenium - 54 patients were treated with Fumaric Acid in addition to intravenous thymus extract and selenium (no concentrations were provided). They showed a faster healing rate than with Fumaric Acid alone. The author determined that Fumaric Acid, thymus extract and selenium have a synergistic effect.	Christ (1999)
in this randomized, double-blind, vehicle-controlled study, 143 patients were treated for up to 13 weeks. Group A received Fumaric Acid tablets with an increasing daily dosing from 105 to 1075 mg + ointment vehicle. Group B received Fumaric Acid tablets + calcipotriol ointment (50 µg/g). Ointments were applied twice daily. Clinical response was assessed using percentage changes in the PASI, from baseline to treatment end. The mean percentage change in the PASI was -76.1% in group B and -51.9% in group A, the difference between treatments was -24.2% (95% Cl from -34.2 to -14.2%; $P < 0.001$). Group B responded more rapidly to treatment. Investigators' and patients' overall efficacy assessments were significantly more favorable for Group B ($P < 0.001$). Group B was prescribed less Fumaric Acid esters than group A. This difference was greatest at the last visit (mean daily dose 529 and 685 mg, respectively; $P = 0.006$). Overall, adverse events in the 2 groups were similar. The authors concluded that the combination of calcipotriol and Fumaric Acid esters is significantly more effective and faster acting than Fumaric Acid ester monotherapy in the treatment of severe plaque psoriasis.	Gollnick et al. (2002)
12 patients received Fumaric Acid esters for severe psoriasis. The mean duration of the psoriasis was 24 years (range 8 – 42). All of the patients failed to respond to topical therapy and/or phototherapy alone. The existing regimen of each patient was substituted with Fumaric Acid esters produced in tablets containing 2 dose levels. Low strength tablets contained 30 mg dimethylfumarate, 67 mg ethylhydrogenfumarate Ca salt, 5 mg ethylhydrogenfumarate Mg salt, and 3 mg ethylhydrogenfumarate Zn salt. The high-dose tablets contained 120 mg dimethylfumarate, 87 mg ethylhydrogenfumarate Ca salt, 5 mg ethylhydrogenfumarate Mg salt, and 3 mg ethylhydrogenfumarate Zn salt. Doses were taken at intervals of 2 weeks or longer and 1 – 3 times a day. Patients were examined every 2 weeks until improvement was noted and then every month. One out of 12 patients discontinued treatment early due to flushing while on the low-dose tablets. The other 11 patients all demonstrated improvement in psoriasis after starting treatment with Fumaric Acid esters. Nine patients received Fumaric Acid esters in combination with other systemic agents and generally enabled the doses of the more hazardous drugs to be reduced. The authors recommended that careful monitoring be used when using Fumaric Acid esters in such combined regimens.	Balasubramaniam et al. (2003)
while ~⅔ of patients treated with Fumaric Acid experienced gastrointestinal symptoms, and ⅓ developed flushing, these authors reported that long-term administration of Fumaric Acid has been associated with a transient increase in liver enzyme levels and with kidney damage.	Hoefnagel et al. (2003)
oral treatment of psoriasis on an outpatient basis, using a preparation containing Fumaric Acid derivatives, was evaluated as initial monotherapy (3 months) and as long-term basic therapy (12-14 months) in 13 and 11 patients, respectively. The course of the disease was analyzed in each individual case. After completion of both parts of the trial, half of the patients that had only responded poorly to conventional antipsoriatic therapy showed a significant improvement which occurred after several weeks of treatment. In 4 patients the medication had to be stopped because of abdominal pain. No severe side effects, particularly of a renal, hepatic or hematological nature, could be established.	Bayard et al. (2004)
clinical experience in Italy; >80% of patients achieved complete remission following 6 months of treatment with dimethylfumarate.	Carboni et al. (2004)

Laxative Effects

Twenty-six constipated patients suffering from a variety of chronic diseases not involving the gastrointestinal tract were given oral doses of 5-30 g Sodium Fumarate; a satisfactory bowel motion resulted in 18 patients. There was much variability of response to a given dose between patients and in the same individual. Doses above 15 g caused unpleasant side effects. No abnormalities were noted in the urine or serum non-protein nitrogen level (Bodansky et al. 1942).

Other Clinical Treatment

Kreuter et al. (2005) investigated Fumaric Acid esters in the treatment of necrobiosis lipoidica (NL). NL is an uncommon granulomatous skin disease with association to diabetes mellitus. Eighteen patients with histopathologically proven NL were used in this non-controlled study. Fumaric Acid esters dosages were given as a standard therapy regimen for psoriasis for at least 6 months. The results were evaluated by clinical and histological scoring, as well as ultrasound assessments. Three patients discontinued therapy with Fumaric Acid esters, while the remaining 15 completed the study. After a mean ± SD treatment period of 7.7 ± 2.9 months, a significant ($p < 0.001$) decrease in the mean ± SD clinical score, from 7.4 ± 1.8 at the beginning to 2.2 ± 1.3 at the end of the therapy, was observed. Significant clinical improvement of NL was accompanied by significant ($P = 0.019$) increase of dermal density as assessed by means of 20-MHZ ultrasound, and significant ($P = 0.011$) reduction of the histological score. Adverse effects were moderate and consisted mainly of gastrointestinal complaints and flushing. During follow-up of at least 6 months, clinical outcome remained stable in all patients. The authors therefore concluded that the study demonstrates that Fumaric Acid esters are beneficial and safe in the treatment of patients with NL.

CASE REPORTS

Stuhlinger et al. (1990) reported on a case where 2 sisters, aged 25 and 29 years, with generalized psoriasis guttata since childhood, developed nausea, upper-abdominal pain, loss of appetite, palpitations and flushes in the course of local and oral administration of Fumaric Acid. Because of these side effects the treatment was discontinued after about 2 weeks, and the symptoms disappeared. But proteinuria and haematuria were subsequently noted, creatinine concentration rose to 2.2 and 2.5 mg/dl, respectively, while creatinine clearance fell to 44 and 27 ml/min, respectively. Examination of urinary sediments and analysis of urinary proteins gave results compatible with tubular-interstitial renal damage. The abnormal renal functions and urinary findings proved reversible within 3 weeks.

Fliegner and Spiegel (1992) reported on a case of fully reversible tubular toxicity with consecutive metabolic osteopathy following systemic Fumaric Acid therapy. A 46-yr-old female patient with a long history of recurrent palmoplantar psoriasis underwent oral treatment with Fumaric Acid in accordance with the Schafer method, preceding attempts at curative treatment with conventional antipsoriatic agents having proved unsatisfactory.

Two months later, the patient began experiencing arthralgia, back pain in the early hours of the morning and myalgia with increasing frequency, progressing to disablement in moving and walking, and finally, to total immobility. Nine months later, it was determined that the reason for these severe disabilities stemmed from hypophophataemic osteomalacia as a result of a complex disturbance of the renal tubular system. The clinical symptoms and the results of laboratory chemistry tests returned to normal as soon as Fumaric Acid medication was discontinued. Two re-exposure attempts confirmed the causal relationship. The authors therefore concluded that Fumaric Acid medication should never be administered without clinical and chemical controls (Fliegner and Spiegel 1992).

Raschka and Koch (1999) reported on the case of a 38 year old woman who was treated with Fumaric Acid (420 mg) for 5 years before she complained of excessive fatigue and weakness. According to the clinical laboratory, she had developed severe proximal tubular damage. Hypophosphatemia, glycosuria and proteinuria persisted although medication was stopped immediately.

Haviv et al. (1999) described a case of a 48-yr old Caucasian female admitted with respiratory distress. Previous medical history was positive for only psoriatic arthritis mutilans beginning at the age of 30-yrs. Medical treatments with glucocorticoids, methotrexate and indomethacin had failed, and the patient underwent bilateral total hip replacement at age 40. Since the age of 39, the patient had been restricted to her home, became a strict vegetarian, and began taking Fumaric Acid tablets 3 times a day. The skin and joint lesions responded to the treatment.

During this period, the patient's physical state gradually deteriorated, and she lost the ability to walk. The patient also experienced loss in weight and height. She eventually developed dyspnoea and was hospitalized. Upon physical examination, it was found that the patient was cachectic. The vital signs were normal, except for tachypnoea, and there was maximum jugular venous distention. The skeletal examination revealed miniature pigeon test, normal size limbs, rosaries of the lower ribs, and extremely fragile bones. Laboratory data disclosed megaloblastic anemia, secondary to B_{12} deficiency, normal liver function tests, normal glucose and electrolytes except for chloride of 116 mmol/L, and normal serum creatinine level. The patient was treated by phosphate loading, and her respiratory capacity improved. However, during a gastrostomy performed for enteral hyperailmentation, she died suddenly. The authors proposed that administration of maleic acid anologue esters in pharmacological doses may have induced a diffuse tubular mitichondrial injury leading to Fanconi syndrome and vitamin D-resistant osteomalacia (Haviv 1999).

Hansson and Thorneby-Andersson (2003) reported a case of a 30-year old healthy male with no history of allergy or skin disease who developed an acute dermatitis. In his profession as an organic chemist, he was exposed to different esters of small organic molecules, among other esters of maleic acid and of Fumaric Acid. Accidentally, his hands had been exposed to a reaction mixture containing dimethyl maleate. He developed a bullous dermatitis on 1 hand and on his left wrist, an erythematous dermatitis with large bullae was noted. After treatment with a topical corticosteroid, the lesions healed. Two weeks later, he again developed an eczematous reaction, displaying erythematous scaling maculae on his left wrist, as well eczema with erythema and a large number of vesicles on the back of his left hand and fingers. These lesions healed after a second treatment with a steroid cream. Two weeks later, he was patch tested with the TRUE test standard series and with the chemicals the patient brought in from his own laboratory. Since the diethyl esters of maleic acid and of Fumaric Acid were available among the patient's chemicals, they were chosen for a comparison of the esters of the 2 acids. The patch tests in the standard series were negative. There were strong reactions to esters of both acids, whereas the free acids as well as maleic anhydride gave negative results. The sensitivity to Fumaric Acid diethyl esters was stronger than that to maleic acid diethyl ester. The patch tests were evaluated after 72 hours, and the reactions were scored.

Guenther et al. (2003) reported on a case of a 68-year-old Caucasian woman who was treated with Fumaric Acid esters (FAE) for 4 days for lichen planus and then developed

generalized pruritic exanthema. This was suspected to be an allergic drug reaction to FAE, and the treatment was discontinued. After 48-72 hours, the exanthema resolved completely. An objective causality assessment revealed that the adverse drug event was probable. As skin testing for diagnostic purposes is not feasible with FAE, the drug-related origin of the exanthema was confirmed by oral rechallenge with FAE. The effectiveness of FAE in the systemic treatment of psoriasis vulgaris has been proven by controlled clinical trials. The compound has been shown to be tolerable and safe even during prolonged treatment. The most frequent adverse effects are gastrointestinal symptoms and flushing, which typically occur 4-6 hours after administration of the drug. Allergic reactions to FAE have not yet been reported. Since the patient was rechallenged with the suspected drug, the authors could confirm the allergic origin of the exanthema. The occurrence of allergic skin reaction should be considered in patients receiving treatment with FAE.

SUMMARY

This report presents available information pertinent to the safety of Fumaric Acid, and its salts and esters as used in cosmetics. Not all Fumaric Acid esters are cosmetic ingredients. For example, salts of dimethyl fumarate and monoethyl fumarate are used in psoriasis treatment, but are not cosmetic ingredients. The salts and esters included in this safety assessment are Disodium Fumarate, Sodium Fumarate, Dibehenyl Fumarate, Di-C12-15 Alkyl Fumarate, Diethylhexyl Fumarate, Diisostearyl Fumarate, Sodium Stearyl Fumarate, and Ferrous Fumarate.

Fumaric Acid is an endogenous compound formed mainly in the citric acid cycle. Fumaric Acid is also a fruit acid, ubiquitous in plants. Human skin naturally produces Fumaric Acid when exposed to sunlight. The salts and esters of Fumaric Acid are known as fumarates and may be derived from succinate by succinate dehydrogenase. Fumarates are then converted by the enzyme fumarase to malates.

Fumaric Acid does not absorb UV light above 290 nm in methanol, acidic methanol or basic methanol solution. Fumaric Acid functions in cosmetics as a fragrance ingredient and pH adjuster; Disodium and Sodium Fumarate are described as buffering agents/ pH adjusters; Dibehenyl Fumarate functions as a nonaqueous viscosity increasing agent; Di-C12-15 Alkyl Fumarate, Diethylhexyl Fumarate, and Diisostearyl Fumarate function as emollient skin-conditioning agents; Sodium Stearyl Fumarate is a binder, bulking agent, and slip modifier; and no function in cosmetics was reported for Ferrous Fumarate.

Fumaric Acid has 4 reported uses at concentrations of 0.0008% - 5%. Di-C12-15 Alkyl Fumarate also has 4 reported uses at concentrations of 0.4% - 5%. In each case specific use concentrations were reported in product categories for which no uses were reported to FDA. Diisostearyl Fumarate has 1 reported use in eye preparations, but no concentration data were available; other uses at concentrations between 1% - 20% were reported in non-coloring hair care and makeup products. Use concentrations were reported for Ferrous Fumarate at 0.0003%.

Fumaric Acid is poorly absorbed after oral intake. However, Fumaric Acid esters are almost completely absorbed in the small intestine. Dimethylfumarate is rapidly hydrolyzed by esterases to monoethylfumarate, which is regarded as the active metabolite. Monomethylfumarate is further metabolized in the citrate cycle into water and carbon dioxide. There is no evidence for a cytochrome P450-dependent metabolism of Fumaric Acid esters. Excretion of metabolites is mainly through breathing, with only small amounts being excreted via urine and feces. Dimethylfumarate has a half-life of about 12 min, and monoethylfumarate has a half-life of 36 h. Peak concentrations of monomethylfumarate in blood are seen between 5 h and 6 h. Dimethylfumarate and free Fumaric Acid do not bind to serum proteins. Monomethylfumarate shows a protein binding of about 50%. The Fumaric Acid concentration in normal human plasma is about 2 µM, with the total body content in a adult human ranging from 8 to 80 g.

Fumaric Acid and dimethyl fumarate have cytotoxic and antiproliferative effects in vitro. Dimethyl fumarate and dimethyl maleate are potent inducers of cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase activity in Hepa 1c1c7 murine hepatoma cells in culture, whereas Fumaric Acid and maleic acids are much less potent. The addition of dimethyl fumarate into the diet of female CD-1 mice and female Sprague-Dawley rats at 0.2% - 0.5% concentrations elevated cytosolic glutathione transferases and quinine reductase activities in a variety of organs, whereas much higher concentrations of Fumaric Acid were only marginally active.

Fumaric Acid has a low chronic toxicity and is a naturally-occurring metabolic intermediate that is already in the food chain as an additive.

Fumaric Acid is hepatoprotective in rat hepatocytes in vitro and in albino rats in vivo.

In short-term animal studies using rabbits, up to 2080 mg/kg bw of Disodium Fumarate daily for 28 days did not result in any mortality. Of 6 rabbits that received up to 3680 mg/kg bw for 17 days, 3 animals died.

Rabbits that received i.v. injections of 50-500 mg/kg Sodium Fumarate every second or third day for 10-32 days had no injurious effect on blood levels of non-protein nitrogen or creatinine, phenosulfolphthalein excretion, or kidney and liver histology. Rabbits that received twice weekly i.p. injections of 60 mg/kg bw of Sodium Fumarate over 17-29 weeks had swelling and congestion of the thyroid gland and atrophy of testes, with low hyaluronidase content. Male rabbits that received 60 mg/kg bw Sodium Fumarate every second day by i.p. injection for 150 days had gonadotropic activity, as well as estrogenic activity, detected in the serum. There was progressive testicular atrophy in all animals.

Rats (14/group) maintained on daily diets containing 0.1% and 1.0% Fumaric Acid for 2 years had no clinical or pathological effects. Eight groups of 14 weanling rats kept on diets containing 0, 0.1 and 1.0% Fumaric Acid and 1.38% Sodium Fumarate for one year (half the groups) or two years had no adverse effects (e.g., rate of weight gain, hemoglobin, blood picture, calcium balance as shown by bone histology, or on the histology of liver, kidney, spleen and stomach). Five groups of 12 male and 12 female rats fed diets containing 0, 0.1, 0.5, 0.8 and 1.2% of Fumaric Acid for 2 years had no effects on growth or food consumption; at the 1.5% level there was a slight increase in mortality rate and some testicular atrophy. Fumaric Acid fed to dogs at 0, 1, 3 and 5% of the diet for 2 years produced no adverse effect on body weight gain, development, hematology, blood sugar and urea levels, organ weights, and gross and histopathological examination of all principal organs and tissues. Rabbits fed diets containing 0 or 6.9% Sodium Fumarate for 150 days had no significant differences from controls in body weight gain, feed consumption, mortality rate, blood counts, blood sugar, non-protein nitrogen level and urine; and organ weights were not significantly different between the groups and histologic examination showed no adverse findings attributable to the diet. In particular, spermatogenesis and testicular structure were unaffected.

Systemic and topical therapies with Fumaric Acid and its derivatives are used in the treatment of psoriasis. Topical application was accompanied by perilesional skin irritation,

macular papular rashes and urticarial reactions. In a guinea pig maximization study, 20 animals were used for immunization: 10 with dimethylfumarate (DMF) and 10 with monoethylfumarate (MEF). Each guinea pig received 1 ml of a compound dissolved in 6 ml phosphate buffer saline and mixed with 6 ml Freund's complete adjuvant: in the nucha (0.4 ml), in front and hind legs (each 0.1 ml), and in both ears (0.1 ml). Another 10 guinea pigs were injected with Freund's complete adjuvant and served as the control animals. The results of the cytotoxicity test demonstrated that DMF was the most toxic derivative. DMF also induced contact-urticarial reactions in contrast to MEF. Challenge experiments 21 days after immunization (open epicutaneous) with Fumaric Acid (400 mM), MEF (100 mM) and DMF (20 mM) in MEF- and DMF-sensitized guinea pigs demonstrated that both MEF and DMF are moderate contact sensitizers. In DMF-sensitized animals cross-reactions with MEF were found. Fumaric Acid was not found to be a sensitizer.

Twelve guinea pigs (male and female) were bred in order to determine whether Fumaric Acid might have an effect on reproduction and lactation. The animals received 1% Fumaric Acid in the diet (~400 mg/kg b.w./day). The exposure period was not reported. There were no detectable toxic effects on growth, reproduction or lactation of the Fumaric Acid-treated guinea pigs.

The drugs, Fumaderm® and Fumaderm® initial, are approved for use in Germany in the treatment of plaque psoriasis. Studies on rats and rabbits exposed to doses approaching levels causing maternal toxicity, yielded no evidence of any teratogenic effect. Embryo-fetal toxicity (growth retardation, mortality) was only observed at doses known to cause maternal toxicity. In one reproduction study on rats, there was no evidence to indicate any effect on fertility.

Fumaric Acid was not mutagenic in several Ames tests and in CHO cells in culture, but was mutagenic in one assay using L5178Y cells in culture. Neither Di-C12-15 Alkyl Fumarate nor Diisostearyl Fumarate were mutagenic in Ames tests.

Fumaric Acid in the diet up to 1.5% was not carcinogenic in male and female Osborne-Mendel rats. Fumaric Acid was found to induce moderate focal hyperplasia of the epidermis. No tumors were formed in female Swiss mice treated once a week with 7,12-dimethylbenz(a)anthracene (1.5% in mineral oil), then with Fumaric Acid twice a week (1% in acetone), for a total of 76 weeks. Fumaric Acid, isolated as the active component of Capsella bursa-pastoris herb, inhibited the solid growth of Ehrlich tumors in mice, and was found to significantly reduce the growth and viability of Ehrlich, MH134, and L1210 mouse tumor cells in culture at concentrations of 0.3 ~ 1.2 mg/mL, with no deleterious effect on monolayer development of mouse and chick embryo cells, but with activity to enhance the recovery of cells from the toxic effects of mitomycin C, aflatoxin B_1, N-methyl-N'-nitro-N-nitrosoguanidine, and potassium 1-methyl-7-[2-(5-nitro-2-furyl) vinyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate. Fumaric Acid inhibited carcinogenesis by potassium 1-methyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (NFN) in male ICR/JCL mice. The administration of Fumaric Acid suppressed the NFN-induced stomach and lung carcinogenesis. The administration of Fumaric Acid effectively suppressed the development of hepatocellular carcinoma, hyperplastic nodules, and hyperplastic areas in the livers of rats fed 3-methyl-4'-(dimethylamino)azobenzene.

No evidence of primary irritation or allergic hypersensitivity was seen in any of the subjects patch tested with 20% aqueous solution of the trade name mixture Unicotrozon C-49 containing 5% Fumaric Acid and 17.5% Fumaris Officinalis Extract that also contains some Fumaric Acid. A leave-on product containing 1% Di-C12-15 Alkyl Fumarate did not demonstrate a potential for eliciting dermal irritation or sensitization.

Diisostearyl Fumarate is a high shine emollient with good conditioning properties. In lip care products, it helps to disperse pigments and is used to decrease feathering and bleeding.

The contact-sensitization potential of a lip gloss containing 20% Diisostearyl Fumarate to human skin by means of maximization assay was evaluated. A total of 26 healthy adults (23 females, 3 males) participated in the study; of which 25 completed the study. No adverse or unexpected reactions were seen in any of the subjects during the induction phase and no instances of contact allergy were recorded during the challenge phase at either 48 or 72-h.

The contact-sensitization potential of a lip gloss containing 17.41% Diisostearyl Fumarate to human skin by means of maximization assay was evaluated. A total of 26 healthy adults (20 - 61 years old) participated in the study; 25 completed the study. The lipgloss containing 17.4% Diisostearyl Fumarate did not possess a detectable contact-sensitizing potential and is not likely to cause contact sensitivity reactions under normal use conditions.

Fumaric Acid and its esters are used in psoriasis treatment, primarily in Europe.

Case reports include reports of nausea, upper-abdominal pain, loss of appetite, palpitations and flushes consistent with those seen in clinical testing; abnormal renal functions and urinary findings appeared generally reversible.

The cis isomer of Fumaric Acid, Maleic Acid, was found safe for use in cosmetics as a pH adjustor.

DISCUSSION

Overall, the CIR Expert Panel considered that the available data, including the role of Fumaric Acid in normal metabolism, animal toxicity data, and clinical experience were adequate to assess the safety of these ingredients as used in cosmetics.

While salts of dimethyl fumarate and monoethyl fumarate are not cosmetic ingredients, they are approved pharmaceuticals in Europe for treatment of psoriasis. As a consequence, they have been evaluated for both sensitization (published) and reproductive and developmental toxicity (unpublished). In both cases, no concern regarding sensitization potential were raised about these compounds.

The CIR Expert Panel recognized that certain ingredients in this group are reportedly used in a given product category, but the concentration of use was not available. For other ingredients in this group, information regarding use concentration for specific product categories was provided, but the number of such products was unknown. In still other cases, an ingredient was not in current use, but may be used in the future. The information available on the types of products and at what concentration indicate a pattern of use, within which some of these ingredients likely would be used.

The available safety test data support that these ingredients can be used safely at concentrations up to 20%.

In the absence of inhalation toxicity data, the Panel determined that these ingredients can be used safely in hair sprays, because the product particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (~38 μm) and pump hair sprays (>80 μm) is large compared to respirable particulate sizes (≤10 μm).

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concluded that Fumaric Acid, Disodium Fumarate, Sodium Fumarate, Dibehenyl Fumarate, Di-C12-15 Alkyl Fumarate,

Diethylhexyl Fumarate, Diisostearyl Fumarate, Sodium Stearyl Fumarate, and Ferrous Fumarate are safe as used in cosmetic formulations in the practices of use given in this Final safety assessment.[1]

REFERENCES

Altmeyer, P.J. et al. 1994. Antipsoriatic effect of Fumaric Acid derivatives. Results of a multicenter double blind study in 100 patients. *J. Am. Acad. Dermatol.;* 30:977-981.

Altmeyer, P. and C. Nuchel. 1996. Systemic therapy of psoriasis. *Dtsch. Med. Wochenschr*; 121:1605-1607.

AMA Laboratories, Inc. 1991. Primary skin irritation Di-C12-14 Alkyl Fumarate (Marrix SF). AMA Ref. No. WPAT91092/PS7199. BCCI.

AMA Laboratories, Inc. 1991. Primary skin irritation Di-C12-14 Alkyl Fumarate (Marrix SF). AMA Ref. No. WPAT91-92/PS7199. BCCI.

AMA Laboratories, Inc. 1991. Primary eye irritation Di-C12-14 Alkyl Fumarate (Marrix SF). AMA Ref. No. WPAT91-92/PE7199. BCCI.

Andersen, F.A., ed. 2007. Final Report of the Safety Assessment of Maleic Acid. *Int. J. Toxicol.* 26(S2):125-130.

Arai, T.1953. Effect of Fumaric Acid on the Yoshida sarcoma. *Gan.*; 44: 361-362.

Arai, T. 1955. Experimental study on the function of the reticuloendothelial system. V. Effects of various hormones and vitamins on the function of the reticuloendothelial system and of the liver. *Tohoku J. Exp. Med.*; 6:113-125.

Balasubramaniam, P. and J. Berth-Jones. 2003. Fumaric Acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. *Br. J. Toxicol.*; 150:741-746.

Bartek Ingredients, Inc. 2007. Fumaric Acid. Website accessed on December 12, 2007; 1 page. http://www.bartek.ca/fumaric_acid.html

Bayard, W. et al. 1987. Peroral long-term treatment of psoriasis using Fumaric Acid derivatives. *Hautartz*; 38:279-285.

Bayard, S. et al. 2004. Inter- and intra- hemispheric processing of visual event-related potentials in the absence of the corpus callosum. *J. Cog. Neurosci.*; 16:401-414.

Bimax Ingredients, Inc. 2007. Fumaric Acid. Website accessed on December 12, 2007. 1 page. http://www.patentstorm.us/patents/6869998-description.html

BiogenIdec. 2008. Summary of product characteristics for Fumaderm® and Fumaderm® initial. Unpublished data submitted by the Council on February 22, 2008. 4 pages. [2]

Bodansky, O.1942. The toxicity and laxative action of Sodium Fumarate. *J. Amer. pharm. Ass., Sci. Ed.*; 31:1

Bournias-Vardiabasis, N. et al. 1983. Detection of teratogens in the *Drosophila* embryonic cell culture test: Assay of 100 chemicals. *Teratol.*; 28:109-122.

Bower D. 1999. Unpublished information on hair spray particle sizes provided at the September 9, 1999 CIR Expert Panel meeting.[2]

Butterfield, J.H. et al. 1986. Extracellular deposition of eosinophil granule major basic protein in lymph nodes of patients with Hodgkin's disease. *Blood*; 68:1250-1256.

Carboni, I. et al. 2004. Fumaric Acid esters in the treatment of psoriasis: an Italian experience. *J. Dermatolog. Treat.*; 15:23-26.

Chemical Land 21. 2007. MSDS on Fumaric Acid and Ferrous Fumarate. http://www.chemicalland21.com/petrochemical/FUMARIC%20ACID.htm and http://www.chemicalland21.com/lifescience/foco/FERROUS%20FUMARATE.htm. Websites accessed on December 12, 2007.

Christ, H.W. 1999. Immunomodulating therapy of psoriasis vulgaris. *Med. Klin. (Munich)*; 94:90-92.

Clinical Research Laboratories, Inc. 2004. Repeated insult patch test of a leave-on product containing 1% Di-C12-15 Alkyl Fumarate. Unpublished data submitted by Personal Care Products Council (Council) on January 22, 2008. 13 pages.[2]

Clinical Research Laboratories, Inc. 2005. Repeated insult patch test of a leave-on product containing 1% Di-C12-15 Alkyl Fumarate. Unpublished data submitted by Council on January 22, 2008. 13 pages. [2]

Consumer Product Testing. 1993. Schercemol DISF Diisostearyl Fumarate: Primary dermal irritation in rabbits, primary ocular irritation in rabbits, acute oral toxicity in rats. Unpublished data submitted by Personal Care Products Council on February 5, 2008.[2]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2007. Concentration of use - Fumaric Acid, Di-C12-15 Alkyl Fumarate, and Diisostearyl Fumarate. Unpublished data submitted by CTFA on August 22, 2007. 2 pages. [2]

Davidson, P.M. 1990. Antimicrobial agents. *Food and Science Technology*; 35:83-138.

De Haan, P. et al. 1994. The risk of sensibilization and contact urticaria upon topical application of Fumaric Acid derivatives. *Dermatol.*; 188:126-130.

Dermtest. 1997. Human patch test on 20% aqueous Unicontrozon C-49 (contains 5% Fumaric Acid and 17.5% Fumaria Officinalis Extract that also contains some Fumaric Acid. Unpublished data submitted by Council on January 9, 2008. 6 pages. [2]

Encyclopedia of Alternative Energy and Sustainable Living Transport Concepts and Designs. 2008. Citric acid cycle. Website accessed on January 22, 2008: http://www.daviddarling.info/encyclopedia/C/citric_acid_cycle.html.

European Chemicals Bureau. 2000. IUCLID Dataset Fumaric Acid. Website accessed on January 9, 2008: http://ecb.jrc.it/IUCLID-DataSheets/110178.pdf.

Food Chemicals Codex. 2008. Monographs: Ferrous Fumarate, Fumaric Acid and Sodium Stearyl Fumarate. Sixth ed. Pp. 363 - 65, 381, 912-13.

Food and Drug Administration (FDA). 2006. Frequency of use of cosmetic ingredients. *FDA database.* Washington, DC:FDA

FDA. 2007. Fumaric Acid and salts of Fumaric Acid (21CFR172.350) and Sodium Stearyl Fumarate (21CFR172.826).

Fitzhugh, O. G. 1947. The comparative chronic toxicities of Fumaric, tartaric, oxalic, and maleic acids. *J. Amer. pharm. Ass., sci. Ed.*; 36: 217 .

Fliegner, L. 1992. Osteomalacia as an apparent side effect of oral Fumaric Acid therapy. *Hautarzt*; 43:554-560.

Gafvert, E. 1994. Allergenic components in modified and unmodified rosin: Chemical characterization and studies of allergenic activity. *Acta. Derm. Venreol*. Suppl.; 184:1-36.

Gollnick, H. et al. 2002. Topical calcipotriol plus oral Fumaric Acid is more effective and faster acting than oral Fumaric Acid monotherapy in the treatment of severe chronic plaque psoriasis vulgaris. *Dermatol.*; 205:46-53.

Gottschalck, T.E. and J. Bailey, eds. 2008. *International Cosmetic Ingredient Dictionary and Handbook*, 12th ed vol 1 and 2. Washington, DC: CTFA.

Guenther, C.H. et al. 2003. Macular exanthema due to Fumaric Acid esters. *Ann. Pharmacother.*; 37:234-236.

Hagedorn, M. et al. 1975. Fumaric Acid monoethylester: Effect on DNA-synthesis and preliminary findings in experimental studies in animals. *Arch. Dermatol. Res.*; 254:67-73.

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

[2] Available for review: Director, CIR, 1101 17th St., NW, Suite 412, Washington, DC 20036

Hansson, C. and K. Thorneby-Andersson. 2003. Stereochemical considerations on concomitant allergic contact dermatitis to ester of the cis-trans isomeric compounds maleic acid and Fumaric Acid. *Skin Pharmacol. Appl. Skin Physiol*.; 16:117-122.

Harrison, J. W. E. 1962. Unpublished report of LaWall and Harrisson Research Laboratories, submitted to WHO.

Haviv, Y.S. et al. 1999. Fumaric Acid ester-induced diffuse renal tubular injury presenting as Fanconi. *Clin. Drug Invest*.; 17:333-335.

Hoefnagel, J.J. et al. (2003). Long-term safety aspects of systemic therapy with Fumaric Acid esters in severe psoriasis. *Br. J. Dermatol*.; 149:363-369.

Ishidate, M. Jr. et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol*.; 22:623-636.

Jensen P.A. and D. Obrien. 1993. Industrial Hygiene. In: *Aerosol Measurement. Principles Techniques and Applications*, eds. K. Willeke, P.A. Baron. New York: John Wiley and Sons, Inc., 538-540.

Johnsen, M.A. 2004. The Influence of Particle Size. *Spray Technology and Marketing*. November:24-27.

Kelloff, G.J. et al. 1994. Chemopreventive drug development: Perspectives and progress. *Cancer Epidmiol. Biomarkers*; 3:85-98.

KGL, Inc. 2006. An evaluation of the contact-sensitization potential of a topical coded product (lip gloss containing 20% Diisostearyl Fumarate) in human skin by means of the maximization assay. Unpublished data submitted by Personal Care Products Council on September 25, 2008.[2]

KGL, Inc. 2007. An evaluation of the contact-sensitization potential of a topical coded product (lipstick containing 17.41% Diisostearyl Fumarate) in human skin by means of the maximization assay. Unpublished data submitted by Personal Care Products Council on September 2, 2008.[2]

Kim, S.H. and F.W. Karasek. 1981. Negative ions of dicarboxylic acids observed by plasma chromatography and atmospheric pressure ionization mass spectrometry. *J. Chromatogr*.; 234:13-23.

Kolbach, D.N. and C. Nieboer. 1992. Fumaric Acid therapy in psoriasis: results and side effects of 2 years of treatment. *J. Am. Acad. Dermatol*.; 27:769-771.

Kreuter, A. et al. 2005. Fumaric Acid esters in necrobiosis lipoidica: results of a prospective noncontrolled study. *Br. J. Dermatol*.; 153:802-807.

Kuroda, K. and M. Akao. 1981. Antitumor and anti-intoxication activities of Fumaric Acid in cultured cells. *JPN J. Cancer Res*.; 72:777-782.

Kuroda, K. 1982. Inhibitory effect of Fumaric Acid on forestomach and lung carcinogenesis by 5-nitrofuran naphthyridine derivative in mice. *J. Natl. Cancer Inst*.; 69:1317-120, 9.

Kuroda, K. 1983. Inhibitory effect of Fumaric Acid on 3-methyl-4'-(dimethylamino)-azobenzene-induced hepatocarcinogenesis in rats. *J. Natl. Cancer Inst.*; 71:855-857.

Kuroda, K. 1986. Fumaric Acid enhances DNA synthesis of rat hepatocytes by counteracting the toxicities of mitomycin C and aflatoxin B1. *Jpn. J. Cancer Res*.; 77:750-758.

Kuroda, K. 1987. Inhibitory effect of Fumaric Acid on hepatocarcinogenisis by thioacetamide in rats. *J. Natl. Cancer Inst*.; 79:1047-1051.

Kuroda, K.1989. Inhibitory effect of Fumaric Acid on 3'-methyl-4-(dimethylamino) azobenzene-induced hepatocarcinogenesis in rats. *Chem. Pharm. Bull.*; 37:1345-1346.

Leberco Celsis Testing. 1996. Acute dermal toxicity limit test (OECD 402) on Marrix SF (Di-C12-15 Alkyl Fumarate). Assay #9617703.

Levey, S., Lasichak, A.G., Brimi, R., et al. 1946. A study to determine the toxicity of Fumaric Acid. *J. Amer. Pharm. Ass., Sci. Ed.*; 35: 298.

Lewis, Ed. 1991. Fumaric Acid. *Dangerous Properties of Industrial Materials*. 7th Ed., Vol. 3.

Locke, A. et al. 1942. *J. Amer. Pharm. Ass*.; 31: 12.

Lonza. 2006. Product specification Fumaric Acid. Unpublished data submitted by Personal Care Products Council on January 8, 2008. 2 pages. [2]

Lubrizol. 2006. Technical data sheet Schercemol TM DISM Ester. Unpublished data submitted by Personal Care Products Council on February 5, 2008.

Lubrizol. 2007. Technical data sheet Schercemol ™ DISF Ester. Unpublished data submitted by Personal Care Products Council on February 5, 2008.

Marshall, L. M., Orten, J. M. and Smith, A. H. 1949. Chromatography of carboxylic acids on a silica gel column with a benzene-ether solven system. *J. biol. Chem*.; 179: 1127.

Mrowietz, U., Christophers, E., and Altmeyer, P. 1999. Treatment of severe psoriasis with Fumaric Acid esters: Scientific background and guidelines for therapeutic use. *Br. J. Dermatol*.; 141:424-429.

National Institute for Occupational Health and Safety (NIOSH). 1986. Registry of Toxic Effects of Chemical Substances.

Neiboer, C., Dehoop, D., van Loenen, A.C. et al. 1989. Systemic therapy with Fumaric Acid derivatives: New possibilities in the treatment of psoriasis. *J. Am. Acad. Dermatol*.; 20:601-608.

Neiboer, C., Dehoop, D., L., Ngendijk, P.N., et al. 1990. Fumaric Acid therapy in psoriasis: A double-blind comparison between Fumaric Acid compound therapy and monotherapy with dimethylfumarate acid ester. *Dermatologica*.; 181:33-37.

Nikko Chemicals Co., Ltd. 2007. Specifications Nikkol NES 203-27 (Sodium C12-15 Pareth Sulfate). Unpublished data submitted by the Council on July 10, 2008.[2]

Nikko Chemicals Co., Ltd. 2008. Manufacturing process Nikkol NES 203-27 (Sodium C12-15 Pareth Sulfate). Unpublished data submitted by the Council on July 10, 2008.[2]

O'Neil, M.J., ed. 2006. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck & Co., Inc., p. 2564.

Packman, E. W., Abbott, D. D. and Harrisson, J. W. E. 1963. Comparative subacute toxicity for rabbits of citric, Fumaric, and tartaric acids. *Toxicol. appl. Pharmacol*.; 5: 163.

Periera, M.A., Barnes, L.H., Rassman, V.L. et al. 1994. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. *Carcinogenesis*; 15:1049-1054.

Personal Care Products Council (Council). 2008. Concentration of use - Ferrous Fumarate. Unpublished data provided by the Council on February 22, 2008. 1 page.[2]

Raab, W. 1984. Psoriasis therapy with Fumaric Acid and Fumaric Acid esters. *Z. Hautkr*.; 59:671-679.

Rao, K.S. and S.H. Mishra. 1997. Antihepatotoxic activity of monomethyl fumarate isolated from *Fumaria indica*. *J. Ethnopharmacol*.; 60:207-213.

Rantanen, T. 2008. The cause of the Chinese sofa/chai dermatitis epidemic is likely to be contact allergy to dimethylfumarate, a novel potent contact sensitizer. *Br. J. Dermatol*.; 159:218-221.

Rapson, W.H., Nazar, M.A., and Bulsky, V.V. 1980. Mutagenicity produced by aqueous chlorination of organic compounds. *Bull Environ. Toxicol*.; 24:590-596.

Raschka, C. and H.J. Koch. 1999. Long-term treatment of psoriasis using Fumaric Acid preparations can be associated with severe proximal tubular damage. *Hum. Exp. Toxicol*.; 18:738-739.

RCC. 2001. Acute oral toxicity study in rats Marrix SF (Di-C12-15 Alkyl Fumarate). RCC Study Number 823432.[2]

RCC. 2002. Contact hypersensitivity in albino guinea pigs, maximization test on Marrix SF (Di-C12-15 Alkyl Fumarate). RCC Study Number 842039.[2]

Research and Development. 1988. Sensitization study on Diisostearyl Malate. Unpublished data submitted by Personal Care Products Council on February 5, 2008.[2]

SafePharm Laboratories. 2008. EX 800185 (Diisostearyl Fumarate) Reverse mutation assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli*. Unpublished data submitted by Council on February 11, 2008. 20 pages. [2]

Saffioti, U., and Shubik, P. 1963. Studies on promoting action in skin carcinogenesis. National Cancer Institute (NCI) Monograph; 10:489-507.

Schelegelmilch, R. and H.U. Wolf. 1986. Mutagenic activity of acetonitrile and Fumaric Acid dinitrile in 3 short-term mutagenicity test systems. *Naunyn-Schmiedeberg's Arch. Pharmacol.;* 332:R21.

Science Lab. 2007a. MSDS on Sodium Stearyl Fumarate. http://www.sciencelab.com/msds.php?msdsId=9925042 Website accessed on December 12, 2007.

Science Lab. 2007b. MSDS on Ferrous Fumarate. http://www.sciencelab.com/msds.php?msdsId=9924052 Website accessed on December 12, 2007.

Sebok, B., Bonnekoh, B., Geisel, J., et al. 1994. Antiproliferative and cytotoxic profiles of antipsoriatic Fumaric Acid derivatives in keratinocyte cultures. *Eur. J. Pharmacol.*; 270:79-87.

Sitek Research Laboratories. 1995. Evaluation of a test article (Marrix SF, Di-C12-15 Alkyl Fumarate) in the Salmonella typhimurium/Escherichia coli plate incorporation/preincubation mutation assay in the presence and absence of aroclor –induced rat liver S-9 with a confirmatory study. Sitek Study No. 0335-2140.[2]

Skaria, A.M. and U. Schmid. 1996. Antipsoriatic effect of Fumaric Acid derivates. *J. Am. Acad. Dermatol*.; 34 (2Pt 1): 323-324.

Smith, C.G.1963. Relationship between cytotoxicity in vitro and whole animal toxicity. *Cancer Chemother. Rep.*; 30:9-12.

Spencer, S.R. 1990. Induction of glutathione transferases and NADPH: Quinone reuctase by Fumaric Acid derivatives in rodent cells and tissues. *Cancer Res*.; 50:7871-7875.

Stephens & Associates, Inc. 1997. Fourteen day cumulative irritation test. Test materials SC 11349-04 and SC11349-05 each contained 5% Di-C12-15 Alkyl Fumarate (trade name Marrix SF). Study number C97-D245. Unpublished data submitted by Personal Care Products Council on April 11, 2008.[2]

Stephens & Associates, Inc. 1998. An evaluation of the safety, effectiveness, comedogenicity, and acnegenicity of a topically applied cosmetic product designed to improve skin moisturization in women. Test material 24334 contained 5% Di-C12-15 Alkyl Fumarate (trade name Marrix SF) Study number C97-0201. Unpublished data submitted by Personal Care Products Council on April 11, 2008.[2]

Stephens & Associates, Inc. 1998. Human repeat insult patch test. Test materials SC 11349-06 and SC11349-07 each contained 5% Di-C12-15 Alkyl Fumarate (trade name Marrix SF). Study number C97-D229. Unpublished data submitted by Personal Care Products Council on April 11, 2008[2]

Stuhlinger, W. 1990. The nephrotoxic effect of therapy with Fumaric Acid esters in psoriasis. *Dtsch Med.Wochenschr*.; 115:1712-1715.

Ullmann's Encyclopedia of Industrial Chemistry. 1996. Maleic and Fumaric Acids. 5th Edition. Vol. A16.

Vandermeeren, M. et al. 1997. Dimethylfumarate is an inhibitor of cytokine-induced E-selectin, VCAM-1, and ICAM-1 expression in human endothelial cells. *Biochem. Biophys. Res. Commun*.; 234:19-23.

Vernot, E.H., MacEwen, J.D., Haun, C.C. et al. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. *Toxicol. Appl. Pharmacol*. 42:417-423.

Wang, C.Y. 1973. Isolation of fumaric-acid succinic-acid isoquercitrin and tiliroside from *Pteridium aquilinum. Phytochem*.; 12:2298-2299.

Weiss, J. M. 1923. Inactive maleic acid as a Food Acidulent. *Ind. Eng. Chem*.; 15: 628.

International Journal of Toxicology
31(Supplement 1) 5S-76S
© The Author(s) 2012
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1091581812447203
http://ijt.sagepub.com
SAGE

Final Report of the Cosmetic Ingredient Review Expert Panel on the Safety Assessment of Dicarboxylic Acids, Salts, and Esters

Monice M. Fiume[1], HBart eldreth[2], Wilma F. Bergfeld[3], Donald V. Belsito[3], Ronald A. Hill[3], Curtis D. Klaassen[3], Daniel Liebler[3], James G. Marks, Jr[3], Ronald C. Shank[3], Thomas J. Slaga[3], Paul W. Snyder[3], and F. Alan Andersen[4]

Abstract

The CIR Expert Panel assessed the safety of dicarboxylic acids and their salts and esters as used in cosmetics. Most dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients, but the functions of most of the salts in cosmetics are not reported. Some of the esters function as skin conditioning or fragrance ingredients, plasticizers, solvents, or emollients. The Expert Panel noted gaps in the available safety data for some of the dicarboxylic acid and their salts and esters in this safety assessment. The available data on many of the ingredients are sufficient, however, and similar structural activity relationships, biologic functions, and cosmetic product usage suggest that the available data may be extrapolated to support the safety of the entire group. The Panel concluded that the ingredients named in this report are safe in the present practices of use and concentration.

Keywords

final report of the cosmetic ingredient review expert panel on the safety assessment of dicarboxylic acids, salts and esters, safety, cosmetics

Introduction

The safety of sebacic acid and other alkyl α,ω-dicarboxylic acids, and their salts, monoesters and diesters as used in cosmetics, has been reviewed by the CIR Expert Panel (the Panel). The dicarboxylic acids are terminally functionalized straight alkyl chains characterized by a separation between the carboxylic acid functional groups of 1 to 10 carbons (1 carbon separation, 3 carbons total (C3) = malonic acid; 2 carbons separation (C4) = succinic acid; 3 carbons separation (C5) = glutaric acid; 4 carbons separation (C6) = adipic acid; 7 carbons separation (C9) = azelaic acid; 8 carbons separation (C10) = sebacic acid; and 10 carbons separation (C12) = dodecanedioic acid). The simple alkyl diesters are the result of the condensation of alkyl dicarboxylic acids and 2 equivalents of alkyl alcohols. The simple alkyl esters (mono- and di-) of these dicarboxylic acids have straight or branched side chains ranging in length from 1 to 18 carbons. Throughout this report, the data are presented by order of acid chain length (ie, beginning with malonic acid and ending with dodecanedioic acid; and beginning with dimethyl malate and ending with diisocetyl dodecanedioate).

This report presents available information in 2 groups, the 12 alkyl dicarboxylic acids/salts and the 44 corresponding (mono- and di-) esters.

The alkyl dicarboxylic acids and salts include:

- malonic acid
- succinic acid
- sodium succinate
- disodium succinate
- glutaric acid
- adipic acid
- azelaic acid

[1] Scientific Analyst/Writer, Cosmetic Ingredient Review
[2] Chemist, Cosmetic Ingredient Review
[3] The 2011 Cosmetic Ingredient Review Expert Panel member
[4] Director, Cosmetic Ingredient Review

Corresponding Author:
F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA
Email: cirinfo@cir-safety.org

- dipotassium azelate
- disodium azelate
- sebacic acid
- disodium sebacate
- dodecanedioic acid.

The esters include:

- diethyl malonate
- decyl succinate
- dimethyl succinate
- diethyl succinate
- dicapryl succinate
- dicetearyl succinate
- diisobutyl succinate
- diethylhexyl succinate
- dimethyl glutarate
- diisobutyl glutarate
- diisostearyl glutarate
- dimethyl adipate
- diethyl adipate
- dipropyl adipate
- dibutyl adipate
- dihexyl adipate
- dicapryl adipate
- di-C12-15 alkyl adipate
- ditridecyl adipate
- dicetyl adipate
- diisopropyl adipate
- diisobutyl adipate
- diethylhexyl adipate
- diisooctyl adipate
- diisononyl adipate
- diisodecyl adipate
- dihexyldecyl adipate
- diheptylundecyl adipate
- dioctyldodecyl adipate
- diisocetyl adipate
- diisostearyl adipate
- isostearyl sebacate
- diethyl sebacate
- dibutyl sebacate
- dicaprylyl/capryl sebacate
- diisopropyl sebacate
- diethylhexyl sebacate
- dibutyloctyl sebacate
- diisooctyl sebacate
- dihexyldecyl sebacate
- dioctyldodecyl sebacate
- diisostearyl sebacate
- dioctyldodecyl dodecanedioate
- diisocetyl dodecanedioate

The acids and their salts included in this report function in cosmetics as pH adjusters, and the esters function as fragrance ingredients, plasticizers, skin-conditioning agents or solvents, and corrosion inhibitors. CAS numbers, definitions, structures and functions for the alkyl dicarboxylic acid, salt, and ester ingredients included in this report are given in Table 1.

A safety assessment of diethylhexyl adipate (often inaccurately named dioctyl adipate)[1] and diisopropyl adipate was published in 1984, with the conclusion that these ingredients are safe as used in cosmetics.[2] The safety of these ingredients was re-reviewed and confirmed in 2005[3] and 2006.[4] Additionally, dibutyl adipate was originally reviewed in 1996, and at that time the available data were found insufficient to support the safety of dibutyl adipate in cosmetic formulations. When re-reviewed in 2006, additional data were made available to address the data needs identified by the CIR Expert Panel, and an amended conclusion was issued stating that dibutyl adipate is safe for use in cosmetic formulations.[5]

In order to focus on the acids and their salts separately from the dicarboxylic acid esters, this report is presented in 2 sections.

Part I: Alkyl Dicarboxylic Acids and their Salts

Chemistry

Method of Manufacture

While many of the alkyl dicarboxylic acids are present in natural sources, commercial production of these acids has historically occurred via alkali pyrolysis of lipids.[6] For example, when castor oil (a lipid which is comprised of approximately 84% ricinoleic acid side chain bearing triglycerides) is pyrolyzed with sodium hydroxide, some of the major products are sebacic acid and 2-octanol (Figure 1).[6] Sodium and potassium salts of the alkyl dicarboxylic acids are readily prepared via addition to the appropriate stoichiometric equivalent/equivalents of sodium hydroxide or potassium hydroxide, respectively.

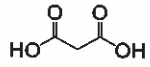
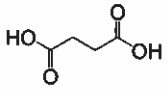
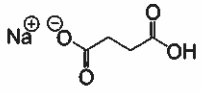
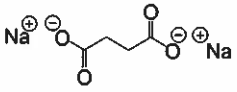
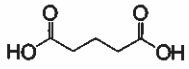
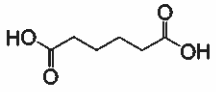
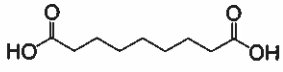
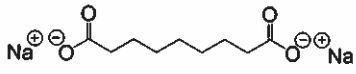
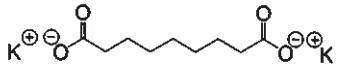
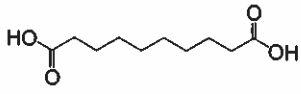
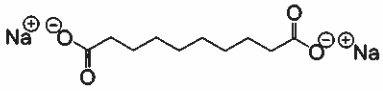
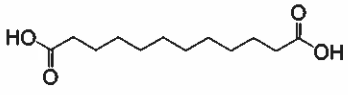
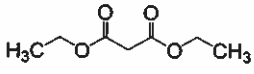
Some of the ingredients in this assessment are tallow derivatives. The CIR accepts the Food and Drug Administration (FDA) determination (21 CFR 700.27(a)) that tallow derivatives are not prohibited cattle materials and may be used in cosmetics.

Malonic acid (C3). Malonic acid, first prepared by malic acid oxidation, is commonly manufactured by more recent methods including the ozonolysis of cyclopentadiene or the air oxidation of 1,3-propanediol.[7]

Succinic acid (C4). Succinic acid is an intermediate of the citric acid cycle and is found in almost all plant and animal cells, although at very low concentrations.[8] Succinic acid is commonly produced synthetically by catalytic (eg, nickel or palladium catalyst) hydrogenation of maleic anhydride.

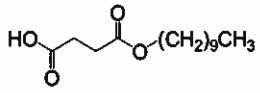
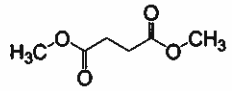
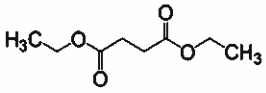
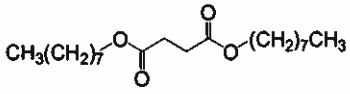
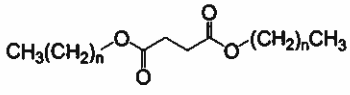
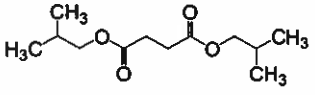
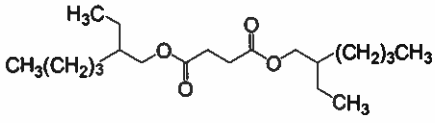
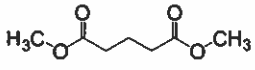
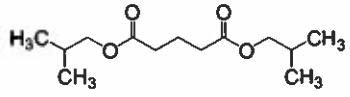
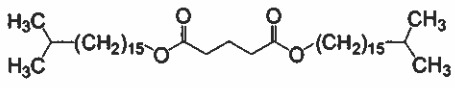
Glutaric (C5) and adipic(C6) acids. Although glutaric acid is often encountered in nature, adipic acid is not commonly encountered in nature. Glutaric and adipic acids were first synthesized by oxidation of castor oil with nitric acid. However, adipic acid is now more commonly manufactured by the

Table 1. Definitions, Functions, and Structures of Dicarboxylic Acid, Sal,t and Ester Ingredients in This Safety Assessment

Ingredient CAS No.	Definition	Function/Functions	Formula/Structure
Dicarboxylic Acids and Metal Salts			
Malonic Acid 141-82-2	Malonic Acid is the organic compound that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Succinic Acid 110-15-6	Succinic Acid is the dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Sodium Succinate 2922-54-5	Sodium Succinate is the sodium salt of succinic acid.	Buffering Agents; pH Adjusters	
Disodium Succinate 150-90-3	Disodium Succinate is the disodium salt of Succinic Acid.	Fragrance Ingredients; Not Reported	
Glutaric Acid 110-94-1	Glutaric Acid is the organic compound that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Adipic Acid 124-04-9	Adipic Acid is the organic dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Azelaic Acid 123-99-9	Azelaic Acid is the dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Disodium Azelate 17265-13-3	Disodium Azelate is the disodium salt of azelaic acid.	Not Reported	
Dipotassium Azelate 19619-43-3	Dipotassium Azelate is the organic salt that conforms to noted structure.	Not Reported	
Sebacic Acid 111-20-6	Sebacic Acid is the organic dicarboxylic acid that conforms to noted structure.	pH Adjusters	
Disodium Sebacate 17265-14-4	Disodium Sebacate is the disodium salt of Sebacic Acid. It conforms to the noted structure.	Not Reported	
Dodecanedioic Acid 693-23-2	Dodecanedioic Acid is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Miscellaneous	
Malonic Diester Ingredient			
Diethyl Malonate 105-53-3	Diethyl Malonate is the organic compound that conforms to noted structure.	Fragrance Ingredients	

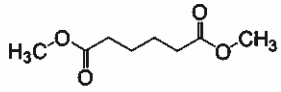
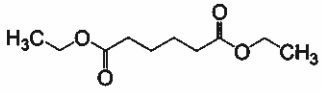
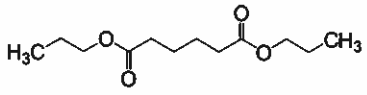
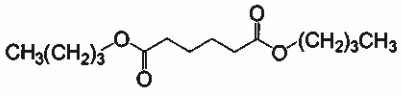
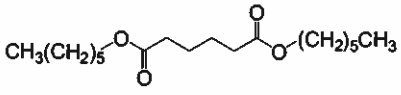
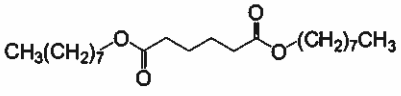
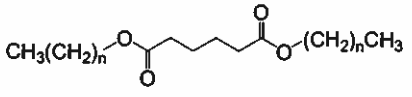
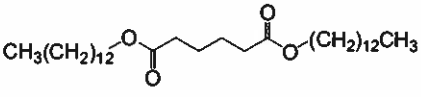
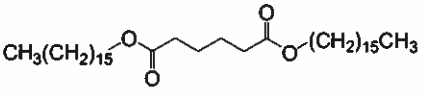
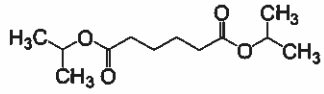
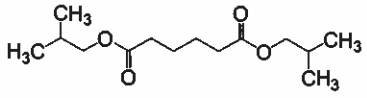
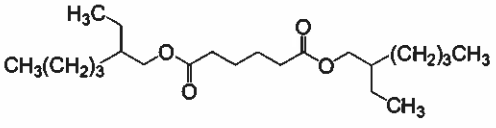
(continued)

Table 1. (continued)

Ingredient CAS No.	Definition	Function/Functions	Formula/Structure
Succinic Ester Ingredients			
Monoester			
Decyl Succinate 54482-22-3 (wrong CAS No. 2530-33-8)	Decyl Succinate is the monoester of decyl alcohol and succinic acid.	Skin-Conditioning Agents - Emollient	
Diesters			
Dimethyl Succinate 106-65-0	Dimethyl Succinate is the diester of methyl alcohol and Succinic Acid.	Nail Polish and Enamel Removers	
Diethyl Succinate 123-25-1	Diethyl Succinate is the diester of ethyl alcohol and Succinic Acid.	Fragrance Ingredients; Plasticizers; Solvents	
Dicapryl Succinate 14491-66-8	Dicapryl Succinate is the organic compound that conforms to noted structure.	Film Formers; Hair Conditioning Agents; Nail Conditioning Agents; Plasticizers; Skin-Conditioning Agents - Emollient	
Dicetearyl Succinate 93280-98-9	Dicetearyl Succinate is the diester of Cetearyl Alcohol and Succinic Acid.	Skin-Conditioning Agents - Miscellaneous	 wherein n=15 or 17
Branched			
Diisobutyl Succinate 925-06-4	Diisobutyl Succinate is the organic compound that conforms to the noted structure.	Plasticizers	
Diethylhexyl Succinate 2915-57-3	Diethylhexyl Succinate is the diester of 2-ethylhexyl alcohol and Succinic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Glutaric Ester Ingredients			
Dimethyl Glutarate 1119-40-0	Dimethyl Glutarate is the diester of methyl alcohol and glutaric acid.	Nail Polish and Enamel Removers	
Branched			
Diisobutyl Glutarate 71195-64-7	Diisobutyl Glutarate is the organic compound that conforms to noted structure.	Plasticizers	
Diisostearyl Glutarate No CAS No.	Diisostearyl Glutarate is the diester of isostearyl alcohol and glutaric acid.	Skin-Conditioning Agents - Emollient	One example of an "iso" 

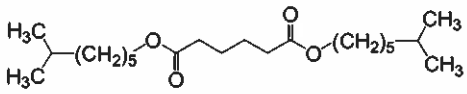
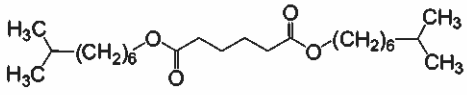
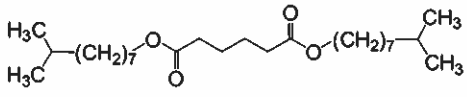
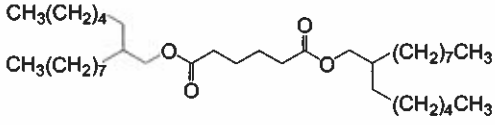
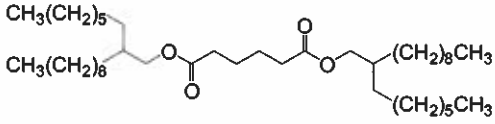
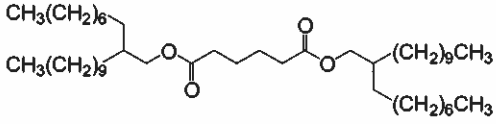
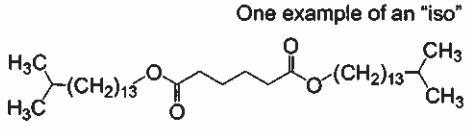
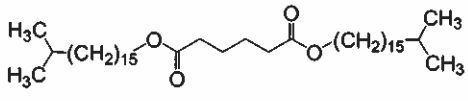
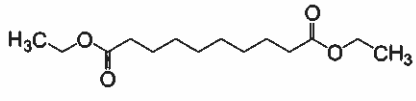
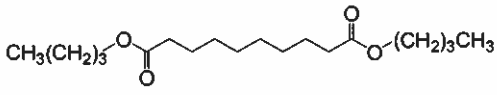
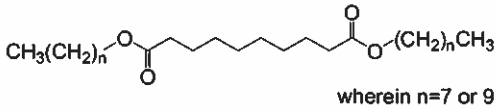
(continued)

Table 1. (continued)

Ingredient CAS No.	Definition	Function/Functions	Formula/Structure
Adipic Ester Ingredients			
Dimethyl Adipate 627-93-0	Dimethyl Adipate is the diester of methyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethyl Adipate 141-28-6	Diethyl Adipate is the diester of ethyl alcohol and adipic acid.	Fragrance Ingredients; Skin-Conditioning Agents - Emollient	
Dipropyl Adipate 106-19-4	Dipropyl Adipate is the diester of propyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dibutyl Adipate 105-99-7	Dibutyl Adipate is the diester of butyl alcohol and adipic acid.	Nail Polish and Enamels; Suntan Gels, Creams, and Liquids	
Dihexyl Adipate 110-33-8	Dihexyl Adipate is the diester of hexyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dicapryl Adipate 105-97-5	Dicapryl Adipate is the diester of capryl alcohol and adipic acid.	Plasticizers	
Di-C12-15 Alkyl Adipate No CAS No.	Di-C12-15 Alkyl Adipate is the diester of C12-15 Alcohols and adipic acid.	Skin-Conditioning Agents - Emollient	 wherein n=11, 12, 13 or 14
Ditridecyl Adipate 16958-92-2	Ditridecyl Adipate is the diester of Tridecyl Alcohol and Adipic Acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dicetyl Adipate 26720-21-8	Dicetyl Adipate is the diester of cetyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient	
Branched			
Diisopropyl Adipate 6938-94-9	Diisopropyl Adipate is the diester of isopropyl alcohol and Adipic Acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diisobutyl Adipate 141-04-8	Diisobutyl Adipate is the diester of isobutyl alcohol and Adipic Acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethylhexyl Adipate 103-23-1	Diethylhexyl Adipate is the diester of a 2-ethylhexyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	

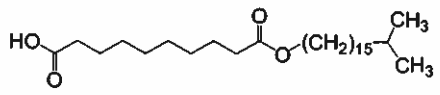
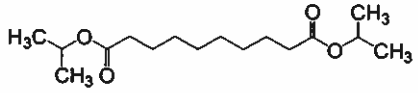
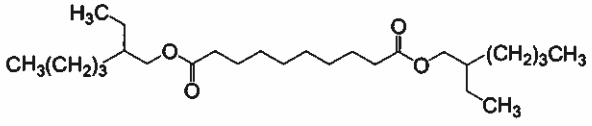
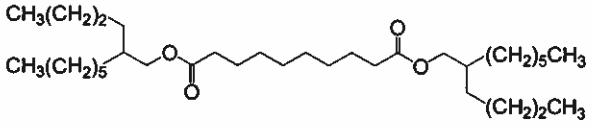
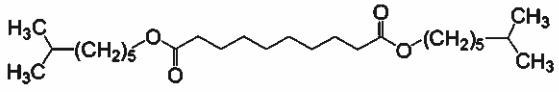
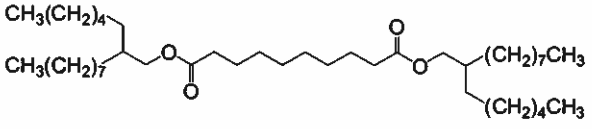
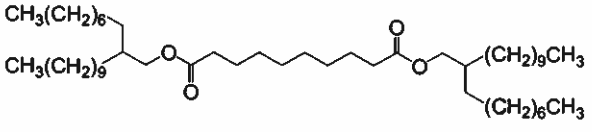
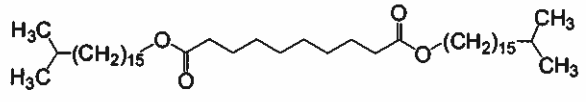
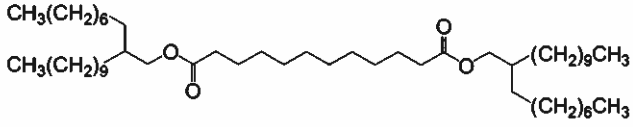
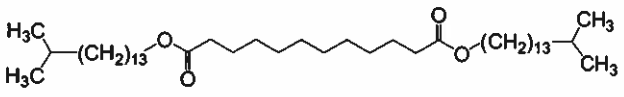
(continued)

Table 1. (continued)

Ingredient CAS No.	Definition	Function/Functions	Formula/Structure
Diisooctyl Adipate 108-63-4	Diisooctyl Adipate is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Diisononyl Adipate 33703-08-1	Diisononyl Adipate is the diester of isononyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Diisodecyl Adipate 27178-16-1	Diisodecyl Adipate is the diester of isodecyl alcohol and Adipic Acid.	Plasticizers; 5kin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Dihexyldecyl Adipate 57533-90-1	Dihexyldecyl Adipate is the diester of hexyldecanol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diheptylundecyl Adipate 155613-91-5	Diheptylundecyl Adipate is the diester of adipic acid and heptylundecanol.	Skin-Conditioning Agents - Emollient; Solvents	
Dioctyldodecyl Adipate 85117-94-8	Dioctyldodecyl Adipate is the diester of octyldodecanol and adipic acid.	Plasticizers; Skin-Conditioning Agents - Emollient	
Diisocetyl Adipate S9686-69-0 *sec:* 58262-41-2	Diisocetyl Adipate is the diester of hexadecyl alcohol and adipic acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Diisostearyl Adipate 62479-36-1	Diisostearyl Adipate is the diester of Isostearyl Alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient	One example of an "iso" 
Sebacic Ester Ingredients			
Diethyl Sebacate 110-40-7	Diethyl Sebacate is the diester of ethyl alcohol and Sebacic Acid	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Dibutyl Sebacate 109-43-3	Dibutyl Sebacate is the diester of butyl alcohol and sebacic acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Dicaprylyl/ Capryl Sebacate No CAS. No.	Dicaprylyl/Capryl Sebacate is the organic compound that conforms generally to the noted structure.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	 wherein n=7 or 9

(continued)

Table 1. (continued)

Ingredient CAS No.	Definition	Function/Functions	Formula/Structure
Branched Monoester			
Isostearyl Sebacate 478273-24-4	Isostearyl Sebacate is the half-ester of isostearyl alcohol and sebacic acid.	Skin-Conditioning Agents - Miscellaneous	One example of an "iso" 
Branched Diesters			
Diisopropyl Sebacate 7491-02-3	Diisopropyl Sebacate is the diester of isopropyl alcohol and Sebacic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethylhexyl Sebacate 122-62-3	Diethylhexyl Sebacate is the diester of 2-ethylhexyl alcohol and Sebacic Acid.	Fragrance Ingredients; Plasticizers; Solvents	
Dibutyloctyl Sebacate 184706-97-6	Dibutyloctyl Sebacate is the diester of butyloctyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diisooctyl Sebacate 10340-41-7	Diisooctyl Sebacate is the organic compound that conforms to noted structure.	Antioxidants; Plasticizers; Skin-Conditioning Agents - Emollient	One example of an "iso" 
Dihexyldecyl Sebacate 359073-59-9	Dihexyldecyl Sebacate is the diester of hexyldecyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dioctyldodecyl Sebacate 69275-01-0	Dioctyldodecyl Sebacate is the diester of octyldodecanol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diisostearyl Sebacate No CAS No.	Diisostearyl Sebacate is the diester of isostearyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient	One example of an "iso" 
Dodecanoic Ester Ingredients			
Dioctyldodecyl Dodecanedioate 129423-55-8	Dioctyldodecyl Dodecanedioate is the diester of octyldodecanol and dodecanedioic acid.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous	
Diisocetyl Dodecanedioate 131252-83-0	Diisocetyl Dodecanedioate is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents	One example of an "iso" 

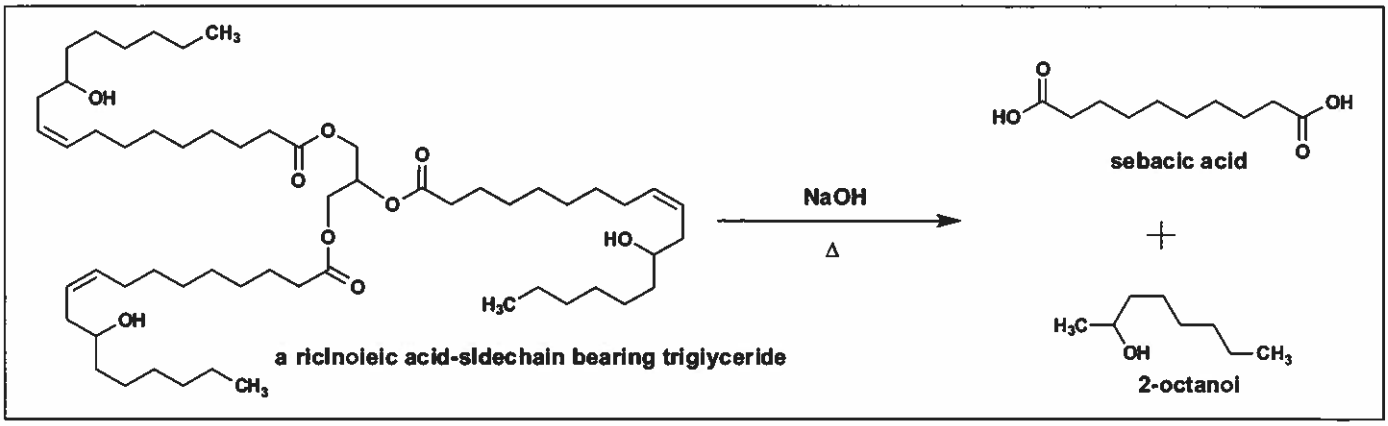


Figure 1. Sebacic acid synthesis from castor oil.

Table 2. Physical and Chemical Properties of the Alkyl Dicarboxylic Acid and Salt Ingredients[14-16]

INCI Name	Malonic Acid	Succinic Acid	Sodium Succinate	Disodium Succinate	Glutaric Acid	Adipic Acid
Appearance	small crystals	colorless prisms	crystalline	crystalline	large monoclinic prisms	white, monoclinic prisms
Molecular Weight (g/mol)	104.06	118.09	140.07	162.05	132.11	146.14
Melting/Boiling Point (°C)	135 (dec.)/ 264 (est.)	185-187/ 235	206 (est.)/ 486 (est.)	156 (est.)/ 426 (est.)	97.5-98/ 302-304	152/265
Density (g/cm^3)	1.63	1.56	–	–	1.429	1.360
Vapor pressure (mm Hg @ 25°C)	0.001 (est.)	0.0000002	7.3 E^{-10} (est.)	8.7 E^{-8} (est.)	0.000003	0.07
Solubility (g/L water @ 25°C)	1520	83	1000 (est.)	31 (est.)	639	30
Log K_{ow}	−0.81	−0.59	−3.98 (est.)	−3.98 (est.)	−0.29	0.08

INCI Name	Azelaic Acid	Disodium Azelate	Dipotassium Azelate	Sebacic Acid	Disodium Sebacate	Dodecanedioic Acid
Appearance	monoclinic prismatic needles	crystalline	crystalline	Monoclinic prismatic tablets	crystalline	–
Molecular Weight (g/mol)	188.22	238.18	264.40	202.25	246.21	230.31
Melting/Boiling Point (°C)	106.5/ 286.5	186 (est.)/ 484 (est.)	186 (est.)/ 484 (est.)	134.5/ 294.5	194/496 (est.)	128/383 (est.)
Density (g/cm^3)	1.0291	–	–	1.207	–	1.16
Vapor pressure (mm Hg @ 25°C)	0.00002 (est.)	1.4 E^{-9} (est.)	1.4 E^{-9} (est.)	0.000007 (est.)	5.9 E^{-10} (est.)	0.000002 (est.)
Solubility (g/L water @ 20°C)	2.4	1000 (est.)	1000 (est.)	1.0	1000 (est.)	0.040
Log K_{ow}	1.57	−3.56 (est.)	−3.56 (est.)	2.19 (est.)	−3.01 (est.)	3.17 (est.)

oxidation of cyclohexane, cyclohexanol, or cyclohexanone, and glutaric acid may be manufactured by ozonolysis of cyclopentene.[9]

Azelaic acid (C9). Azelaic acid, first detected in rancid fats, was originally produced via nitric acid oxidation of oleic acid.[10] Azelaic acid is a naturally occurring dicarboxylic acid that can be found in dietary sources, such as whole grains.[11] Azelaic acid is commonly manufactured by oxidative cleavage of oleic acid (obtained from grease or tallow) with chromic acid, nitric acid, or by ozonolysis.[10,7]

Sebacic acid (C10). Sebacic acid was originally isolated from distillation products of beef tallow. More recently, however, sebacic acid has been manufactured via alkali pyrolysis of castor oil, as mentioned above and drawn in Figure 1, or by alkali pyrolysis of ricinoleic acid.[12,7]

Dodecanedioic acid (C12). Dodecanedioic acid can be manufactured by fermentation of long-chain alkanes with a specific strain of *Candida tropicalis*.[13] Another method of manufacture involves the nitric acid oxidation of a mixture of cyclododecanone and cyclododecanol.[7]

Physical and Chemical Properties. Table 2 lists the physical and chemical properties of the dicarboxylic acids and salts. Figure 2 presents the relationship between molecular weight of these ingredients and the octanol/water partition coefficient expressed as log K_{ow}.

The alkyl dicarboxylic acids vary considerably in their physical properties. The shorter chain (malonic, succinic, and glutaric) members are crystalline solids, very water soluble, and have limited solubility in organic solvents. As the chain length increases through adipic to dodecanedioic, water solubility decreases sharply (although still soluble in hot water). In other words, the water solubility of these acids is inversely proportional to their chain length. There is a marked alternation in melting point with changes in carbon number from even to odd.[7] Odd members (eg, malonic acid and glutaric acid) exhibit lower melting points and higher solubility than even carbon number alkyl dicarboxylic acids (eg, succinic acid and adipic acid). These alternating effects are believed to be the result of the inability of odd carbon number

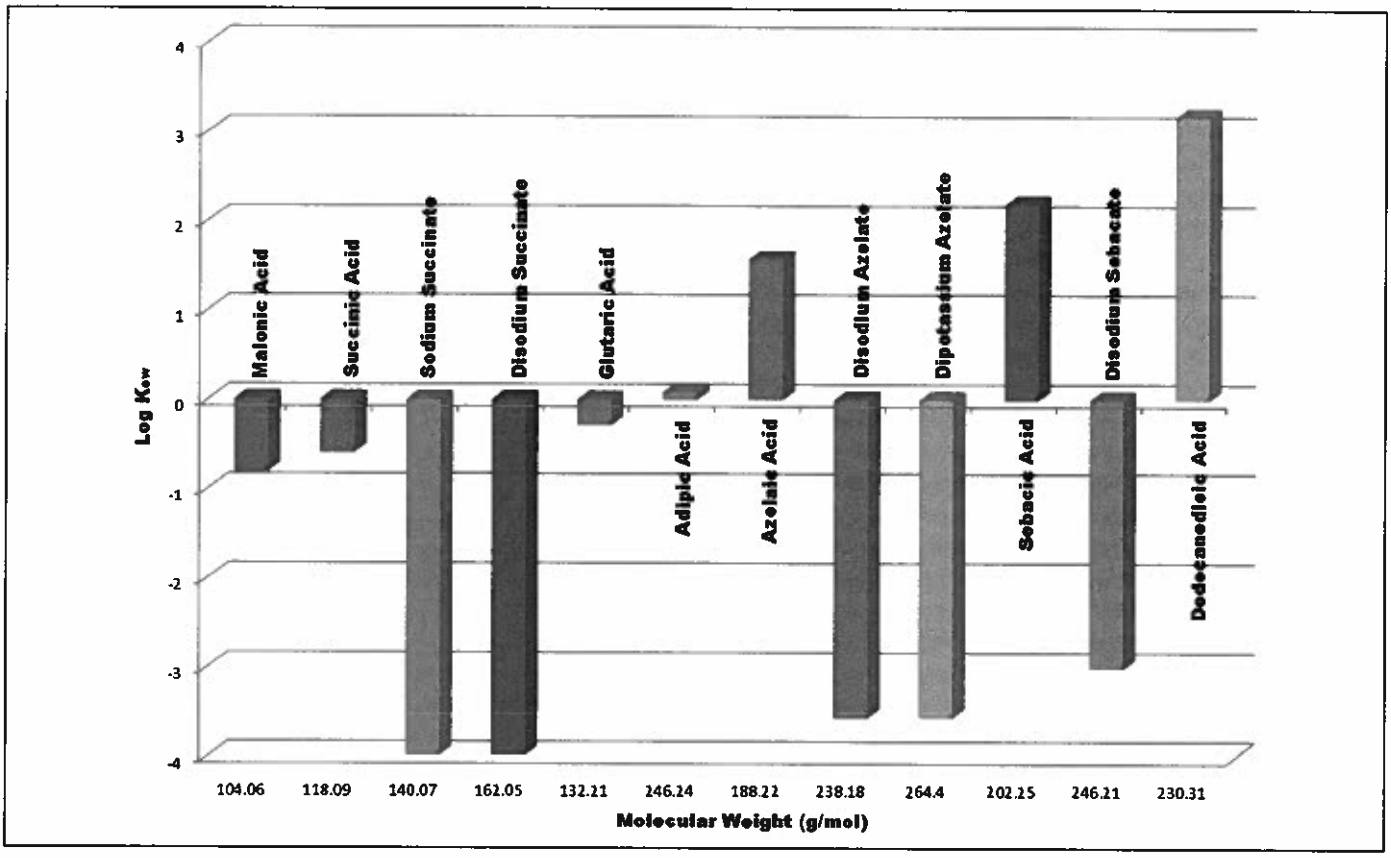


Figure 2. Dicarboxylic acids and their Salts; Log K_{ow} vs molecular weight

compounds to assume an in-plane orientation of both carboxyl groups with respect to the hydrocarbon chain.

Dicarboxylic acids react with Brønsted-Lowry bases (eg, sodium hydroxide) to form carboxylate salts (eg, sodium succinate or disodium succinate). Dicarboxylic acids also react with alcohols to give mono- and di-esters, such as those in this report.

Analytical Methods

Succinic acid. Methods used to analyze succinic acid include acidimetric titration for acidity; comparison with platinum–cobalt (Pt-Co) standard calibrated solutions for color; oxidation with potassium permanganate for detection of unsaturated compounds; atomic absorption or plasma spectroscopy for metals; and titration with silver nitrate or barium chloride for chloride or sulfate detection, respectively.[7] Small concentrations of succinic acid can be detected by common instrumentation such as gas–liquid chromatography (GLC) and polarography.

Adipic acid. Adipic acid can be extracted from a water sample and analyzed by gas chromatography/mass spectrometry.[17]

Sebacic acid. Gas chromatography can be used to identify sebacic acid in air.[18]

Diisopropyl adipate and diethylhexyl adipate. Diisopropyl adipate and diethylhexyl adipate can be identified through standard infrared (IR) spectroscopy. Gas–liquid chromatography, liquid–liquid extraction, mass spectrometry, and high-pressure liquid chromatography (HPLC) are also methods of analysis for the adipates.[2]

Ultraviolet Absorption. The dicarboxylic acids and their salts included in this review would not be expected to have any meaningful ultraviolet (UV) absorption. Except for the acid functional group, these ingredients do not possess any conjugated π bonds or nonbonding electrons. The π bonds and nonbonding electrons in the acid functional group are not part of any conjugated systems. Accordingly, these ingredients are unlikely to absorb light within the UVA-UVB spectrum at a detectable molar absorptivity.

Use

Cosmetic

The ingredients included in this safety assessment have a variety of functions in cosmetics.[19] The majority of the dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients. The functions of most of the salts are not reported, but it is stated that sodium succinate functions as a buffering agent or pH adjuster. The functions of all ingredients are listed in Table 1.

Six of the 12 dicarboxylic acids and their salts included in this safety assessment are reported to be used in cosmetic

Table 3. Frequency[20] and Concentration[21,23] of Use by Duration and Exposure—Dicarboxylic Acids and Their Salts

	No. of Uses	Conc. of Use (%)	No. of Uses	Conc. of Use (%)	No. of Uses	Conc. of Use (%)
	Succinic Acid		Sodium Succinate		Disodium Succinate	
Totals	4	0.001-26	7	NR	45	0.0005-0.4
Duration of Use						
Leave-On	*2*	*0.001-0.2*	*3*	*NR*	*38*	*0.005-0.4*
Rinse Off	*2*	*0.001-26*	*4*	*NR*	*7*	*0.0005*
Exposure Type						
Eye Area	NR	NR	NR	NR	4	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR
Dermal Contact	2	0.01-26	5	NR	40	0.0005-0.4
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR
Hair, Noncoloring	2	0.001-0.2	2	NR	5	NR
Hair, coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	0.2	1	NR	NR	NR
Bath products	NR	26	1	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR
	Adipic Acid		Azelaic Acid		Sebacic Acid	
Totals	25	0.000001-18	9	0.007-10	12	0.0009-1
Duration of Use						
Leave-On	*2*	*0.000001*	*7*	*0.007-0.3*	*9*	*0.0009-0.03*
Rinse Off	*23*	*0.5-18*	*2*	*10*	*3*	*0.001-1*
Exposure Type						
Eye Area	NR	0.000001	NR	NR	NR	NR
Possible Ingestion	NR	0.000001	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR
Dermal Contact	1	0.000001-18	25	0.007-10	12	0.0009-1
Deodorant (Underarm)	NR	NR	NR	NR	NR	0.0009
Hair, noncoloring	24	0.5	NR	NR	NR	NR
Hair, coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	–	NR	NR	NR	1	0.04
Bath products	1	15-18	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR

Abbreviation: NR, not reported to be used.

formulations. The frequency of use of the acids and salts, as supplied to the FDA by industry in 2010 as part of the Voluntary Cosmetic Registration Program (VCRP),[20] and the concentration of use, as supplied by industry in response to Personal Care Products Council (Council) surveys in 2009[21] and 2010,[22,23] are found in Table 3.

For the dicarboxylic acids and their salts, disodium succinate has the greatest number of reported uses, with a total of 45. The acid with the highest concentration of use is succinic acid, with a use concentration of up to 26%; use at this concentration is in a bath product that will be diluted for use. The highest leave-on concentration is 0.4% disodium succinate, with dermal contact exposure.

Some of the ingredients are applied around the eye, can possibly be ingested, or involve mucous membrane exposure, and some are used in underarm deodorants. None are reported to be used in baby products.

The dicarboxylic acids and their salts are in the European Union (EU) inventory of cosmetic ingredients.[24]

Noncosmetic

Many of the dicarboxylic acids and their salts are used in foods as direct or indirect food additives. The alkyl dicarboxylic acids are unusually versatile because of their 2 carboxyl groups.[9] This enables many additional types of useful reactions, particularly the manufacture of polymers (eg, nylon). The most common uses include functions as plasticizers, lubricants, and building blocks in the manufacture of polyesters, polyamides, and other plastics. The alkyl dicarboxylic acid salts are used to synthesize cyclic ketones, including commercially used macrocyclic musk compounds.[25]

Malonic acid. Malonic acid is a useful intermediate in the manufacture of barbiturates.[14]

Succinic acid. Succinic acid is listed by the FDA as a food additive that is generally recognized as safe (GRAS).[26] Succinic acid is also utilized in detergents, pigments, toners, cement

additives, soldering fluxes, and as an intermediate in the synthesis of a number of pharmaceutical products.[7]

Adipic acid. Adipic acid is listed as a GRAS food additive by the FDA.[27] Adipic acid has several industrial uses in the production of adhesives, plasticizers, gelatinizing agents, hydraulic fluids, lubricants, emollients, polyurethane foams, leather tanning, and urethane.[7] However, the bulk of the industrial production of adipic acid is driven by its usefulness in the manufacture of nylon-6,6 (in combination with 1,6-hexanediamine).

Azelaic acid. FDA has approved azelaic acid for use in treating acne and rosacea. A skin cream containing 20% (w/w) azelaic acid is indicated for the topical treatment of mild-to-moderate inflammatory acne vulgaris,[28] and a gel containing 15% azelaic acid is approved for treating rosacea.[29] These drugs are available by prescription only. (As a reference point, azelaic acid is reported to be used in cosmetics at 0.3% in leave-on and 10% in rinse-off formulations that have dermal exposure.[23])

Azelaic acid is used in the manufacture of plasticizers, lubricants, and greases. Azelaic acid was identified as a molecule that accumulated at elevated levels in some parts of plants and was shown to be able to enhance the resistance of plants to infections.[30]

Sebacic acid. Before 1973, sebacic acid was widely used in the United States, as an aromatic in food.[31]

Sebacic acid is used in resorbable polymer systems that deliver chemotherapeutic agents (eg, cisplatin, carboplatin) that are implanted at the site of tumors to provide for sustained release of the drugs.[32] Sebacic acid and its derivatives have a variety of industrial uses as plasticizers, lubricants, diffusion pump oils, candles, and as intermediates in the synthesis of polyamides and various alkyd resins.[7]

Dodecanedioic acid. Dodecanedioic acid is used in the production of nylon (nylon-6,12), polyamides, coatings, adhesives, greases, polyesters, dyestuffs, detergents, flame retardants, and fragrances.[33]

Diethyl malonate. Diethyl malonate finds great utility as the starting material in malonic ester synthesis, a classic organic chemistry reaction wherein a very wide variety of esters can be synthesized.[25]

Diisobutyl adipate. Diisobutyl adipate is considered by FDA to be a Prior-Sanctioned Food Ingredients, Plasticizer (21 CFR § 181.27).

Diethylhexyl adipate. Diethylhexyl adipate is used as a plasticizer for polyvinyl chloride (PVC) plastics.[34]

Diethyl sebacate. Before 1973, diethyl sebacate was widely used in the United States, as an aromatic in food.[31]

Dibutyl sebacate. Dibutyl sebacate is a component of PVC.[35]

Toxicokinetics

Dicarboxylic acids are natural metabolic products of the ω-oxidation of monocarboxylic acids when the β-oxidation of free fatty acids is impaired.[36] Under normal physiological conditions, dicarboxylic acids are rapidly β-oxidized, resulting in very low cellular concentrations and practically nondetectable concentrations in the plasma.[37] Medium-chain dicarboxylic acids (up to 12 carbon atoms) are β-oxidized in mitochondria and peroxisomes. Oxidation of odd- and even-numbered chains proceeds to different end points. Odd-chain dicarboxylic acids are β-oxidized, giving acetyl-CoA and malonic acid (C3). Oxidation can then go no further, and malonic acid is the starter of fatty acid synthesis. Even-chain carboxylic acids are completely oxidized and produce succinyl-CoA, a gluconeogenic substrate, as an intermediate metabolite. Dicarboxylic acids are more polar than their esters, therefore they will diffuse less readily through normal cell membranes.[38]

Malonic Acid. Malonic acid can be activated to malonyl-CoA and undergoes decarboxylation to acetyl-CoA by various mammalian tissues.[39]

Adipic Acid Nonhuman. Adipic acid metabolism was studied using fasted male albino rats.[40] In 1 study, in which the rats were given a single oral dose, by gavage, with 50 mg radioactive adipic acid (labeled on C1 or C2), 70% of the radioactivity was exhaled as carbon dioxide in 24 hours. Adipic acid and the metabolites urea, glutamic acid, lactic acid, β-ketoadipic acid, and citric acid were recovered in the urine. Very little radioactivity was found in the tissues. Fasted male rats were also given a single dose of a solution containing 50 mg radioactive adipic acid (labeled on C1), by gavage, in conjunction with the intraperitoneal (ip) injection of 2 mL of 0.5 mol/L sodium malonate. After 24 hours, both radioactive adipic acid and succinic acid were found in the urine, which the researchers stated was an indication that adipic acid underwent β oxidation. In a study in which the rats were fed 25 mg radioactive adipic acid (labeled on C1) and 100 mg γ-phenyl-α-aminobutyric acid, followed by a 48-hour urine collection, it was determined that acetate is a metabolite of adipic acid. Finally, rats were given radioactive sodium bicarbonate with nonradioactive adipic acid. Radioactive citric acid was formed, which suggested that carbon dioxide interacted with a metabolite of adipic acid. (Details not specified.)

Two rats were dosed orally by gavage with 2.43 g/kg partially neutralized adipic acid for 28 days. In the urine, 67% of the dose was recovered unchanged. There was no change in excretion pattern over time during the study.

Rabbits were dosed orally by gavage ($n = 4$) or by intravenous (iv) administration ($n = 2$) with 2.43 g/kg partially neutralized adipic acid for 2 days. Following oral administration, 53% to 61% of the dose was recovered unchanged in the urine. With iv administration, 59% to 71% was recovered unchanged in the urine. In another study using rabbits, animals were given a subcutaneous (sc) dose of 2000 mg adipic acid; 3 rabbits were

given a single dose, 1 was dosed on days 1 and 5, and 1 was dosed on days 1, 5, 9, 13, and 15. On average, 61% of the dose was recovered unchanged in the urine. There was an increase in urinary oxalic acid concentrations.

A female dog was fed either 150 mg/kg body weight (bw) adipic acid (in 2 feedings) for 5 days or 750 mg/kg bw (in 2 feedings) for 7 days. In the urine, 18% and 63.6% of the low and high doses, respectively, were recovered unchanged.

Rabbits (number not stated) were given up to 4 sc injections of ≤2000 mg sodium adipate.[41] An average of 61% of the dose was recovered unchanged in the urine. Oxalic acid was increased in the urine.

Human. In a study in which 1 participant was given 33 mg/kg bw sodium adipate, orally, for 5 days (10 g total), 6.76% of the dose was recovered in the urine. In another study in which 1 person was given 100 mg/kg bw adipic acid for 10 days (70 g total), 61% of the dose was recovered in the urine. Administration of 19.0 g adipic acid over 5 days or 23.4 g over 6 or 9 days (1 participant per dose) resulted in 53% of the administered dose recovered in the urine.

C9 to C12 Dicarboxylic Acids Nonhuman. Groups of 30 male Wistar rats were dosed orally, by gavage, with azelaic (C9), sebacic (C10), undecanedioic (C11), or dodecanedioic (C12) acid.[42] Ten rats in each group were dosed with 20, 50, or 100 mg of the respective acid. Blood, urine, and feces from the treated rats were analyzed and compared to the blank control obtained from untreated rats. (None of the C9-C12 acids were found in the blank controls.) In urine, approximately 2.5% of azelaic, 2.1% of sebacic, 1.8% of undecanedioic, and 1.6% of dodecanedioic acid was recovered after 5 days; the amount recovered was not affected by dosage. The dicarboxylic acids were not excreted in conjugated form. None of the C9-C12 dicarboxylic acids were recovered in the feces. In the plasma, dicarboxylic acid catabolites that were 2-, 4-, or 6-carbons shorter than the corresponding dicarboxylic acid were detected.

Human. Groups of 3 male and 2 female participants were also dosed with azelaic, sebacic, undecanedioic, or dodecanedioic acid orally, in gelatin capsules, once a week for 5 weeks.[42] The dose administered increased each week, from 0.5 g at week 1 to 5.0 g at week 5. None of the C9-C12 acids were found in the blank control samples of blood, urine, and feces obtained from nontreated humans. In urine, approximately 60% of azelaic, 17% of sebacic, 5% of undecanedioic, and 0.1% of dodecanedioic acid were recovered after 12 hours; the amount recovered was not affected by dosage. At 24 hours, the amounts recovered were not much increased. Initially, undecanedioic and dodecanedioic acid administration raised the urinary pH to a value of 7.4 to 8.5; the pH returned to normal within 3 to 6 hours. The dicarboxylic acids were not excreted in conjugated form. None of the C9-C12 dicarboxylic acids were recovered in the feces. In the plasma, dicarboxylic acid catabolites that were 2, 4, or 6 carbons shorter than the corresponding dicarboxylic acid were detected. Plasma levels of azelaic acid peaked at 2 hours, while the levels of the other 3 acids peaked at 3 hours. Recovery in the plasma was greatest for azelaic acid, 74.6 μg/mL with the 5 g dose, and the amount detected decreased with increasing chain length.

Azelaic Acid. Azelaic acid is a dietary constituent found in whole grain cereals and animal products.[43] It can be formed endogenously from longer chain dicarboxylic acids, metabolism of oleic acid, and Ψ-oxidation of monocarboxylic acids.[44] Endogenous plasma concentration and daily urinary excretion of azelaic acid are highly dependent on dietary intake. Azelaic acid crosses the blood–brain barrier.[45]

A group of 25 male Wistar rats were dosed orally, by gavage, with 100 μCi of [1,9-^{14}C]azelaic acid, and the animals were killed at various intervals 1 to 96 hours after dosing.[42] After 12 and 48 hours, 13% and 14.5% of the radioactivity was found in expired carbon dioxide, respectively. Approximately 40% of the radioactivity was recovered in the urine over 5 days. The C7 and C5 dicarboxylic acid metabolites were found in the urine up to 72 hours after dosing. Very little was recovered in the feces. Labeled dicarboxylic acids were present in the blood for up to 72 hours and consisted mainly of dicarboxylic acid metabolites. Radioactivity was found in all tissues, with the highest levels present in the liver, lungs, and kidneys after 12 hours. Tissue radioactivity levels then decreased slowly in all organs except adipose tissue, in which case increasing levels were still seen at 96 hours. Approximately 90% of the radioactivity found in the tissues was present in the lipids, and it was essentially localized in the fatty acid portion of the triglycerides and of the phospholipids. Traces of C9, C5, and C7 dicarboxylic acids were detected in the first 24 hours.

Sebacic Acid. Sebacic acid is oxidized to water and carbon dioxide, passing through acetyl-CoA and succinyl-CoA formation.[46]

Disodium Sebacate Nonhuman. Disodium sebacate, 80 and 160 mg with 25 μCi of (1,10) [^{14}C]sebacic acid tracer, was administered by a single iv injection to 14 male Wistar rats, and blood samples were obtained at various intervals 5 to 320 minutes after dosing.[46] The plasma half-life of radioactive disodium sebacate was 37.86 and 39.82 minutes for the 80 and 160 mg dose groups, respectively. The apparent volume of distribution was 2.65 mL/100 g body wt.

In a second experiment, a group of 4 male Wistar rats were given a single-dose 160 mg disodium sebacate with 25 μCi sebacic acid tracer by iv injection, and expired carbon dioxide, urine, and feces were collected. The carbon dioxide half-life for radioactive sebacate was 93.64 minutes; 25% of the administered dose was expired in carbon dioxide. A total of 34.6% of sebacate was recovered in the urine in 24 hours, while 5.08% suberic acid (C8) was recovered in the same time frame. Most of the excretion occurred in the first 4 hours. Radioactivity was not found in the feces.

In the third experiment, groups of 10 male Wistar rats were also given 160 mg disodium sebacate with 25 μCi sebacic acid

tracer by iv injection, and the animals were sacrificed at various intervals from 30 to 360 minutes after dosing. The amount of radioactivity in various organs was analyzed. No appreciable radioactivity was found in the body. Sebacate appeared to be in an absorption phase in fat 1 hour after dosing, but no radioactivity was found in the body after 24 hours.

The pharmacokinetics of disodium sebacate was studied in male and female Wistar rats.[47] Sebacate was administered either ip, 6 doses of 10 to 320 mg, or orally, 2 doses of 80 or 60 mg. Plasma concentrations of sebacate and urinary concentrations of sebacate and its products of β-oxidation (suberic and adipic acids) were measured using GLC/mass spectrometry. Both renal and nonrenal elimination parameters were obtained. The sebacate half-life was 31.5 minutes. The tissue elimination rate was 0.0122/min, and the overall volume of distribution was 26.817 mL/100 g. The renal clearance was 0.291 mL/min per 100 g, which was much less than the value of the glomerular filtration rate (GFR) of approximately 1 mL/min/100 g reported elsewhere, suggesting the presence of sebacate reabsorption from the ultrafiltrate. Sebacate renal clearance was found to be a concentration-independent function, suggesting the presence of a passive back diffusion. The relative bioavailability of the oral route compared to the ip route was 69.09%, showing an extensive absorption of the compound.

Human. The metabolism and excretion of disodium sebacate was studied in 7 fasting male participants that were given a continuous steady infusion of 20 g unlabeled disodium sebacate over 480 minutes.[48] At 240 minutes into the infusion, (1,10)[C^{14}]sebacic acid was infused simultaneously as a tracer (sp act 0.416 μCi/min). The was a gradual increase in the amount of radioactivity expired in carbon dioxide for the first 300 minutes; the value remained elevated for an additional 120 minutes before declining. At 24 hours, 11.38 mmol sebacate was recovered in the urine, as well as 2.04 mmol suberic acid and 1.11 mmol adipic acid, which was less than 15% of the dose administered. The serum concentration of unlabeled sebacate reached a plateau after 270 minutes of infusion. In all, 10% to 15% of serum radioactivity was found in the aqueous fraction of serum extracts. The renal clearance rate was 5.67 mL/min. The overall tissue uptake of unlabeled sebacate was 180 μmol/min, and the apparent distribution volume was 12.46 L. The percentage oxidation of sebacate was 6.14%.

The pharmacokinetic profile of disodium sebacate during a short-time infusion (5 hours at 10 g/h) was also studied in 7 male participants.[49] Sebacate in serum and urine was measured by HPLC. The apparent volume of distribution of sebacate was 8.39 L, and the plasma fractional removal rate constant was 0.0086/min.

Six male participants were given a single iv bolus of 1 g disodium sebacate, while another 6 received 10 g of sebacate in 500 mL of distilled water, iv, at a rate of 3.33 g/h over 3 hours.[50] For the group given a bolus dose, the distribution phase had a short half-life, 0.34 hours, and a rapid elimination, 2.045/h. For the group given the 3 hours infusion, 12% of the dose was excreted as sebacic acid in 24 hours; suberic acid (C8) and adipic acid were also present in the urine.

Dodecanedioic Acid. A group of 25 male Wistar rats were dosed orally, by gavage, with 100 μCi of [10,11-^{3}H]dodecanedioic acid, and the animals were killed at various intervals 1 to 96 hours after dosing.[42] Approximately 50% of the radioactivity was recovered in the urine over 5 days. The C10, C8, and C6 dicarboxylic acid metabolites were found up to 72 hours after dosing. Only 2% of the radioactivity was recovered in the feces. Labeled dicarboxylic acids were present in the blood for up to 72 hours and consisted mainly of dicarboxylic acid metabolites. Radioactivity was found in all tissues, with the highest levels present in the liver, lungs, and kidneys after 24 hours. Tissue radioactivity levels then decreased slowly in all organs except adipose tissue, in which case an increase in radioactivity was still seen at 96 hours. Radioactivity levels were 20% to 40% lower in the lipid extracts of the tissues than in the residual matter.^{3}H was distributed in the whole molecule, not only the fatty acid portion, of the phospholipid and triglyceride fractions. Traces of C12, C10, C8, and C6 dicarboxylic acids were detected in the first 24 hours.

Male Wistar rats were given an iv bolus of 800 μmol/kg disodium dodecanedioic acid.[51] The apparent volume of distribution was 0.248 L/kg, and the plasma half-life was 12.47 minutes. The renal clearance was 0.00051 L/kg/min, while systemic clearance was 0.0138 L/kg/min. Only 3% to 5% of the dose was recovered in the urine.

Percutaneous Absorption

Azelaic acid. The in vitro percutaneous absorption of a 15% azelaic acid gel through human skin, prior to or after the application of 3 different moisturizer formulations, was determined.[52] All doses were applied as 5 μL/cm^2. The second dose was applied 15 minutes after the first. [^{14}C]Azelaic acid had a finite dose absorption profile, with a rise to peak penetration followed by a slow but steady decline. In vitro, 70% of the azelaic acid diffused into the reservoir solution over 48 hours. The application of a moisturizer, and whether it was applied prior to or following azelaic acid administration, did not have a statistically significant effect on the penetration of azelaic acid. However, there was a trend toward greater percutaneous penetration and mass distribution with the application of a moisturizer lotion prior to the azelaic acid gel.

The percutaneous absorption of azelaic acid was determined using 6 male participants. A total of 5 g of a cream containing 20% azelaic acid was applied to the face (1 g), chest (2 g), and upper back (2 g) of each participant, giving an area dose of approximate 5 mg cream/cm^2 skin. The test areas were covered 1 hour after dosing with cotton tissues and washed 24 hours after dosing. After 1 week, 100 mL of an aqueous microcrystalline suspension containing 1 g azelaic acid was given orally to each participant. Urinary excretion of unchanged azelaic acid was measured after each dose. Following dermal application, 1.29% of the dose was recovered unchanged in the urine in 24

hours, and a total of 2.2% was recovered by day 3. Following oral administration, 61.2% of the dose was recovered within 4 hours; excretion was complete at this point. Assuming similar rates and pathways in biotransformation following both routes of exposure, percutaneous absorption of azelaic acid was determined to be 3.6% of the dermally applied dose.[53]

Toxicological Studies

Single Dose (Acute) Toxicity

The acute oral, dermal, inhalation, and parenteral toxicity of the dicarboxylic acids and some of the salts are summarized in Table 4.[40,54-59] The oral LD_{50} values of the dicarboxylic acids had a wide range, for example, adipic acid had values for rats ranging from 940 mg/kg to greater than the highest dose tested (11 000 mg/kg). Most reported values for the acids were >200 mg/kg. The reported dermal LD_{50} values ranged from >6000 mg/kg dodecanedioic acid to >10 000 mg/kg glutaric acid.

Repeat Dose Toxicity

Cellular effects. Dicarboxylic acids have a cytotoxic effect on the abnormally hyperactive and malignant epidermal melanocytes. Dicarboxylic acids, C8 to C13, have been shown to inhibit mitochondrial oxidoreductases,[62] and they have been shown to reversibly inhibit microsomal NADPH and cytochrome P450 reductase.[63] Medium chain length dicarboxylic acids are also competitive inhibitors of tyrosinase in vitro.

Adipic acid. The effect of adipic acid on primary keratinocyte cultures was evaluated using epidermal cells from neonatal NMRI mice.[64] Concentrations of $\leq$30 mmol/L did not inhibit ^{3}H-thymidine incorporation or affect DNA synthesis, while 40 and 50 mmol/L inhibited both of these parameters. No effect on labeling indices was observed with 1 to 30 mmol/L adipic acid.

Azelaic acid. Azelaic acid, a naturally occurring competitive inhibitor of tyrosinase, has a cytotoxic effect on malignant melanocytes.[65] Azelaic acid is also a competitive inhibitor of a number of oxidoreductive enzymes, enzymes involved in DNA synthesis, and of oxidoreductases of the respiratory chain.[66] It has been reported that, in vitro, azelaic acid has time- and dose-dependent, reversible, and antiproliferative and cytotoxic effects on a number of tumoral cell lines. Azelaic acid had no effect on normal cell lines.

Disodium azelate. Disodium azelate inhibited cell proliferation and affected viability of Cloudman and Harding-Passey murine melanomata at concentrations $\geq 10^{-2}$ mol/L when incubated over a 3-day period.[62] The mitochondria were the prime target of action.

The effect of disodium azelate on primary keratinocyte cultures was evaluated using epidermal cells from neonatal NMRI mice.[64] A dose-dependent inhibition of ^{3}H-thymidine incorporation into DNA, ranging from 50% inhibition with 20 mmol/L to 90% inhibition with 50 mmol/L disodium azelate, was observed following a 12-hour incubation period. Concentrations of 1 and 10 mmol/L did not affect DNA synthesis, but a marked reduction was seen with 20 to 50 mmol/L. The effects on DNA synthesis were time dependent, with the maximum inhibitory effect observed at 4 hours; this effect was reversible. RNA and protein synthesis were also inhibited during the first 4 hours of incubation with 50 mmol/L disodium azelate. Cellular structure was altered upon incubation with disodium azelate, primarily affecting mitochondria and the rough endoplasmic reticulum. These effects were also reversible.

Dodecanedioic acid. The disodium salt of dodecanedioic acid inhibited cell proliferation and affected viability of Cloudman and Harding-Passey murine melanomata at concentrations $\geq 10^{-2}$ mol/L, when incubated over a 3-day period.[62] The mitochondria were the prime target of action.

Animal studies. Most of the available animal studies were oral exposures, although limited inhalation studies were available for adipic acid.

Adipic acid Oral. Groups of 6 male Sprague-Dawley rats were dosed orally (method not specified) with 3600 to 5600 mg/kg bw adipic acid as an 18.6% to 24.9% solution in saline for 14 days.[40] Three animals of the 3600 mg/kg bw group, 5 of the 4000 mg/kg bw group, and all of the 4500 to 5600 mg/kg bw groups died prior to study termination. Signs of toxicity included depressed activity, labored respiration, ataxia, and convulsions. No gross findings were noted at necropsy at study termination.

Groups of 5 rats were dosed with 0 or 3000 mg/kg bw of a neutralized 20% adipic acid solution orally, by gavage, for 4 weeks.[49] A nonsignificant decrease in bw gain was observed. In a 4-week study in which a group of 3 rats was dosed orally, by gavage, with 2400 mg/kg bw adipic acid, no significant toxicological effects were noted.

In a 4-week dietary study in which groups of 17 to 20 female rats were fed 0 to 40 mg/d (0-435 mg/kg bw per d) adipic acid, no effects were reported.[49] The no-observable adverse effect level (NOAEL) was >435 mg/kg bw per d. In a 5-week dietary study in which groups of 15 to 18 male rats were fed 0 to 800 mg/d (0-13 333 mg/kg bw/d) decreased bw gains, an unkempt appearance, and diarrhea were observed for the animals fed 800 mg/d the first 3 weeks. In another 5-week dietary study in which groups of 4 rats, gender not specified, were fed 100 or 200 mg/d (310-922 mg/kg bw/d) of a 20% adipic acid solution in ethanol, 5 days/week, no signs of toxicity were observed.

Ten rats were dosed orally, method not specified, with 199 mg/d (638-1332 mg/kg bw/d) sodium adipate, 5 days/week for 9 weeks.[49] No toxicological effects were observed.

A group of 5 guinea pigs, gender not specified, were dosed orally using capsules with 400 mg/d (682-942 mg/kg bw/d) adipic acid for 5 days, followed by dosing with 600 mg/d (1032-1739 mg/kg bw/d), 5 days/week for 5 weeks.[49] No signs of toxicity were observed.

No toxicity was observed in a study in which pigs were fed 1% adipic acid in the diet for 7 days.[49]

Table 4. Acute Toxicity—Dicarboxylic Acids and Their Salts

Animals	No./Gender/Group	Dose	median lethal dose/conc.	Reference
ORAL				
Succinic Acid				
rats	not specified	not specified	2260 mg/kg	60
Sodium Succinate				
rats	4 males, 4 females	0.5-8 g/kg	8 g/kg	58
Glutaric Acid				
rats	5/dose, male and female	50% aqueous solution	2750 mg/kg	55
Adipic Acid				
mice	13 males	1500-2500 mg/kg of a 6% suspension in 0.5% methyl cellulose	1900 mg/kg	40
mice	not specified	not specified	4175 mg/kg	40
mice	not specified	not specified	4200 mg/kg	40
rats	M/F, no. not specified	20% in corn oil	5050 mg/kg	55
rats	5 or 10 males	100-3000 mg/kg (n=5) or 5000 mg/kg (n=10) adipic acid in 0.85% saline	940 mg/kg	40
Wistar rats	not specified	not specified	approx. 3600 mg/kg	40
rats	10 males	5000 mg/kg of a 33.3% suspension in 0.85% saline	grater than highest dose tested	40
rats	5 males, 5 females	14.7-10 000 mg/kg as a 14.7-50% suspension in carboxymethyl cellulose (CMC)	5560 mg/kg	40
rats	not specified	10 000 mg/kg	greater than highest dose tested	40
rat and rabbit	not specified	not specified	greater than highest dose tested	40
rabbits	not specified	2430 or 4860 mg/kg of a 20% partially neutralized soln (75% sodium adipate)	>2430 and <4860 mg/kg	40
Adipic/Glutaric/Succinic Mixture (percentages not given)				
rats	10 males	5000-7500 mg/kg aqueous	6829 mg/kg	55
Azelaic Acid				
Wistar rats	6 males 6 females	500-4000 mg/kg	greater than highest dose tested	59
New Zealand rabbits	6 males 6 females	500-4000 mg/kg	greater than highest dose tested	59
Disodium Sebacate				
Wistar rats	4 males, 4 females	0-5000 mg/kg	greater than highest dose tested	54
New Zealand rabbits	4 males, 4 females	0-6000 mg/kg	greater than highest dose tested	54
Dodecanedioic Acid				
rats	m/f; no. not specified	not specified	>3000 mg/kg	56
DERMAL				
Glutaric Acid				
rabbits	1 rabbit/group; M/F	50% aqueous solution	>10 000 mg/kg	55
Adipic Acid				
rabbits	1- 2/group; male and female	5010 (n=1) or 7940 mg/kg (n=2) 40% adipic acid in corn oil, with occlusion	greater than highest dose tested	40
Adipic/Glutaric/Succinic Mixture (percentages not given)				
rats	not specified	not specified	>200 mg/kg	55
New Zealand white rabbits	not specified	40% aqueous solution; 24 hours occlusive exposure	>7940 mg/kg	55
Dodecanedioic Acid				
albino rabbits	males; no. not specified	not specified	>6000 mg/kg	56
INHALATION				
Adipic Acid				
rats	20/group; males and females	5.4 or 7.7 mg/L; head/nose-only exposure; $MMAD_{50}$ <3.5 μm	greater than highest dose tested	57
Adipic/Glutaric/Succinic Mixture				
rats	20, gender not specified	4 hours exposure' percentages not given	>0.03 mg/L	55

(continued)

Table 4. (continued)

Animals	No./Gender/Group	Dose	median lethal dose/conc.	Reference
rats	Crl:Cd/BR; 42 males	5.9 mg/L; 4 hours nose-only exposure; 66.0% dimethyl glutarate; 16.5% dimethyl succinate; 17.0% dimethyl adipate	anterior and posterior nasal passages were affected; nasal lesions distributed along inspiratory airflow routes; lesions in posterior nasal cavity were less severe	61
PARENTERAL				
Disodium Succinate				
mice	not specified	iv	4500 mg/kg	60
Adipic Acid				
mouse	not specified	ip, 0.681-50% solution in 0.5% CMC	approx. 170 mg/kg	40
mouse	not specified	ip, 600 and 900 mg/kg aqueous	600 mg/kg	40
mouse	not specified	ip admin	4000 mg/kg	40
rats	7 males	ip, 200-350 mg/kg	275 mg/kg	40
mouse	not specified	iv, 650-700 mg/kg 2% solution	680 mg/kg	40
rabbit	not specified	iv, 2430 mg/kg 20% soln, partially neutralized	2430 mg/kg	40
Disodium Azelate				
rats	6 males, 6 females	ip, 0-1198 mg/kg	greater than highest dose tested	59
rabbits	6 males, 6 females	ip, 0-1198 mg/kg	greater than highest dose tested	59
Disodium Sebacate				
Wistar rats	4 males, 4 females	ip, 0-7000 mg/kg	5500 mg/kg; dehydration and ascites formation was noted	54
New Zealand rabbits	4 males, 4 females	ip, 0-8000 mg/kg	6000 mg/kg; dehydration and ascites formation was noted	54
Wistar rats	10	iv, 0-1000 mg/kg	560 mg/kg; dehydration and ascites formation was noted	54
New Zealand rabbits	10	iv, 0-1800 mg/kg	1400 mg/kg; dehydration and ascites formation was noted	54

Groups of 8 to 10 male rats were given 0, 420, 840, 1700, or 3400 mg/kg bw/d sodium adipate for 19 weeks in a protein deficient diet.[57] Animals were killed after either 7 weeks or at study termination. For unexplained reasons, only 5 to 7 animals/group survived until study termination. Rats of the 3400 mg/kg bw/d group had decreased bw gains and decreased bws. (Statistical significance not stated.) Slight effects were seen in the liver, and the NOAEL was 3333 mg/kg bw.

Groups of 13 to 15 male and female rats were fed a diet containing 0, 1600, or 3200 mg/kg bw/d adipic acid for 33 weeks.[40] Rats were killed at various intervals throughout the study. Ten of 14 rats fed 3200 mg/kg bw/d died during weeks 0 to 4; surviving rats had decreased weight gains during this time. However, at study termination, bws were for surviving animals of this group were similar to controls. Slight effects were seen in the liver. (Statistical significance not stated.)

In a 2-year study, groups of 20 male rats were fed a diet containing 0%, 0.1%, 1%, 3%, and 5% adipic acid (equiv. to 0, 75, 750, 2250, and 3750 mg/kg bw/d), and groups of 10 and 19 females were fed 0% and 1% adipic acid, respectively.[49] Weight gains of male rats fed 3% and 5% adipic acid were significantly less than controls. There were no significant toxicological findings upon gross or microscopic observation. The NOAEL was 1% adipic acid for male and female rats.

The effect of adipic acid on hepatic peroxisome proliferation was evaluated in an in vivo study in which 4 male F344 rats were fed chow containing 2% adipic acid dissolved in alcohol.[67] After 3 weeks of dosing, the animals were killed. Adipic acid did not induce peroxisome proliferation and did not affect relative liver to bws.

Inhalation

Mice were exposed to 460 mg/m^3 adipic acid dust for 1.5 mos, or to 13 or 129 mg/m^3 adipic acid for 4 mos (details not given).[40] Decreased weight gain, altered oxidase activity, and upper respiratory tract, liver, kidney, and central nervous system effects were observed.

Two male and 2 female rats were exposed to 126 mg/m^3 adipic acid dust for 15 days, 6 h/d.[49] No signs of toxicity were observed, and no gross or microscopic findings were noted at necropsy.

Sodium succinate. The oral toxicity of sodium succinate was evaluated using F344 rats.[58] Groups of 10 males and 10 females were given 0, 0.3, 0.6, 1.25, 2.5, 5 or 10% sodium succinate in the drinking water for 13 weeks. All animals were killed at the termination of dosing. Body weight gains of animals of the

10% group were significantly decreased, and all animals of this group died by week 4. These animals were extremely emaciated; however, no compound-related microscopic lesions were found. Body weight gains were decreased in animals given ≥2.5% sodium succinate, as compared to controls. No toxicological treatment-related effects were observed.

Glutaric acid. Groups of 15 male and 15 female Sprague Dawley rats were fed a diet containing 0% to 2% glutaric acid for 90 days.[55] Body weight gains were decreased for males and statistically significantly decreased for females of the 2% group. No differences were noted between test and control animals in hematology, clinical chemistry, or urinalysis. There were no microscopic findings or organ weight changes attributable to the test substance. There was no treatment-related mortality. The NOAEL was ≥1%, and the LOAEL was 2% glutaric acid.

Four male and 4 female Beagle dogs were fed a diet containing 0% to 5% glutaric acid for 90 days.[68] Decreased bws, accompanied by reduced feed consumption, were observed for the males and females of the 5% group and females of the 3% group. No other treatment-related effects were observed. The NOAEL was ≥2% and the LOAEL was 3%.

Adipic/glutaric/succinic acid mixture. Groups of 15 male and 15 female rats were dosed orally, by gavage, for 90 days with 0% to 30% of a mixture that contained 4% adipic, 16% glutaric, and 5% succinic acid.[55] The vehicle was deionized water, and the dosing volume was 10 mL/kg. Two males and 1 female of the 30% group died, and the deaths were considered dose-related. Also in this group, bws were reduced for males and females, and feed consumption was statistically significantly reduced in males. An increased incidence of labored breathing and rales was noted. The urine pH was statistically significantly reduced in both males and females dosed with 30% of the mixture. In the 10% group, bw gains were slightly, but not statistically significantly, reduced in females and feed consumption was statistically significantly reduced in males. The NOAEL was 3% and the LOAEL was 10%.

Azelaic acid. Groups of 15 male and 15 female Wistar rats were fed a diet containing 140 or 280 mg/kg bw azelaic acid for 180 days, and a control group of 10 males and 10 females was given untreated feed.[59] No significant toxicological effects were observed. Growth was similar between test and control groups, as were the microscopic examinations and clinical chemistry parameters. The researchers found similar, negative, results when groups of 10 male and 10 female New Zealand rabbits were fed diets containing 0, 200, or 400 mg/kg bw azelaic acid for 180 days.

Disodium sebacate. Groups of 10 male and 10 female Wistar rats were fed a diet containing 0, 500, or 1000 mg/kg bw disodium sebacate for 6 mos, after which time they were killed and necropsied.[54] Growth was similar between test and control groups, as were the microscopic examinations and clinical chemistry parameters. The researchers found similar, negative, results when groups of 10 male and 10 female New Zealand rabbits were fed diets containing 0, 750, or 1000 mg/kg bw disodium sebacate for 6 mos.

Ocular Irritation

Ocular irritation studies are summarized in Table 5.

Succinic acid. The ocular irritation potential of succinic acid was evaluated using albino rabbits.[55] Undiluted test material, 0.005 mL, was applied to the center of the cornea. The eyes were not rinsed. Succinic acid was a severe eye irritant, with necrosis visible upon staining. The score for ocular irritation, on a scale of 1 to 10, was 8.

Glutaric acid. A Draize ocular irritation study was performed in which 100 mg of glutaric acid was instilled in the eyes of 3 rabbits and the eyes were rinsed 24 hours after application.[55] Glutaric acid was irritating to rabbit eyes, with a primary irritation index (PII) of 35.2/110. Mild erythema, slight edema, and slight dullness were still present after 7 days.

Adipic acid. The ocular irritation of adipic acid was evaluated using groups of 2 albino rabbits.[55] Ten or 57.1 mg of adipic acid was placed in the eye of each rabbit, and the eye of 1 animal in each group was rinsed. With 10 mg followed by rinsing, mild conjunctival irritation was observed; and the eye was normal within 3 days. In the unrinsed eye, mild conjunctival irritation and a minimal iritic effect were observed; minimal conjunctival irritation was still observed after 7 days and the eye was normal after 14 days. With instillation of 57.1 mg adipic acid followed by rinsing, moderate to mild conjunctival irritation and transient mild opacity were observed; the eye was normal in 3 days. In the unrinsed eye, moderate to mild conjunctival irritation, mild opacity of the cornea, and a minimal iritic effect were observed; the eye was normal at day 7. However, other studies have reported that adipic acid produced severe irritation in rabbit eyes, and the signs of irritation were still present after 8 days.[40]

Adipic/glutaric/succinic acid mixture. The ocular irritation potential of a mixture of adipic, glutaric, and succinic acid, percentages not specified, was evaluated using 2 male albino rabbits.[55] One-tenth milliliter of the test substance was instilled in the conjunctival sac of each animal, and the eye of 1 animal, but not the other, was rinsed. The contralateral eye served as the negative control. Mild to severe conjunctivitis was observed in on both the rinsed and unrinsed rabbit eyes. Both eyes were normal within 21 days.

Dodecanedioic acid. In studies using rabbits that evaluated the ocular irritation of dodecanedioic acid, slight irritation was reported in 1 study, with a PII of 11.96/110, and small areas of corneal opacity and mild conjunctival irritation were seen in the other study.[56] Details were not provided.

Table 5. Ocular Irritation—Dicarboxylic Acids

Concentration	Animals	Procedure	Results	Reference
Succinic Acid				
not specified	not specified	ocular irritation study (details not specified)	severe ocular irritant	55
Glutaric Acid				
not specified	not specified	ocular irritation study (details not specified)	moderate ocular irritant	55
Adipic Acid				
undiluted	2 albino rabbits	10 or 57.1 mg placed in eye; eye of 1 animal rinsed	10 mg: mild conjunctival irritation in the rinsed and unrinsed eyes; the rinsed eye was normal at 3 days and the unrinsed eye was normal at 14 days; 57.1 mg: mild conjunctival irritation with transient corneal opacity in the rinsed eye; the eye was normal by day 3; moderate to mild conjunctival irritation with mild corneal opacity and iritic effects in the unrinsed eye; the eye was normal at day 7	55
undiluted	6 rabbits; gender not specified	0.1 mL instilled into the eye; eyes were not rinsed	severely irritating - primary irritation index of 41.5/110; irritated conjunctiva and scar formation, increased corneal opacity and iridal inflammation; not cleared by day 8	69
undiluted	3 rabbits; gender not specified	100m g instilled following GLP; acute eye irritation/corrosion test	severe irritation; corneal opacity and iridal irritation; cleared within 16 days	69
undiluted	2 rabbits; gender not specified	50 mg placed in eye; eyes were not rinsed	severely irritating; corneal opacity still present at day 8	69
Dodecanedioic Acid				
not specified	male rabbits, no. not specified	ocular irritation study (GLP; details not provided)	slight irritant; irritation index 11.96/110	56
not specified	rabbits; no./gender not specified	ocular irritation study (details not provided)	small area of corneal opacity and mild conjunctival irritation; cleared within 7 days	56

Dermal Irritation/Sensitization

Most of the available dermal irritation and sensitization data regarding alkyl dicarboxylic acids and their salts were from animal studies. These animal data are summarized in Table 6. Human data were available for adipic and azelaic acids only.

Succinic acid. Succinic acid was a slight irritant to rabbit skin.[55] Details were not provided.

Glutaric acid. The dermal irritation potential of glutaric acid was determined using 2 male and 4 female New Zealand white rabbits.[53] A 0.5 g aliquot of glutaric acid was applied to the clipped skin on the back of the rabbits. The test site was scored for irritation after 3 minutes, and the site was then washed. The test material was then applied to 2 other test sites, which were covered with a rubber wrap. The sites were examined at 1 hour and 4 hours, and the site was washed after both examinations. The sites were then evaluated at 24 and 48 hours after application. Slight erythema was seen in 1 rabbit throughout the study. Irritation was not observed in the other rabbits.

Adipic Acid Nonhuman. A dermal irritation study was performed in which 500 mg of 50% aqueous adipic acid was applied under an occlusive patch to a 5 cm x 5 cm area of intact and abraded skin of 6 rabbits for 24 hours.[38] With intact skin, an erythema score of 2 to 3/4 was reported, with clearing by day 3. With abraded skin, mild to severe erythema and edema were reported, which cleared by day 7.

Adipic acid, undiluted or as an 80% aqueous paste, was applied occlusively to the backs of ears of rabbits for 24 hours. Two rabbits were used per group. No irritation was observed on the backs of animals. Erythema was observed on the ear, with clearing by 72 hours. In another study in which adipic acid was applied occlusively for 24 hours, irritation was not observed. Details were not provided.

A semi-occlusive application of 500 mg of a paste of 50% adipic acid in propylene glycol to 6 rabbits produced slight to mild irritation in 3 of the rabbits. A semi-occlusive application of undiluted adipic acid was not corrosive. Adipic acid, 50% in propylene glycol, was not irritating to a group of 10 guinea pigs.

Table 6. Dermal Irritation and Sensitization—Dicarboxylic Acids

Dose/Conc.	Animals	Procedure	Results	Reference
IRRITATION				
Succinic Acid				
not specified	rabbits, no./gender not specified	irritation studies (details not provided)	slight to mild irritation	55
Glutaric Acid				
not specified	rabbits, no./gender not specified	irritation studies (details not provided)	slight irritation	55
Adipic Acid				
500 mg of 50% aqueous	6 rabbits	occlusive application to a 5 cm x 5 cm area of abraded or intact skin for 24 hours	intact skin: erythema (score 2-3/4), cleared by day 3; abraded skin: mild to severe erythema and edema (2/4 at 24 hours; 0-2 at 72 hours), cleared by day 7	40
undiluted or 80% aqueous paste	2 rabbits/group	occlusive application to intact skin on the back and the ear for 20 hours	no irritation on the back; erythema on the ear at 24 hours (score of 2/4), with clearing by 72 hours	40
not specified	rabbits, no./gender not specified	occlusive application for 24 hours	not irritating	40
undiluted or 50% paste in propylene glycol (PG)	6 rabbits	semi-occlusive application of 500 mg for 24 hours	slight to mild irritation in 3/6 rabbits with 50%; no corrosion with undiluted test material	40
50% in PG	10 guinea pigs, gender not specified	applied to intact skin	no irritation	40
Succinic/Glutaric/Adipic Acids Mixture (percentages not specified)				
not given	guinea pigs, no./gender not specified	irritation study (details not provided)	no to mild irritation	57
Dodecanedioic Acid				
not specified	male rabbits, no. not specified	irritation study; 4 hours exposure (GLP; details not provided)	not an irritant; irritation index 0/8	56
0.5 g	male rabbits, no. not specified	FHSA procedures	not an irritant	56
SENSITIZATION				
Adipic Acid				
induction: 0.1 mL of 1.0% aqueous soln; challenge: 0.05 mL of 50 and 25% in PG	10 guinea pigs/group	induction: 4 sacral intradermal injections, 1/week; challenge: dermal application after a 2-week rest period	very mild to no irritation; no sensitization	40
Succinic/Glutaric/Adipic Acids Mixture (percentages not specified)				
not given	guinea pigs, no./gender not specified	sensitization study (details not provided)	not a sensitizer	57
Dodecanedioic Acid				
induction: 0.5%; challenge: 25 and 50%	female guinea pigs, no. not specified	Magnusson-Kligman maximization test (intracutaneous admin at induction; dermal admin at challenge)	not a sensitizer	56

The sensitization potential of adipic acid was evaluated using groups of 10 guinea pigs. For induction, 0.1 mL of 1% aqueous adipic acid was given as a sacral intradermal injection, once a week for 4 weeks. After a 2-week nontreatment period, the dermal challenge was performed with 0.05 mL of 50 and 25% adipic acid in propylene glycol. Adipic acid produced very mild or no irritation and it was not a sensitizer.

Human. In 2 case reports involving occupational exposure to adipic acid, positive sensitization reactions were reported with follow-up testing.[38]

Adipic/glutaric/succinic acid mixture. A mixture of adipic, glutaric, and succinic acid (percentages not specified) was evaluated for irritation and for sensitization using groups of 10 male

guinea pigs.[53] The primary irritation potential was evaluated by applying 0.05 mL of an 8 or 80% suspension in dimethyl phthalate to the shaved, intact skin on the shoulder of the animals. The sensitization potential was also evaluated, using 4 sacral intradermal injections of 0.1 mL of a 1% suspension for induction. After a 13-day nontreatment period, a dermal challenge was performed with 0.05 mL of an 8% and 80% suspension of the mixture. Ten previously untreated guinea pigs were exposed to the same challenge applications as the test animals. In the test for primary irritation, the 8% suspension produced no irritation, and no to mild irritation was observed 24 hours after exposure to the 80% suspension. No sensitization was observed at either dose.

Azelaic acid. The cumulative irritation potential of a 15% azelaic acid gel (prescription formulation; vehicle not identified) was determined in a study using 31 female and 2 male participants.[64] (During the study, 1 participant withdrew for personal reasons.) White petrolatum was used as a negative control. Azelaic acid and petrolatum, 0.2 g of each, were applied under occlusion to 2 cm x 2 cm sites on the back of each participant 3 times per week for 3 weeks. Weekday patches were removed after 24 hours, while the patches applied on Fridays were removed after 72 hours. The test sites were evaluated 15 to 30 minutes after removal of the patch, and then a new patch was applied. Application was discontinued if severe irritation, which was designated by a maximum erythema score of 3, was observed. A 15% azelaic acid gel was statistically significantly more irritating than the negative control, with a mean cumulative irritancy index of 1.05/3. Individual reaction scores for the test article ranged from 0 to 3, and 5 participants discontinued patching with azelaic acid due to an irritation score ≥ 3. Cumulative irritancy increased with successive patching. The researchers noted that since the vehicle used for azelaic acid was not tested, there was uncertainty as to whether the vehicle components affected the irritation scores.

Twice daily application of a cream containing 20% azelaic acid has been reported to cause erythema, irritation, pruritus, dryness, scaling, and burning.[65]

Dodecanedioic acid. Dodecanedioic acid was not an irritant to rabbit skin in a 4-hour exposure study or upon application of 0.5 g.[54] In a maximization study using female guinea pigs, 0.5% dodecanedioic acid was injected intracutaneously at induction and 25 and 50% was used for the dermal challenge. Dodecanedioic acid was not a sensitizer.

Mucosal Irritation

Succinic acid. Succinic acid has been considered to be an exacerbating factor in ulcerative colitis, therefore its influence on rat colonic mucosa in terms of mucosal blood flow and superoxide generation was investigated.[66] The left side of the colon of 5 male and 5 female rats was exposed, and 0.9% to 5% succinic acid in physiological saline was instilled into the colonic lumen. A segment of the colon was then ligated as to not include the mesenteric blood vessel. Mucosal blood flow decreased with all dose levels. Microscopically, the higher the concentration of succinic acid, the greater was the erosion formation in the colonic mucosa. Significant polymorphonuclear cell infiltration superoxide generation from colon tissue was observed with 0.01% succinic acid, as compared to higher or lower concentrations. Succinic acid, at fecal concentrations found in active stage ulcerative colitis, appears to be implicated in mucosal injury, mediated by a decrease in colonic mucosal blood flow and infiltration of superoxide-generating polymorphonuclear cells into the mucosa.

Reproductive and Developmental Toxicity

Malonic Acid. Malonic acid, 0.1%, reduced the pH of sperm suspensions from 7.5 to 4.5 to 5.5 and it rendered human spermatozoa immotile within 30 minutes.[67] A concentration of 1.0% reduced the pH to 1.5 to 3.0 and was almost instantaneously spermicidal.

Succinic Acid. Thirty ovariectomized female rats were given daily sc injections of 5.0 mg/d succinic acid for 3 weeks.[53] Ten females were used as controls. Daily vaginal smears were similar for test and control animals. Microscopically, no changes were seen in the uterine horn, cervix, or vagina of the animals.

Glutaric Acid. The reproductive toxicity of glutaric acid was evaluated using groups of 25 female rats.[53] The animals were dosed orally, by gavage, with 0, 125, 400, or 1300 mg/kg glutaric acid on days 6 to 15 of gestation, and the animals were killed on day 20 of gestation. No toxicological or reproductive effects were observed for the 125 mg/kg group. In the 400 mg/kg group, salivation, rales, and nasal discharge were observed. One dam of the 1300 mg/kg group died on day 10 of gestation, and 1 was killed due to moribund condition on day 13 of gestation. Mean bw gains were decreased in the 1300 mg/kg group during dosing, but bw gains in this group were normal post-dosing. Clinical signs of toxicity in the 1300 mg/kg group included salivation, rales, nasal discharge, and staining around the mouth, nares, and anogenital area. No adverse effects on pregnancy and no teratogenic effects were reported at any of the dose levels. There was a significant increase in resorptions in the 1300 mg/kg group compared to controls, but the value was within normal expected limits and, therefore, not considered biologically meaningful.

Groups of 18 gravid female New Zealand white rabbits were dosed orally, by gavage, on days 6 to 18 of gestation with 0, 50, 160, or 500 mg/kg glutaric acid, and the animals were killed on day 29 of gestation.[53] No test-article related mortality occurred. There were no clinical signs of toxicity, and bws were not affected. No embryotoxic, teratogenic, or adverse reproductive effects were reported.

Adipic Acid. Groups of 20 to 24 gravid albino CD-1 mice were dosed orally, by gavage, with 0, 2.6, 12, 56, or 263 mg/kg bw

adipic acid on days 6 to 15 of gestation.[38] All animals were killed on day 17 of gestation. No reproductive, developmental, or maternal effects were observed, and the NOAEL for maternal and developmental toxicity was 263 mg/kg bw. Similar results were obtained in a study in which gravid Wistar rats were dosed orally, by gavage, with 0, 2.9, 13, 62, or 288 mg/kg bw adipic acid on days 6 to 15 of gestation. The NOAEL for maternal and developmental toxicity was 288 mg/kg bw.[38]

Groups of 21 to 24 gravid hamsters were dosed orally, by gavage, with 0, 2.9, 5, 44, or 205 mg/kg bw adipic acid on days 6 to 10 of gestation. A significant increase in resorption per implant site was observed with 205 mg/kg bw adipic acid, resulting in a decreased number of live fetuses. (This decrease was not evaluated statistically.) No other effects were reported.[38]

Groups of 10 to 14 gravid Dutch-belted rabbits were dosed by oral intubation with 0, 2.5, 12, 54, or 250 mg/kg bw adipic acid on days 6 to 18 of gestation. No reproductive, developmental, or maternal effects were observed. The NOAEL for maternal toxicity was ≥250 mg/kg bw and for developmental toxicity was 250 mg/kg bw.[38]

Azelaic Acid. Reproductive and teratogenic effects of azelaic acid were evaluated using Wistar rats and New Zealand rabbits.[57] A group of 20 gravid rats was fed a diet containing 140 mg/kg bw/d azelaic acid, and a control group of 10 gravid rats was given untreated feed. Half of each group was killed and necropsied on day 19 of gestation, and the remaining animals continued dosing for 3 mos. The day of gestation that dosing started is not clear. No gross or microscopic lesions were observed for the uteri, placentas, or ovaries. There were no differences in reproductive, teratogenic, or developmental effects between treated and control groups, nor were there any differences in fetal weights of the live fetuses. Similar results were seen using groups of 20 gravid rabbits fed 200 mg/kg bw/d azelaic acid; 10 untreated gravid rabbits were used as a negative control group.

Embryotoxic effects were observed in oral studies with rats receiving 2500 mg/kg bw/d of azelaic acid.[42] Similar effects were observed in studies in rabbits given 150 to 500 mg/kg bw/d and in monkeys given 500 mg/kg bw/d. The doses at which these effects were noted were all within toxic dose ranges for the dams. No teratogenic effects were observed. (Details were not provided.)

Disodium Sebacate. Reproductive, teratogenic, and developmental effects of disodium sebacate were evaluated using Wistar rats and New Zealand rabbits.[57] Groups of 20 gravid rats were fed a diet containing 0 or 500 mg/kg bw/d disodium sebacate, and groups of 20 gravid rabbits were fed 0 or 1000 mg/kg bw. Half of each group was killed and necropsied on day 19 of gestation, and the remaining animals continued dosing for 3 mos. The day of gestation that dosing started is not clear. No gross or microscopic lesions were observed for the uteri, placentas, or ovaries. There were no differences in reproductive or developmental effects between treated and control groups, nor were there any differences in fetal weights of the live fetuses.

Dodecanedioic Acid. The reproductive toxicity of 0 to 1000 mg/kg bw dodecanedioic acid was evaluated in an OECD combined repeated doe and reproductive/developmental toxicity screening test using male and female Crl:CD:BR rats.[54] The no-observable effect level (NOEL) for reproductive and developmental toxicity was 1000 mg/kg bw.

Sodium Salt of Adipic, Azelaic, Sebacic, and Dodecanedioic Acids. The influence of the sodium salt of some dicarboxylic acids (adipic acid, azelaic acid, sebacic acid, dodecanedioic acid) on both spontaneous and evoked muscle activity of the uterine horns of 35 female Wistar rats (250-300 g) has been studied in vitro.[68] Spontaneous activity of uterine muscle was inhibited by dicarboxylic salts causing the total abolition of mechanical events at concentrations of 24, 32, 40, and 64×10^{-3} mol/L. Dicarboxylic salts antagonized the maximal isometric contraction of the uterine horn induced by administration of acetylcholine, oxytocin or prostaglandins (PGF_2-α). The amount of antagonism was dependent upon the concentration of dicarboxylic salt used. Dicarboxylic salts had an specific inhibitory effect on the uterine horn which progressively increased with their chain length. The results suggested that the inhibitory effects of dicarboxylic salts on smooth muscle could be due to a cellular membrane hyperpolarization.

Genotoxicity

Available genotoxicity studies are summarized in Table 7.

In Vitro

Malonic acid. Malonic acid, 3333 μg/plate, was not mutagenic in a National Toxicology Program (NTP) preincubation assay, with or without metabolic activation.[70]

Succinic acid. The genotoxic potential of succinic acid was evaluated in an Ames test and in a chromosomal aberration study using a Chinese hamster fibroblast cell line.[71] Succinic acid, at a concentration of ≤5.0 mg/plate in phosphate buffer, was not mutagenic in the Ames test. (Whether metabolic activation was used is not stated.) Concentrations of ≤1.0 mg/mL in saline were not genotoxic in the chromosomal aberration assay.

Disodium succinate. The genotoxic potential of disodium succinate was evaluated in an Ames test and in a chromosomal aberration study using a Chinese hamster fibroblast cell line.[71] In the Ames test, disodium succinate was not mutagenic at concentration up to 5.0 mg/plate in phosphate buffer. (Whether metabolic activation was used is not stated.) Equivocal genotoxic results were obtained in the chromosome aberration assay of ≤15.0 mg/mL disodium succinate in saline using Chinese hamster fibroblast cells.

Disodium succinate, ≤10 mg/plate, was negative in another Ames test, with and without metabolic activation.[77]

Table 7. Genotoxicity Studies—Dicarboxylic Acids

Concentration	Vehicle	Procedure	Test System	Results	Reference
In Vitro					
Malonic Acid					
≤3333 µg/plate	water	NTP preincubation assay, ± metabolic activation	*S typhimurium TA100, TA1535, TA97, TA98*	negative	70
Succinic Acid					
≤5 mg/plate	phosphate buffer	Ames test	*S typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98	negative	71
≤1.0 mg/mL	saline	chromosomal aberration assay	Chinese hamster fibroblasts cells	negative	71
Sodium Succinate					
≤10 µg/plate	distilled water	Ames test, ± metabolic activation	*S typhimurium TA97, TA102*	negative	72
Disodium Succinate					
≤5 mg/plate	phosphate buffer	Ames test	*S typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98*	negative	71
≤10 000 µg/plate	distilled water	Ames test, ± metabolic activation	*S typhimurium* TA97, TA102	negative	60
≤15.0 mg/mL	saline	chromosomal aberration assay	Chinese hamster fibroblasts cells	equivocal	71
Glutaric Acid					
0-5000 µg/plate	not specified	Ames test, ± metabolic activation	*S typhimurium* TA98, TA100, TA153S, TA 1537, TA1538	negative	55
0-8295 µg/mL	not specified	mouse lymphoma assay, ±metabolic activation	L5178Y/ TK cells	negative with neutral pH	55
0-12.5 mg/mL w/ out; 0-26.3 mg/ mL w/met. act.	DMSO	transformation assay, ± metabolic activation	Balb/c-3T3 cells	positive, ± activation	55
≤10 000 µg/plate	water	NTP preincubation assay, ± metabolic activation	*S typhimurium TA100, TA1535, TA97, TA98*	negative	73
Adipic Acid					
≤10 000 µg/plate	DMSO	NTP preincubation assay, ± metabolic activation	*S typhimurium TA100, TA1535, TA97, TA98*	negative	74
≤10 mg/plate	not specified	Ames test, ± metabolic activation	*S typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E coli* WP2	negative	40
≤5 mg/plate	not specified	Ames test, ± metabolic activation	*S typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E coli* WP2uvrA	negative	40
≤200 mg/L	not specified	Ames test, without metabolic activation	*S typhimurium* TA1530, G-46	negative	40
≤200 mg/L	not specified	yeast gene mutation assay, without metabolic activation	*S. cerevisiae* D-3	negative	40
≤2000 µg/plate	DMSO	mouse lymphoma assay, ± metabolic activation	*L5178Y/TK ± cells*	negative	75
≤200 mg/L	not specified	cytogenetic assay, without metabolic activation	human embryonic lung fibroblasts	negative	40
≤1000 µg/mL	not specified	viral enhanced cell transformation assay	Syrian hamster ovary cells	negative	40
Adipic/Glutaric/Succinic Acid Mixture					
0-3000 µg/plate	50% aqueous solution	Ames test, ± metabolic activation	*S typhimurium* TA98, TA100, TA1535, TA 1537, TA1538	negative	55
≤5000 µg/mL	not specified	unscheduled DNA synthesis	F344 rat hepatocytes	negative	55
≤2500 µg/mL	not specified	HGPRT assay, without metabolic activation	not specified	negative	55
≤3500 µg/mL	not specified	HGPRT assay, with metabolic activation	not specified	negative	55

(continued)

Table 7. (continued)

Concentration	Vehicle	Procedure	Test System	Results	Reference
≤1500 μg/plate	distilled water	transformation assay, without metabolic activation	CHO cells	negative	55
≤2500 μg/plate	distilled water	transformation assay, with metabolic activation	CHO cells	positive at 2000 μg/mL	55
Azelaic Acid					
20%	cream	Ames test; no details	not specified	negative	44
20%	cream	HGRPT test; no details	Chinese hamster ovary cells	negative	55
20%	cream	human lymphocyte test, no details	human lymphocytes	negative	55
Sebacic Acid					
≤5000 μg/plate	DMSO	Ames test, ± metabolic activation	*S typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E coli* WP2	negative	76
Dodecanedioic Acid					
10-5000 μg/plate	not specified	Ames test, ± metabolic activation	*S typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	56
In Vivo					
Glutaric Acid					
0, 800 mg/kg	distilled water	micronucleus assay	4 male and 4 female CD-1 mice/group	negative	55
Adipic Acid					
≤375 mg/kg; 1 or 5 doses	not specified	cytogenetic assay; animals dosed orally by gavage	male rats	negative	40
5000 mg/kg (1 dose); 2500 mg/kg (5 doses)	not specified	cytogenetic assay; animals dosed orally by gavage	male rats	negative	40
≤375 mg/kg; 1 or 5 doses	not specified	dominant lethal assay; animals dosed orally be gavage	male rats	negative	40
5000 mg/kg (1 dose); 2500 mg/kg (5 doses)	not specified	dominant lethal assay; animals dosed orally be gavage	male rats	negative	40
Adipic/Glutaric/Succinic Acid Mixture					
2750 mg/kg (males), 1375 mg/kg (females)	not specified	cytogenetic assay; animals dosed orally by gavage	male and female Sprague Dawley rats; 1 dose	negative	55
Azelaic Acid					
20%	cream	dominant lethal assay	mice	negative	55
Dodecanedioic Acid					
≤5000 mg/kg	not specified	micronucleus assay	Crl:CD-1(CR)BR mice	negative	56

Glutaric acid. Glutaric acid was evaluated in vitro in a standard Ames assay, the L5178Y/TK ± mouse lymphoma assay with and without metabolic activation, and the mammalian in vitro Balb/c-3T3 cell transformation assay with and without metabolic activation.[78] The Ames tests were negative. However, the cell transformation assay was positive both in the presence and absence of metabolic activation and the results in the mouse lymphoma assay were dependent upon pH of the culture medium. The researchers stated that the variable response in the mouse lymphoma assay and the positive effect in the cell transformation assay may have been an indirect effect of other factors (such as the pH or osmolarity of the media in which the cells were exposed), rather than a direct effect of glutaric acid.

Adipic acid. Adipic acid was evaluated in a number of Ames assays using *Salmonella typhimurium* and *Escherichia coli*; results were negative, with or without metabolic activation, at concentrations as high as 10 000 mg/plate.[40,74,75] Negative results were also obtained in an Ames test with 0 to 200 mg/L adipic acid using *S typhimurium* TA1530 and G-46 without metabolic activation.[40] Results were negative in a yeast gene mutation assay using *Saccharomyces cerevisiae* without metabolic activation at concentrations ≤200 mg/L. A mouse lymphoma assay using L5178Y/TK ± cells was negative with and without metabolic activation at concentrations of ≤2000 μg/plate,[75] as was a cytogenetic assay using human embryonic lung fibroblast cells with ≤200 mg/L adipic acid.[40] In a viral enhanced cell transformation assay using

Syrian hamster embryo cells at doses of 62 to 1000 μg/mL adipic acid, results were negative.

Adipic/glutaric/succinic acid mixture. A mixture of adipic, glutaric, and succinic acid, percentages not specified, tested as a 50% aqueous solution, was not mutagenic in an Ames assay using *S typhimurium*, with or without metabolic activation, at concentrations of ≤300 μg/plate.[55] Negative results were also obtained in an unscheduled DNA synthesis assay at concentrations of ≤5000 μg/plate using rat hepatocytes and in an HGPRT assay at concentrations of ≤2500 μg/plate, without, and of ≤3500 μg/plate, with, metabolic activation. In an *in* vitro transformation assay using Chinese hamster ovary (CHO) cells at concentrations of ≤1500 μg/mL without and ≤2500 μg/mL with metabolic activation, positive results were obtained with, but not without, metabolic activation at 2000 μg/plate.

Azelaic acid. Azelaic acid, 20%, was not mutagenic or genotoxic in an Ames assay, HGPRT test in CHO cells, or human lymphocyte test.[44] Details were not provided.

Dodecanedioic acid. Dodecanedioic acid was not mutagenic in an Ames assay at concentrations of ≤5000 μg/plate, with and without metabolic activation.[56] Toxicity occurred at ≥500 μg/plate.

In Vivo

Glutaric acid. Glutaric acid was evaluated in a mammalian micronucleus cytogenetic assay in mice.[78] Glutaric acid was not genotoxic in this assay. (Details not specified.)

Adipic acid. Adipic acid was not genotoxic in in vivo cytogenetic assays using chromosomes from rats dosed orally, by gavage, with a single dose of 5000 mg/kg bw or daily for 5 days with 2500 mg/kg bw.[40] Adipic acid was also not genotoxic in dominant lethal studies with doses up to 5000 mg/kg bw.

Adipic/glutaric/succinic acid mixture. A mixture of adipic, glutaric, and succinic acid, percentages to specified, was not genotoxic in vivo using male and female Sprague Dawley rats dosed orally by gavage with 2750 and 1375 mg/kg of the mixture, respectively.[55]

Azelaic acid. Azelaic acid was not genotoxic in a dominant lethal assay in mice.[44] (Details not specified.)

Dodecanedioic acid. Dodecanedioic acid, ≤5000 mg/kg bw, was not mutagenic in a micronucleus assay using mice.[56]

Carcinogenicity

Sodium Succinate. Groups of 50 male and 50 female F344 rats were given drinking water containing 0, 1, or 2% sodium succinate for 2 years, and the carcinogenic potential was determined.[58] Dosing was discontinued after 104 weeks, and, after a 9-week recovery period, the rats were killed. Body weights of the high-dose animals were decreased by 10% as compared to controls. There were no statistically significant differences in overall tumor incidence or mean survival time between treated and control animals. An increase in the incidence of C-cell adenoma/carcinoma of the thyroid in females of the 2% group, and a positive trend in the occurrence of this tumor, was considered a function of experimental variability and not related to dosing. Sodium succinate was not toxic or carcinogenic to male or female F344 rats when given in the drinking water for 2 years.

Adipic Acid. Adipic acid was not carcinogenic in the 2-year chronic oral toxicity study (described previously) in which groups of 20 male rats were fed diets containing 0%, 0.1%, 1%, 3%, and 5% adipic acid, and groups of 10 and 19 females were fed 0% and 1% adipic acid, respectively.[57]

Tumor Promotion

Succinic acid, sodium succinate, disodium succinate. The promotion of urinary bladder carcinogenesis by sodium succinate was evaluated using male F344 rats.[79] Groups of 16 male F344 rats were given 5% succinic acid, sodium succinate, or disodium succinate with 0.05% *N*-butyl-*N*- (4-hydroxybutyl)nitrosamine (BBN) in the drinking water for 4 weeks, followed by dietary administration of 5% of the respective test article without BBN for 32 weeks. Negative controls were given water with BBN only and untreated feed. Groups of 8 male F344 rats followed the same protocol without the addition of BBN to the drinking water, as did a group of non-BBN-treated negative controls. The animals were killed at week 37.

In the BBN-pretreated groups, many rats given sodium or disodium succinate developed hematuria towards the end of the study. There were no statistically significant differences in body or organ weights between the control and test groups. (Information on organ and bws was not provided for the non-BBN groups.) Large tumors were found on the urinary bladders of the BBN-pretreated animals given sodium and disodium succinate; tiny lesions were found in the control or succinic acid BBN-pretreated animals. The incidence and number of urinary bladder carcinomas and papillomas and of papillary or nodular hyperplasia (preneoplastic lesions) were statistically significantly increased in the sodium and disodium succinate BBN-pretreated groups as compared to the succinic acid and control BBN-pretreated groups. The incidence and numbers observed in the sodium and disodium succinate groups were not statistically significantly different from each other. An association between tumor area and sodium intake was noted. Urinary bladder lesions were not observed in any of the animals that were not pretreated with BBN. Urinary pH and electrolyte concentrations were affected by dosing with sodium or disodium succinate with BBN, as compared to the control and succinic acid groups, and statistically significant differences between these 2 groups were observed as well.

The researchers also evaluated cell proliferation and DNA synthesis in the urinary bladder epithelium. Groups of 20 male F344 rats were given 5% succinic acid, sodium succinate, or

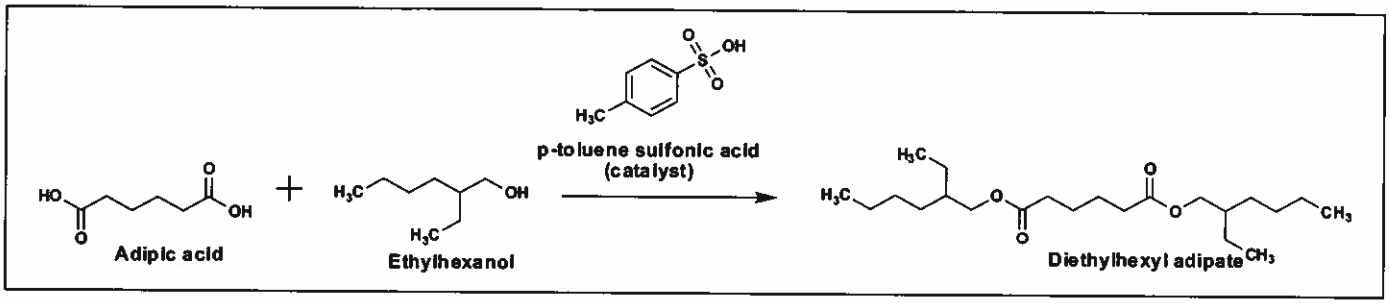


Figure 3. Diethylhexyl adipate synthesis from adipic acid.

disodium succinate in the feed, without BBN pretreatment for 8 weeks. Negative controls were given basal diet. Five rats per group were given an ip injection of 50 mg/kg bw 5-bromo-2'-deoxyuridine (BrdU) 1 hour prior to being killed. Compared to control values, BrdU uptake was statistically significantly increased by increased disodium succinate and was increased, but not in a statistically significant manner, by sodium succinate. Succinic acid did not have any effect on DNA synthesis. Microscopically, simple hyperplasia was observed in the urinary bladders of animals given sodium and disodium succinate. The appearance of the urinary bladder epithelial surface was altered by sodium and disodium succinate. Spermidine/spermine N^1-acetyltransferase activity in the urinary bladder epithelium was increased for disodium succinate, but not sodium succinate, when compared to controls. Urinary pH and electrolyte concentrations were affected as described previously.

Part II: Esters of alkyl Dicarboxylic Acids Chemistry

Method of Manufacture

Alkyl dicarboxylic acids are easily esterified with the appropriate alcohol, with our without acid or metal catalyst (Fischer esterification).[9] For example, diethylhexyl adipate can be manufactured from adipic acid and ethylhexanol with an acid catalyst (Figure 3).

Diethyl malonate. Malonic acid esters can be produced either by cobalt-catalyzed alkoxycarbonylation of chloroacetates with carbon monoxide in the presence of the appropriate alcohol, or by hydrolysis of cyanoacetic acid followed by esterification with the respective alcohol.[39] Diethyl malonate is prepared from chloroacetic acid and sodium cyanide followed by esterification with ethanol and sulfuric acid.[80]

Diisopropyl adipate. Diisopropyl adipate is produced by esterification of adipic acid with an excess of isopropanol. The excess alcohol is removed by vacuum stripping and the ester is then alkali-refined and filtered.[2]

Dibutyl adipate. Adipic acid is esterified with butyl alcohol by a continuous distillation process.[81]

Diethylhexyl adipate. Diethylhexyl adipate can be prepared by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst such as sulfuric acid or *para*-toluenesulfonic acid (Figure 2).[17] Purification of the reaction product includes removal of the catalyst, alkali refining, and stripping.[2]

Alkyl succinates. Succinic anhydride reacts readily with alcohols to give monoesters of succinic acid (eg, decyl succinate from decanol), which are readily further esterified to the diesters by Fischer methods.[7] Dimethyl succinate can be produced from methanol and succinic anhydride or succinic acid, or by hydrogenation of dimethyl maleate. Diethyl succinate can be prepared by the same methods (from ethanol or diethyl maleate).

Chemical and Physical Properties

Table 8 lists the properties of the alkyl dicarboxylic acid esters. Figure 4 shows the relationship between molecular weight and the octanol water partitioning coefficient, expressed as log K_{ow}, for these ingredients.

The diesters, in contrast to the free acids, are much more lipid soluble and more difficult to dissolve in water. The mono-esters, by definition, are hybrids of the acids and diesters, but their physical properties are much more closely related to the diesters.

Short-chain alkyl (ie, methyl, isopropyl, and butyl) mono- and diesters are more soluble in water, less lipophilic, and relatively more volatile than the corresponding longer chain alkyl (ie, C8-C13 alcohol) esters.[21] Most esters with molecular weights greater than 340 have boiling points greater than 300°C and are relatively nonvolatile and lipophilic (log K_{ow} >7).

Impurities

Diethyl malonate. Diethyl malonate is a colorless organic liquid with an ester like odor.[39] The purity is typically > 99 %. Impurities from the production process include ethanol (ca. 0.1 % w/w), ethyl acetate (ca. 0.05 % w/w), and ethyl methyl malonate (ca. 0.05 % w/w).

Dibutyl adipate. Impurities are generally not found due to the manufacturing process, but available data demonstrate that arsenic levels are below a detection limit of 1 ppm, heavy metals (as lead) are below a detection limit of 10 ppm, and sulfated ash is below a detection limit of 0.1%.[81]

Table 8. Physical and Chemical Properties of the Mono- and Dicarboxylic Acid Esters[14-16]

INCI Name	Diethyl Malonate	Decyl Succinate	Dimethyl Succinate	Diethyl Succinate	Dicapryl Succinate	Dicetearyl Succinate	Diisobutyl Succinate
Appearance	colorless liquid	–	–	liquid	–	–	liquid
Molecular Weight (g/mol)	160.17	258.35	146.14	174.19	342.51	566-623	230.30
Melting/Boiling Point (°C)	−50/ 198 199	96/377 (est.)	19.5/196.1	−21.3/217.7	14 (est.)/ 375 (est.)	–/–	−48 (est.)/ 216
Density (g/cm^3)	1.055	1.002 (est.)	1.1	1.04	0.94 (est.)	–	0.967
Vapor pressure (mm Hg @ 25°C)	0.269	0.000001 (est.)	0.4 (est.)	0.126	0.000008 (est.)	–	0.019 (est.)
Solubility (g/L water @ 25°C)	20	20 (est.)	50 (est.)	10 (est.)	0.0015 (est.)	–	0.60 (est.)
Log K_{ow}	0.96	4.57 (est.)	0.26 (est.)	1.28 (est.)	7.39 (est.)	–	3.00 (est.)

INCI Name	Diethylhexyl Succinate	Dimethyl Glutarate	Diisobutyl Glutarate	Diisostearyl Glutarate	Dimethyl Adipate	Diethyl Adipate	Dipropyl Adipate
Appearance	–	liquid	–	–	–	–	230.30
Molecular Weight (g/mol)	342.51	160.17	244.33	637.07	174.19	202.25	−15.7/ 274 (est.)
Melting/Boiling Point (°C)	−12 (est.)/ 359 (est.)	42.5/ 214.2	−38 (est.)/ 237	212 (est.)/ 600 (est.)	210/229 (est.)	24-26/ 248-249	0.98
Density (g/cm^3)	0.933	1.0876	0.97 (est.)	–	1.062	1.08	0.0055 (est.)
Vapor pressure (mm Hg @ 25°C)	0.00002	0.185 (est.)	0.008 (est.)	7.8 E^{-13} (est.)	0.073 (est.)	0.027 (est.)	0.62 (est.)
Solubility (g/L water @ 25°C)	0.002 (est.)	27 (est.)	0.29 (est.)	1.16 E^{-16} (est.)	14 (est.)	2.8 (est.)	2.99 (est.)
Log K_{ow}	7.08 (est.)	0.57 (est.)	3.44 (est.)	17.5 (est.)	0.95 (est.)	1.97 (est.)	

INCI Name	Dibutyl Adipate	Dihexyl Adipate	Dicapryl Adipate	Di-C12-15 Alkyl Adipate	Ditridecyl Adipate	Dicetyl Adipate	Diisopropyl Adipate
Appearance	–	liquid	–	–	–	–	Liquid
Molecular Weight (g/mol)	258.35	314.46	426.67	482-567	510.83	594.99	230.30
Melting/Boiling Point (°C)	37.5/300 (est.)	−8/351 (est.)	26.5-27.1/ 442 (est.)	–/–	45.9/503 (est.)	56.5-57/ 559 (est.)	−1.1/253 (est.)
Density (g/cm^3)	0.96	0.95 (est.)	0.92 (est.)	–	0.91 (est.)	0.897 (est.)	0.982 (est.)
Vapor pressure (mm Hg @ 25°C)	0.0011 (est.)	0.00004 (est.)	0.00000005 (est.)	–	3.0 E^{-10} (est.)	1.5 E^{-12} (est.)	0.0192 (est.)
Solubility (g/L water @ 25°C)	0.14 (est.)	0.0082 (est.)	0.000041 (est.)	–	0.0000011 (est.)	0.00000005 (est.)	0.78 (est.)
Log K_{ow}	4.0 (est.)	6.0 (est.)	10.1 (est.)	–	13.8 (est.)	17 (est.)	2.68 (est.)

iNCI Name	Diisobutyl Adipate	Diethylhexyl Adipate	Diisooctyl Adipate	Diisononyl Adipate	Diisodecyl Adipate	Dihexyldecyl Adipate	
Appearance	liquid	liquid	–	–	–	–	
Molecular Weight (g/mol)	258.35	370.57	370.57	398.62	426.67	594.99	
Melting/Boiling Point (°C)	−20/278-280	−67.8/390	9 (est.)/ 382 (est.)	56 (est.)/ 230	51 (est.)/ 426 (est.)	181 (est.)/ 548 (est.)	
Density (g/cm^3)	0.95	0.925	0.93 (est.)	–	–	0.896 (est.)	
Vapor pressure (mm Hg @ 25°C)	0.0036 (est.)	0.0000009	0.000004 (est.)	3.3 E^{-6} (est.)	1.9 E^{-6} (est.)	4.6 E^{-12} (est.)	
Solubility (g/L water @ 25°C)	0.18	0.00078	0.00067 (est.)	4.0 E^{-5} (est.)	5.2 E^{-6} (est.)	0.00000006 (est.)	
Log K_{ow}	3.70 (est.)	6.11	7.77 (est.)	9.24 (est.)	10.1 (est.)	16.6 (est.)	

(continued)

Table 8. (continued)

INCI Name	Diheptylundecyl Adipate	Dioctyldodecyl Adipate	Diisocetyl Adipate	Diisostearyl Adipate	Diethyl Sebacate	Dibutyl Sebacate
Appearance	–	–	–	–	liquid	liquid
Molecular Weight (g/mol)	651.10	707.20	594.99	651.10	258.35	314.46
Melting/Boiling Point (°C)	229 (est.)/ 584 (est.)	267 (est.)/ 619 (est.)	181 (est.)/ 565 (est.)	229 (est.)/ 611 (est.)	5/298	−10/ 344 345
Density (g/cm^3)	0.892 (est.)	0.888 (est.)	0.896 (est.)	–	0.969 (est.)	0.94
Vapor pressure (mm Hg @ 25°C)	1.26 E^{-13} (est.)	3.17 E^{-15} (est.)	1.4 E^{-11} (est.)	2.4 E^{-13} (est.)	0.00054 (est.)	0.00004 (est.)
Solubility (g/L water @ 25°C)	9.8 E^{-9} (est.)	2.1 E^{-9} (est.)	4.0 E^{-12} (est.)	3.6 E^{-14} (est.)	0.15 (est.)	0.0085 (est.)
Log K_{ow}	18.7 (est.)	20.9 (est.)	16.0 (est.)	17.9 (est.)	3.92 (est.)	5.96 (est.)

INCI Name	Dicaprylyl/Capryl Sebacate	Isostearyl Sebacate	Diisopropyl Sebacate	Diethylhexyl Sebacate	Dibutyloctyl Sebacate	Diisooctyl Sebacate
Appearance	–	–	–	–	–	–
Molecular Weight (g/mol)	426-482	454.73	286.41	426.67	538.89	426.67
Melting/Boiling Point (°C)	–/–	215 (est.)/ 545 (est.)	−7 (est.)/ 308 (est.)	−48/436 (est.)	135 (est.)/ 510 (est.)	51 (est.)/ 428 (est.)
Density (g/cm^3)	–	0.929 (est.)	0.953 (est.)	0.91	0.901 (est.)	0.916 (est.)
Vapor pressure (mm Hg @ 25°C)	–	2.5 E^{-13} (est.)	0.0007 (est.)	8.7 E^{-8} (est.)	1.6 E^{-10} (est.)	1.6 E^{-7} (est.)
Solubility (g/L water @ 25°C)	–	0.0013 (est.)	0.046	0.00006 (est.)	0.0000006 (est.)	0.00006 (est.)
Log K_{ow}	–	11.2 (est.)	4.63 (est.)	9.72 (est.)	14.1 (est.)	9.72 (est.)

INCI Name	Dihexyldecyl Sebacate	Dioctyldodecyl Sebacate	Diisostearyl Sebacate	Dioctyldodecyl Dodecanedioate	Diisocetyl Dodecanedioate
Appearance	–	–	–	–	–
Molecular Weight (g/mol)	651.10	763.31	707.20	791.36	679.15
Melting/Boiling Point (°C)	229 (est.)/ 584 (est.)	299 (est.)/ 652 (est.)	268 (est.)/ 568 (est.)	314 (est.)/ 668 (est.)	247 (est.)/ 635 (est.)
Density (g/cm^3)	0.892 (est.)	0.885 (est.)	–	0.884 (est.)	–
Vapor pressure (mm Hg @ 25°C)	1.3 E^{-13} (est.)	7.4 E^{-17} (est.)	4.8 E^{-15} (est.)	1.1 E^{-17} (est.)	3.6 E^{-14} (est.)
Solubility (g/L water @ 25°C)	0.0000001 (est.)	6.8 E^{-10} (est.)	3.2 E^{-16} (est.)	3.6 E^{-10} (est.)	3.4 E^{-15} (est.)
Log K_{ow}	18.4 (est.)	22.6 (est.)	19.9 (est.)	23.7 (est.)	18.9 (est.)

"(est.)" = estimated value by EPI Suite
"(dec.)" = some decomposition occurred
"–" = Value not found
"E^{-13}" = divided by 10^{13}

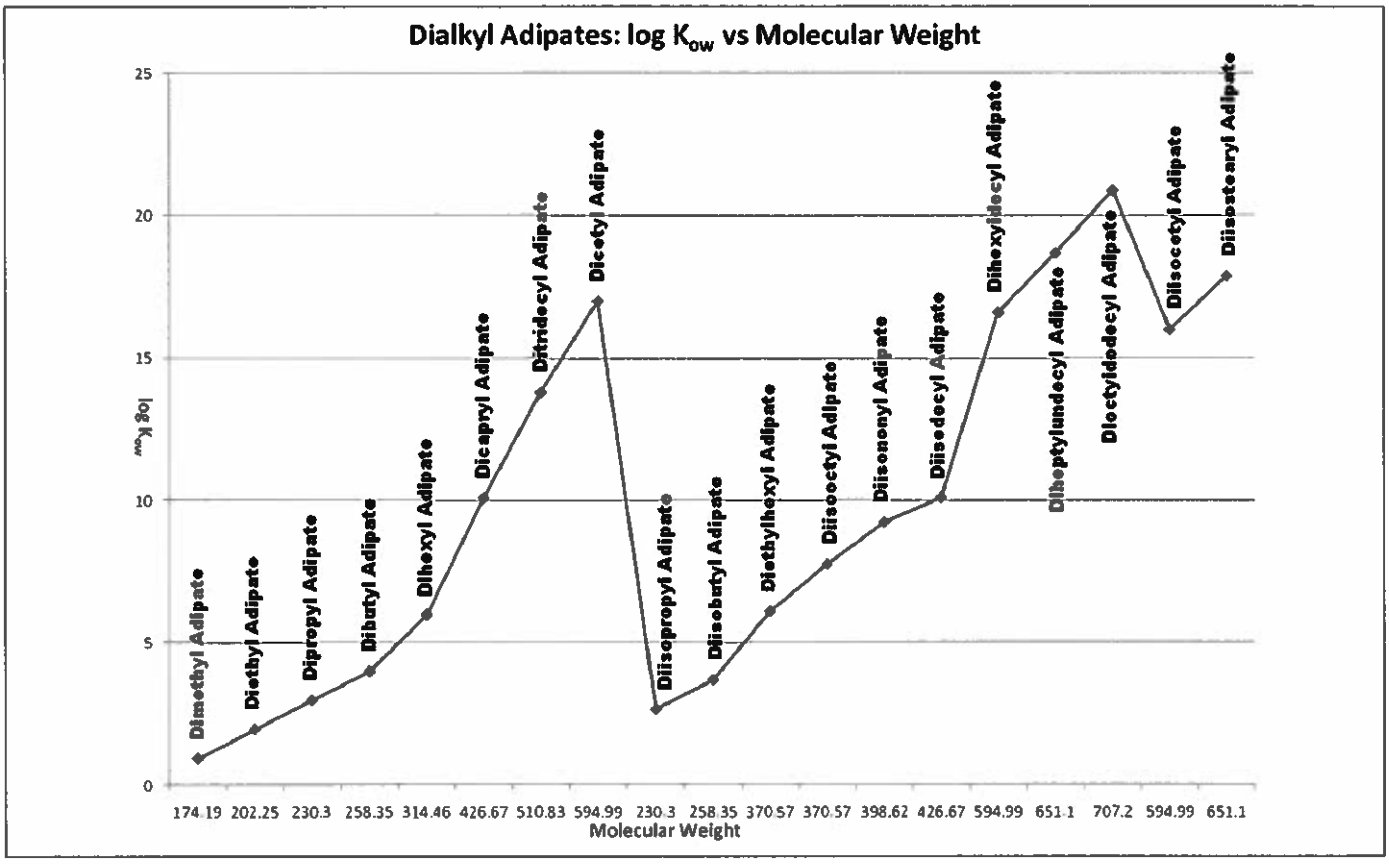


Figure 4. Example of the effects of chain length and branching on solubility. Log K_{ow} vs molecular weight

Diisopropyl adipate and diethylhexyl adipate. Diisopropyl adipate and diethylhexyl adipate are considered stable; however, hydrolysis of the ester groupings may occur in the presence of aqueous acids or bases. No known impurities occur in either diisopropyl adipate or diethylhexyl adipate, although the acid values imply the presence of adipic acid or of the monoester in both.[2]

Diethylhexyl adipate is commercially available with the following specifications: purity—99% to 99.9%; acidity—0.25 µg/100g max; moisture—0.05% to 0.10% max.[17]

Diisopropyl sebacate. A supplier reported that the expected impurities in diisopropyl sebacate are the starting material sebacic acid, <0.3%, and isopropyl alcohol, <0.2%.[82]

Ultraviolet Absorption

The alkyl dicarboxylic acid esters included in this review would not be expected to have any meaningful UV absorption. Except for the acid and ester functional groups, these ingredients do not possess any conjugated π bonds or nonbonding electrons. The π bonds and nonbonding electrons in the acid and ester functional groups are not part of any conjugated systems. Accordingly, these ingredients are unlikely to absorb light within the UVA-UVB spectrum at a detectable molar absorptivity.

Use

Cosmetic

The ingredients included in this safety assessment have a variety of functions in cosmetics.[19] For the esters, some of the common functions include skin conditioning agents, fragrance ingredients, plasticizers, solvents, and emollients. The functions of all ingredients are listed in Table 1.

A total of 24 of the 44 esters included in this safety assessment are reported to be used in cosmetic formulations. The frequency of use of the esters, with the exception of dibutyl, diisopropyl, and diethylhexyl adipate, which have previously been reviewed, as supplied to the FDA by industry in 2010 as part of the Voluntary Cosmetic Registration Program (VCRP),[20] and the concentration of use, as supplied by industry in response to Personal Care Products Council (Council) surveys in 2009[21] and 2010,[22,23] are found in Table 9. The 2010 and historical use data for the 3 previously reviewed esters are found in Table 10. The 20 esters not currently reported to be used are listed in Table 11.

Diisopropyl adipate has the greatest number of current uses, with 70 reported. The highest concentration of use is for dimethyl glutarate, 15% in a dermal rinse-off product. The ingredients with the highest leave-on use concentrations, which are all dermal contact exposures, are diethylhexyl adipate, 14%, diisostearyl adipate, 10%, and diisopropyl sebacate, 10%.

Some of the alkyl dicarboxylic acid ester ingredients are applied around the eye, can possibly be ingested, or involve mucous membrane exposure, and some are used in underarm deodorants. None are reported to be used in baby products.

Dicapryl and diethylhexyl succinate, dibutyl, dicapryl, diisopropyl, diisobutyl, and diethylhexyl adipate, diisopropyl, diethylhexyl, and dioctyldodecyl sebacate, and dioctyldodecyl and diisocetyl dodecanedioate are used in hair sprays, and effects on the lungs that may be induced by aerosolized products containing this ingredient, are of concern.

The aerosol properties that determine deposition in the respiratory system are particle size and density. The parameter most closely associated with deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of $\leq 10\mu m$ are respirable. Particles with a d_a from 0.1 to 10 μm settle in the upper respiratory tract and particles with a $d_a < 0.1$ μm settle in the lower respiratory tract.[86,87]

Particle diameters of 60 to 80 μm and ≥80 μm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[88] In practice, aerosols should have at least 99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 μm.[89] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable. Alkyl dicarboxylic acids esters are in the European Union (EU) inventory of cosmetic ingredients.[24]

Noncosmetic

Many of the dicarboxylic acids esters are used in foods as direct or indirect food additives.[9] The diesters have widespread use as lubricants, plasticizers, and solvents.[86]

Toxicokinetics

The simple alkyl di-esters are the result of the condensation of alkyl dicarboxylic acids and 2 equivalents of alkyl alcohols. These ingredients can be metabolized via hydrolysis back to the parent alcohol, the monoester, and the parent dicarboxylic acid (Figure 5). Previous safety assessments conducted by the Panel have addressed the safety of cetyl, methyl, isostearyl, myristyl, and behenyl alcohol.[90,200]

Metabolism of diesters in animals is expected to occur, initially, via enzymatic hydrolysis, leading to the corresponding dicarboxylic acids and the corresponding linear or branched alcohol.[87] These dicarboxylic acids and alcohols can be further metabolized or conjugated to polar products that are excreted in urine. However, other studies have shown that enzymatic hydrolysis of at least some diesters may be incomplete and result, instead, in the production of monoesters.[91]

Diethyl malonate. Diethyl malonate is hydrolyzed via a 2-step reaction to malonic acid and the corresponding alcohol, ethanol.[39]

Dimethyl malonate, which is not listed in the *International Cosmetic Ingredient Dictionary*, has similar physico-chemical properties and hydrolyzes in the same manner to malonic acid and methanol. Because of this similarity, data on dimethyl malonate are included in subsequent sections of part II to provide read-across data.

Distribution of diethyl malonate (and dimethyl malonate) is likely to occur in the water compartments, and accumulation in fat is unlikely based on physical and chemical properties. Both esters are likely to be metabolized by unspecific (serine-) esterases of different tissues, in particular, in the liver to the mono- esters and then to malonic acid and ethanol (or methanol). The hydrolysis product is likely to be metabolized via physiological pathways, such as the tricarboxylic acid cycle, as they are part of the normal intermediate metabolism. Both are assumed to readily absorb via mucous membranes.

In Vitro—Nonhuman

The percutaneous absorption of radiolabeled diethyl malonate was determined in vitro using skin from Yorkshire pigs.[39] [2-^{14}C]Diethyl malonate was applied either undiluted (100 μg/cm^2) or diluted in ethanol at 12.5 mg/mL with an applied dose of 100 μg/cm^2 or as 0.5 mg/mL with an applied dose of 4 μg/cm^2. At 50 hours, with undiluted diethyl malonate, 8.8% of the radioactivity was found in the skin and 3% was in the receptor fluid. With 100 μg in ethanol, 13% of the radioactivity was found in the skin and 6% in the receptor fluid and with 4 μg in ethanol, 30% was found in the skin and 10% in the receptor fluid. Absorption appeared to be enhanced with ethanol.

The percutaneous absorption of 1 mg/cm^2 [2-^{14}C]diethyl malonate in 10 μl acetone was determined in vitro also using skin from Yorkshire pigs. At 24 hours, 0.2% to 1.6% of the diethyl malonate was found in the receptor fluid, 0.2% to 0.9% was found in the skin, and 0.6% to 0.7% was found on the skin surface. Skin mediated hydrolysis amounted to 15% to 35% of the applied dose. In the receptor fluid, 20% to 21% of the applied dose was present as hydrolysis products. In the skin and on the skin surface, 3% to 5% and 2% to 4%, respectively, of the applied dose was present as hydrolysis products.

In Vivo—Nonhuman

The percutaneous penetration of radiolabeled diethyl malonate was studied in vivo in the following animal models: athymic nude mouse, human, and pig skin grafted to athymic nude mice, in weanling pigs, and in hairless dogs.[39] [$^{2\text{-}14}$C]Diethyl malonate was applied at a dose of 0.1 mg/cm^2 for 24 hours to a 1.27 cm^2 area of mouse skin, or for 48 hours to a 25 cm^2 area of pigs and hairless dogs using nonoccluded applications. According to the authors, the percutaneous absorption, was estimated from the recovery of radioactivity in urine and feces and corrected for the recovery observed after parenteral (sc)

Table 9. Frequency[20] and Concentration[21,23] of Use by Duration and Exposure—Esters of Dicarboxylic Acids

	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)
	Diethyl Malonate		Dimethyl Succinate		Dicapryl Succinate		Diethylhexyl Succinate		Dimethyl Glutarate		Dimethyl Adipate	
Totals	NR	0.004-0.02	12	0.002-5	12	NR	38	0.02-6	13	0.5-15	12	0.2
Duration of Use												
Leave-On	*NR*	*0.02*	*NR*	*0.002*	*9*	*NR*	*34*	*0.02-6*	*NR*	*NR*	*NR*	*NR*
Rinse Off	*NR*	*0.004-0.01*	*12*	*0.2-5*	*NR*	*NR*	*4*	*3-5*	*13*	*0.5-15*	*12*	*0.2*
Exposure Type												
Eye Area	NR	NR	NR	0.002	NR	NR	1	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	3	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	1	NR	NR	1	NR	NR	NR	NR
Dermal Contact	NR	0.004-0.02	NR	0.002-5	8	NR	34	1-6	NR	15	NR	NR
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Noncoloring	NR	NR	NR	NR	1	NR	4	0.02-5	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	12	0.2	NR	NR	NR	NR	13	0.5	12	0.2
Mucous Membrane	NR	NR	NR	NR	2	NR	1	NR	NR	NR	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Dihexyl Adipate		Dicapryl Adipate		Diisobutyl Adipate		Diisodecyl Adipate		Diheptylundecyl Adipate		Dioctyldodecyl Adipate	
Totals	1	3	43	NR	22	0.001-3	1	NR	NR	6	3	NR
Duration of Use												
Leave-On	*1*	*NR*	*38*	*NR*	*22*	*0.001-3*	*1*	*NR*	*NR*	*6*	*3*	*NR*
Rinse Off	*NR*	*3*	*5*	*NR*	*NR*	*0.002-0.5*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type												
Eye Area	NR	3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	NR
Inhalation	NR	NR	1	NR	S	0.05-3	NR	NR	NR	NR	NR	NR
Dermal Contact	1	3	43	NR	8	0.002-3	1	NR	NR	6	3	NR
Deodorant (Underarm)	NR	NR	30	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Noncoloring	NR	NR	NR	NR	5	0.05-0.2	NR	NR	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	9	0.001-0.7	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	0.009	NR	NR	NR	NR	NR	NR
Bath Products	NR	NR	5	NR	NR	0.5	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 9. (continued)

	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)	No. Uses	Conc. of Use (%)
	Diethyl Malonate		Dimethyl Succinate		Dicapryl Succinate		Diethylhexyl Succinate		Dimethyl Glutarate		Dimethyl Adipate	
	Diisostearyl Adipate		Isostearyl Sebacate		Diethyl Sebacate		Diisostearyl Sebacate		Diisopropyl Sebacate		Diethylhexyl Sebacate	
Totals	6	3-10		0.005-0.7	NR	1.5	NR	0.005-0.7	30	0.06-10	13	0.5-5
Duration of Use												
Leave-On	*4*	*10*		*0.005-0.7*	*NR*	*1.5*	*NR*	*0.005-0.7*	*29*	*0.06-10*	*13*	*0.5-5*
Rinse Off	*2*	*3*		*NR*	*NR*	*NR*	*NR*	*NR*	*1*	*2*	*NR*	*1*
Exposure Type												
Eye Area	NR	NR		NR	NR	NR	NR	NR	1	NR	4	NR
Possible Ingestion	4	10		NR	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	NR	NR		NR	NR	NR	NR	NR	1	NR	NR	1
Dermal Contact	6	3-10		0.005-0.7	NR	1.5	NR	0.005-0.7	23	0.06-10	11	0.5-5
Deodorant (Underarm)	NR	NR		NR	NR	NR	NR	NR	4	1	NR	0.5
Hair, Noncoloring	NR	NR		NR	NR	NR	NR	NR	6	8	2	NR
Hair, Coloring	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR		NR	NR	NR	NR	NR	1	0.08	NR	NR
Mucous Membrane	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Bath Products	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
	Diisooctyl Sebacate		Dioctyldodecyl Sebacate		Dioctyldodecyl Dodecanedioate		Diisocetyl Dodecanedioate					
Totals	NR	1-3	NR	3-8	5	6	2	0.9-7				
Duration of Use												
Leave-On	*NR*	*1-3*	*NR*	*3-8*	*5*	*6*	*2*	*0.9-7*				
Rinse Off	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*				
Exposure Type												
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR				
Possible Ingestion	NR	NR	NR	8	1	6	NR	NR				
Inhalation	NR	NR	NR	3-5	2	NR	NR	0.9-3				
Dermal Contact	NR	1-3	NR	3-8	5	6	2	0.9-7				
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR				
Hair, Noncoloring	NR	NR	NR	NR	NR	NR	NR	NR				
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR				
Nail	NR	NR	NR	NR	NR	NR	NR	NR				
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR				
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR				
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR				

NR - not reported to be used

Table 10. Current and Historical Frequency and Concentration of Use According to Duration and Type of Exposure—Previously Reviewed Esters

	Dibutyl Adipate						Diisopropyl Adipate					
	# of Uses			Conc. of Use (%)			# of Uses			Conc. of Use (%)		
data year	*1994*[85]	*2002*[5]	*2010*[20]	*1996*[85]	*2002*[5]	*2010*[23]	*1981*[2]	*2002*[4]	*2010*[20]	*1981*[2]	*2003*[4]	*2010*[23]
Totals	1	NR	6	NR	5-8	NR	112	66	70	≤0.1-25	0.01-15	0.005-8
Duration of Use												
Leave-On	*1*	*NR*	*6*	*NR*	*5-8*	*NR*	92	60	64	*≤0.1-25*	*0.01-15*	*0.005-8*
Rinse Off	*NR*	*NR*	*0*	*NR*	*NR*	*NR*	20	6	6	*≤0.1-26*	*001-8*	*2-7*
Exposure Type												
Eye Area	NR	NR	2	NR	NR	NR	2	NR	2	1-25	NR	1
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	1	NR	NR	NR
Inhalation	1	NR	2	NR	NR	NR	47	33	21	0.1-25	1-15	0.005-8
Dermal Contact	1	NR	3	NR	8	NR	102	62	50	≤0.1-25	0.01-15	0.005-8
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	6	NR	0.01	NR
Hair—Noncoloring	NR	NR	NR	NR	NR	NR	10	3	17	≤0.1-5	0.1-3	0.5-3
Hair—Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	1	NR	5	NR	NR	1	NR	NR	3	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	1	NR	NR	0.1-1	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	8	6	1	1-25	5-8	2
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Diethylhexyl Adipate					
	# of Uses			Conc. of Use (%)		
data year	*1981*[2]	*2002*[4]	*2010*[20]	*1981*[2]	*2003*[4]	*2010*[23]
Totals	27	49	48	≤0.1-25	0.4-38	0.6-14
Duration of Use						
Leave-On	*21*	*44*	*39*	*≤0.1-10*	*0.4-38*	*0.9-14*
Rinse Off	6	5	9	*1-25*	*NR*	*0.6*
Exposure Type						
Eye Area	NR	2	3	NR	0.4-2	NR
Possible Ingestion	5	1	1	1-5	NR	NR
Inhalation	6	5	5	1-5	NR	NR
Dermal Contact	25	47	43	≤0.1-25	0.4-38	0.6-14
Deodorant (underarm)	1	NR	NR	0.1-1	8	0.9
Hair—Noncoloring	NR	NR	1	NR	NR	NR
Hair—Coloring	NR	NR	NR	NR	NR	NR
Nail	2	2	4	1-5	NR	2-3
Mucous Membrane	NR	4	1	NR	NR	NR
Bath Products	4	NR	NR	10-25	NR	NR
Baby Products	NR	NR	1	NR	NR	NR

NR - not reported to be used

administration. Absorption was 15% in nude mice, 4 % in human skin grafted to nude mice, 6% in pig skin grafted to nude mice, 2.5% in pigs, and 4% in dogs.

In Vitro - Human

An in vitro skin absorption study was performed using diethyl malonate, no vehicle given.[39] Human cadaver split thickness skin was used in flow through cells. Diethyl malonate (4 µl) was applied to the skin samples. After 24 hours, 16% of the applied dose had penetrated through the skin. The maximum flux rate was reached after 5 hours and amounted to 280 µg/h (350 µg/cm^2/h); the mean penetration rate was 99 µg/h (120 µg/cm2/h). Much of the test substance, 45 to 50%, evaporated from the skin, and 34 to 39% remained on the skin.

Ditridecyl adipate. The percutaneous absorption of [^{14}C]ditridecyl adipate was determined using groups of 10 male and 10 female Sprague-Dawley rats that were untreated or that had previously been exposed to unoccluded dermal applications of 0 or 2000 mg/kg bw ditridecyl adipate, 5 days/week for 13 weeks.[92] (This study is described in the section on 'Subchronic Dermal Toxicity'). A single 58 µl dose of 2000 mg/kg bw [^{14}C]ditridecyl adipate was applied topically (size of test site not specified), and urine and feces were collected for 4 days. In the previously untreated rats, a total of 11.6 and 10.6% of the [^{14}C] solution was absorbed by male and female rats, respectively, over 4 days. Approximately 63 and 52% of the absorbed dose (7.4 and 5.5% of the applied dose, respectively) was found in the tissues of males and females, respectively. A total of 3.5% to 4.7% of the applied dose was recovered in the urine and 0.4% to 0.7% in the feces of previously untreated rats. The values for the animals previously dosed with 2000 mg/kg bw ditridecyl adipate were not statistically significantly different from the controls. In the previously dosed animals, a total of 10.8 and 9.1% of the dose

Table 11. Ingredients Not Reported to Be Used

Dicarboxylic Acids and Their Salts
Malonic Acid
Glutaric Acid
Disodium Azelate
Dipotassium Azelate
Disodium Sebacate
Dodecanedioic Acid
Esters of Dicarboxylic Acids
Decyl Succinate
Diethyl Succinate
Dicetearyl Succinate
Diisobutyl Succinate
Diisobutyl Glutarate
Diisostearyl Glutarate
Diethyl Adipate
Dipropyl Adipate
Di-C 12-15 Alkyl Adipate
Ditridecyl Adipate
Dicetyl Adipate
Diisooctyl Adipate
Diisononyl Adipate
Dihexyldecyl Adipate
Diisocetyl Adipate
Dibutyl Sebacate
Dicaprylyl/Capryl Sebacate
Dibutyloctyl Sebacate
Dihexyldecyl Sebacate
Diisostearyl Sebacate

was absorbed by males and females, respectively, over the 4 days, with approximately 87 and 81% of the absorbed dose (9.4 and 7.4% of the applied dose, respectively) found in the tissues of the male and female rats, respectively. A total of 0.7% to 1.3% of the [^{14}C] was recovered in the urine and 0.4% to 0.6% in the feces. Based on the radioactivity recovered in the urine, the bioavailability of ditridecyl adipate was 2% to 6%, and previous dosing did not significantly affect absorption.

Diethylhexyl Adipate In Vitro. The in vitro hydrolysis of diethylhexyl adipate (and mono-(2-ethylheyxl) adipate [MEHA]) using tissue preparations from the liver, pancreas, and small intestine of 2 rats was examined, as were the effects of diethylhexyl adipate on serum and hepatic enzymatic activities in vitro.[93] Diethylhexyl adipate was readily hydrolyzed to MEHA or adipic acid by each tissue preparation. The formation of adipic acid was rapid and approximately the same for all 3 tissues, while the formation of MEHA was rapid only in pancreatic tissue and was negligible in the intestine. The rate of hydrolysis from MEHA to adipic acid was greater that than from diethylhexyl adipate and the highest activity was found in intestinal tissue. In examining the effects on serum and hepatic enzymes, only N-demethylase activity was considerably inhibited by diethylhexyl adipate.

In Vivo—Nonhuman

The elimination, distribution, and metabolism of diethylhexyl adipate was investigated using male Wistar rats.[93] In these studies, diethylhexyl adipate was labeled at the carbonyl carbon. In elimination studies, 2 rats were dosed by gavage with 500 mg/kg bw [^{14}C]diethylhexyl adipate (1.26 μCi/rat) as a saturated solution in dimethyl sulfoxide (DMSO), and respired carbon dioxide, urine, and feces were collected for 2 days. At 24 hours after dosing, 86% of the administered dose was excreted, and at 48 hours, more than 98% of the dose was excreted. In 1 animal, 44.8% of the dose was excreted in expired carbon dioxide and 33.9% in the urine at the 48 hours measurement, while in the other rat, 21.1% and of the dose was excreted in expired carbon dioxide and 52.2% in the urine. Little (1.4 or 5% of the dose) was excreted in the feces.

In the distribution study, 3 rats per group were given a single dose as described above. The animals were killed at various intervals, and blood, organ, and tissue samples were collected. Not taking into account the stomach and intestines, the greatest levels of radioactivity, as a percent of dose administered, were found in the liver, kidney, blood, muscle, and adipose tissue. These values ranged from 0.34% to 8.21% at 6 hours, with the greatest percentage found in the adipose tissue, and from 0.54% to 3.44% at 12 hours, with the greatest percentage found in the muscle. In most tissues, the amount of residual radioactivity reached a peak by 6 hours, except for the liver, kidneys, testicles, and muscle, which reached a peak at 12 hours. The researchers stated that the elimination of radioactivity from the tissues and organs was very rapid, and there was no specific organ affinity.

The metabolism of diethylhexyl adipate was examined in rats dosed orally, by gavage, with 100 mg of nonlabeled diethylhexyl adipate as a 5% solution in DMSO. A control group was dosed with vehicle only. The rats were killed 1, 3, or 6 hours after dosing. The metabolites were determined using GLC. Diethylhexyl adipate was rapidly hydrolyzed to adipic acid, the main intermediate metabolite, and MEHA. In the urine, adipic acid was detected at 1 hours, and excretion as adipic acid in the urine reached 20% to 30% at 6 hours. Diethylhexyl adipate and MEHA were not detected in the urine. Adipic acid only also was detected in the blood and the liver, with constant excretion of 0.5% to 0.7% of the dose in the blood and excretion in the liver increasing with time, with 2% to 3.3% excreted in the liver at 6 hours. In the stomach, diethylhexyl adipate, adipic acid, and MEHA were found. The concentrations of diethylhexyl adipate declined rapidly, while the levels of adipic acid (9% to 10%) and MEHA (6% to 11.5%) peaked at 3 hours. Adipic acid, but not MEHA, was found in the intestine and increased with time, reaching 19% at 6 hours.

The absorption, distribution, and elimination of diethylhexyl adipate was examined using radioactive labeling on the acid [carbonyl-^{14}C] (specific activity 39.5 mCi/mmol) or the alcohol [2-ethylhexyl-1-^{14}C] (44.1 mCi/mmol).[34] The researchers used both DMSO and commercial corn oil as vehicles for all tests, since the were of the opinion that DMSO is an

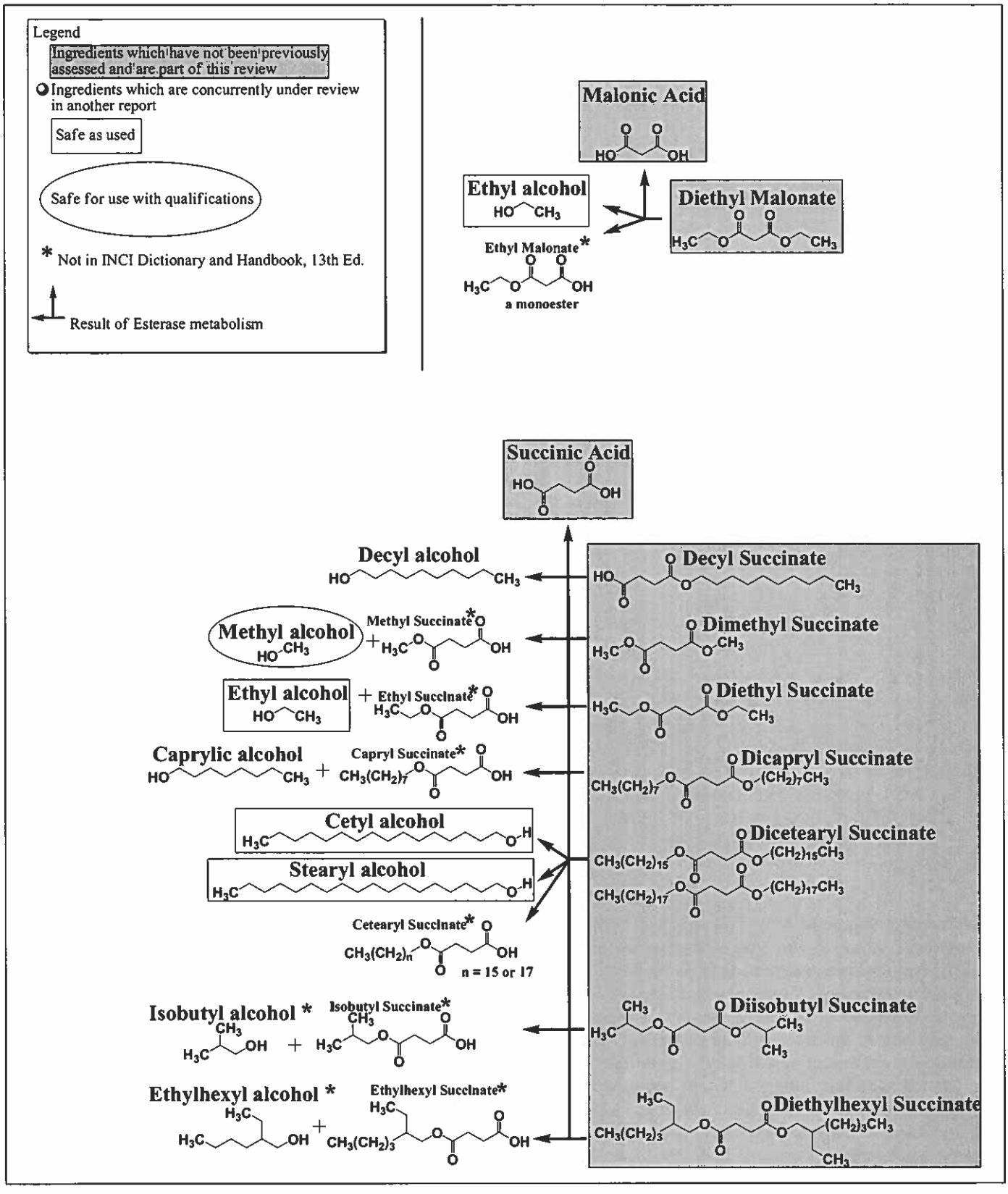


Figure 5A. Map of the malonic and succinic ester ingredients in this assessment and associated esterase metabolites.

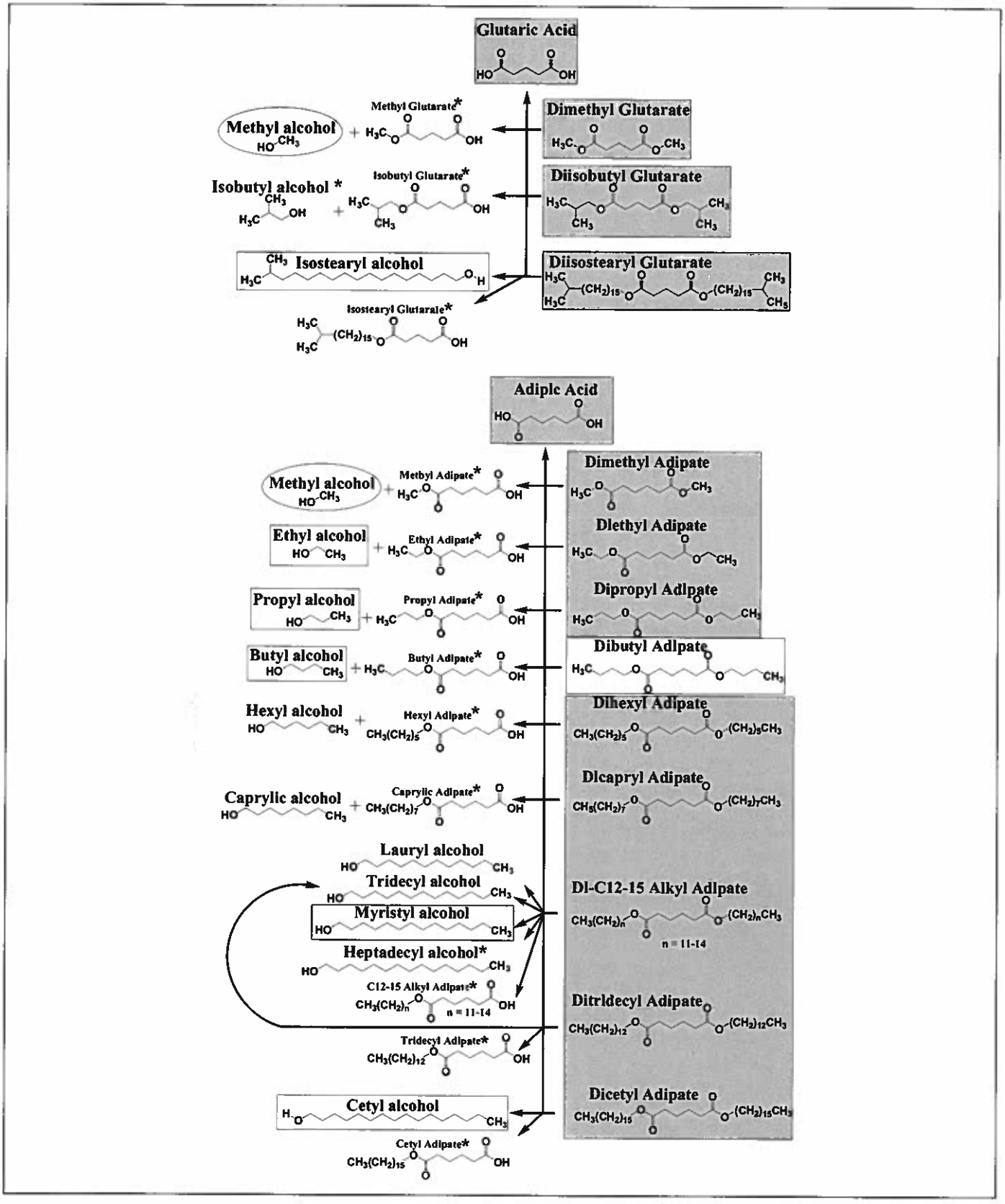


Figure 5B. Map of the glutaric and straight-chain adipic ester ingredients in this assessment and associated esterase metabolites.

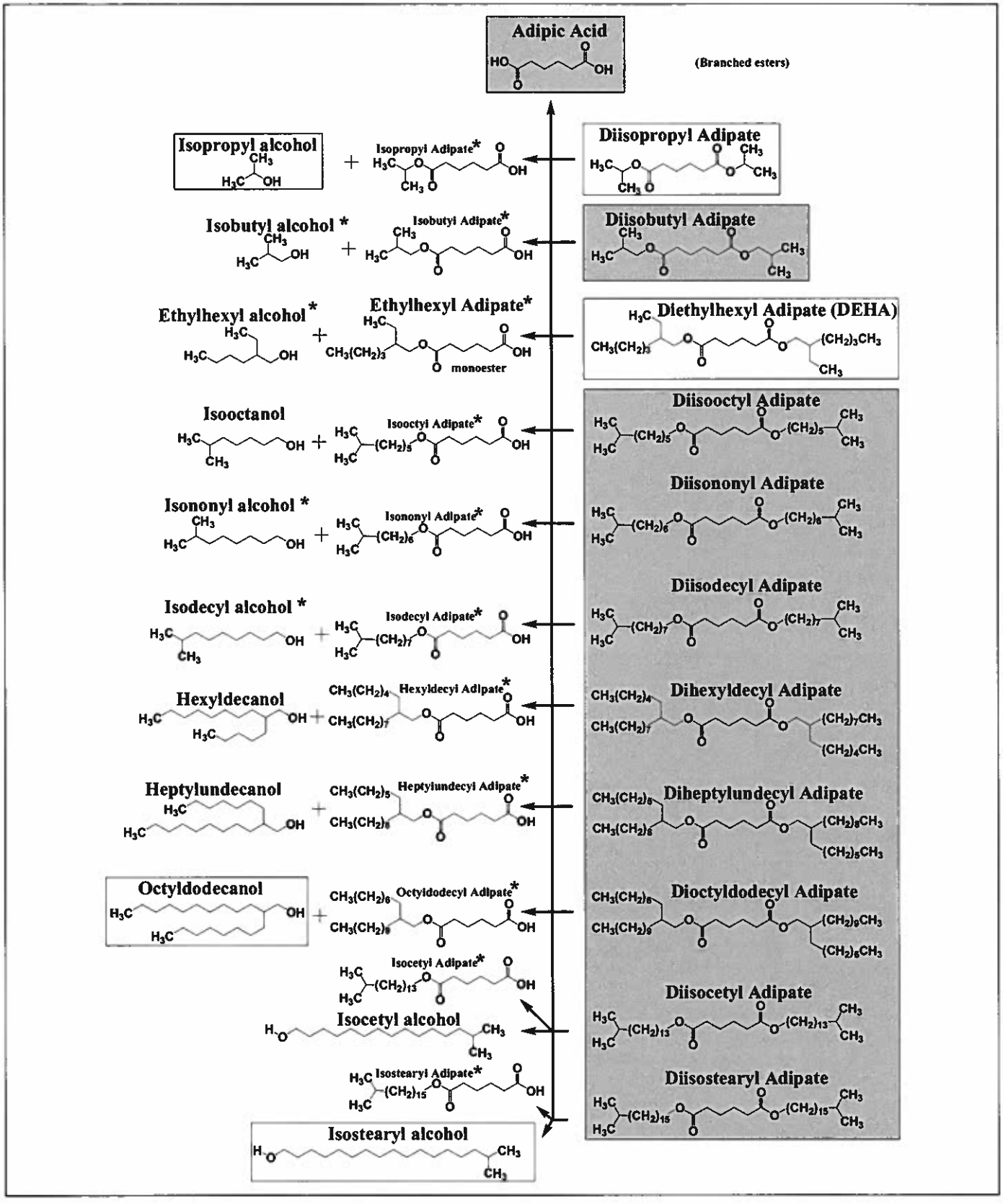


Figure 5C. Map of the branched chain adipic ester ingredients in this assessment and associated esterase metabolites.

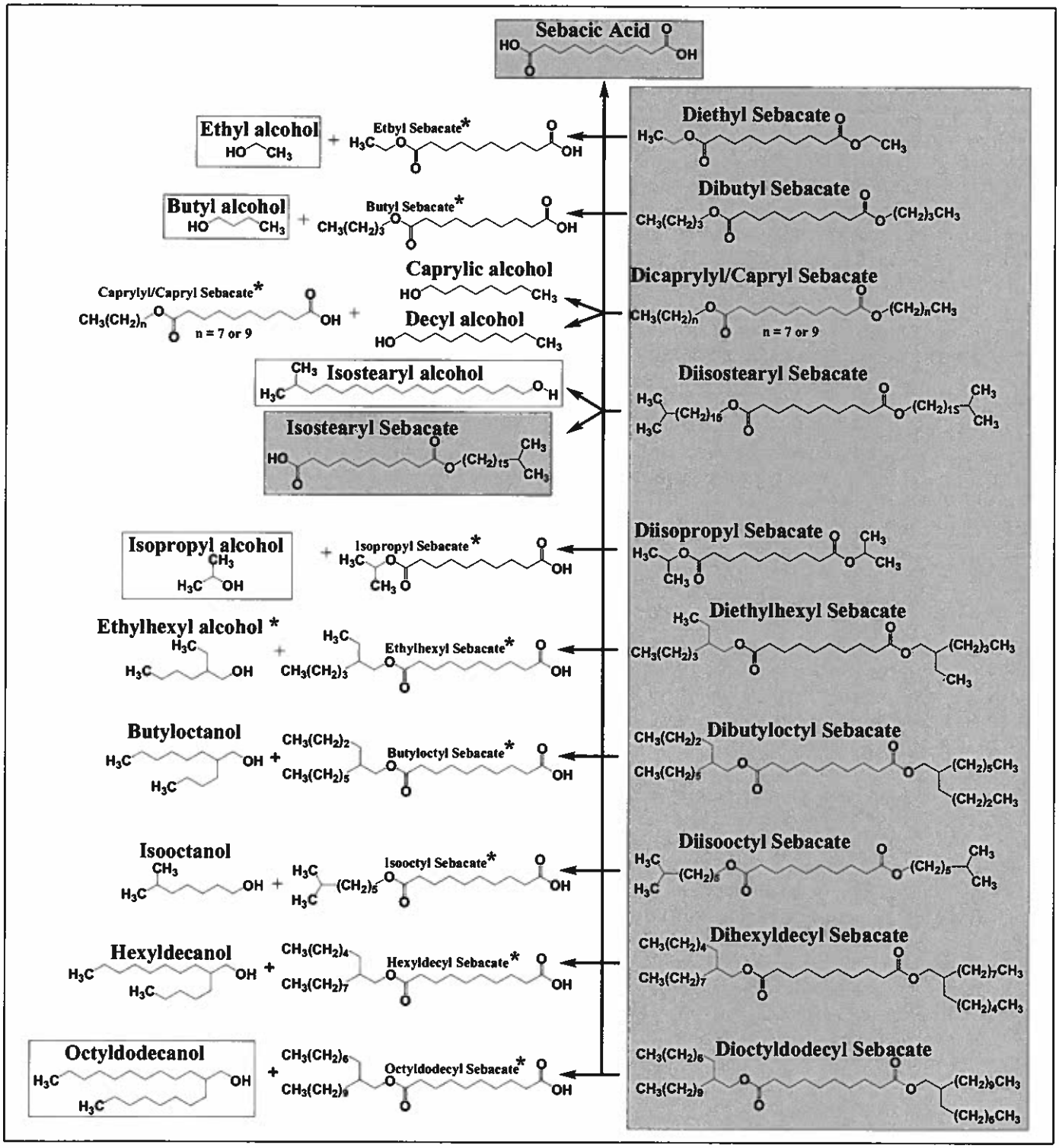


Figure 5D. Map of the sebacic and dodecanedioic ester ingredients in this assessment and associated esterase metabolites.

active penetrant and carrier of other substances through tissue membranes. It was also their opinion that a fat-soluble substance, such as diethylhexyl adipate, is more realistically studied dissolved in corn oil. The following groups of animals were dosed with 84.3 µg (9 µCi) [carbonyl ^{14}C]diethylhexyl adipate or 84.3 µg (10 µCi) [2-ethylhexyl-1-^{14}C]diethylhexyl adipate in both vehicles: 12 male NMRI mice were dosed iv and killed at intervals from 5 minutes to 4 days after dosing; 10 male NMRI mice were dosed intragastrically (i.g.) and killed at intervals from 20 minutes to 4 days after dosing; 12 gravid NMRI mice were dosed iv or i.g. on day 17 of gestation and killed at intervals from 20 minutes to 24 hours. Six male rats were dosed i.g. with 843 µg (90 µCi) [carbonyl ^{14}C]diethylhexyl adipate or 843 µg (100 µCi) [2-ethylhexyl-1-^{14}C]diethylhexyl adipate and killed at intervals from 20 minutes to 4 hours. Whole body autoradiography was used to determine tissue distribution.

Following dosing with [carbonyl-^{14}C]diethylhexyl adipate, distribution was similar in male mice, male rats, and gravid mice. The amount of radioactivity in the tissues peaked at a later time following i.g. dosing as compared to iv dosing. The presence of radioactivity in the gastrointestinal tract following i.v dosing indicated biliary excretion. Four hours following both iv and i.g. dosing, the greatest uptake of radioactivity was found in the liver, bone marrow, brown fat, adrenal cortex, kidneys, and a few other tissues. At 24 hours after i.g. dosing, significant levels of radioactivity remained in several tissues, including the liver, of both rats and mice. In gravid mice, a "remarkable strong uptake" of radioactivity in the corpora lutea of the ovary was observed at all time intervals with both iv and i.g. dosing, and some radioactivity was found in the fetal intestine, liver, and bone marrow.

Similar distribution patterns were seen following dosing with [2-ethylhexyl-1-^{14}C]diethylhexyl adipate as were seen with [carbonyl-^{14}C]diethylhexyl adipate. Following i.g. dosing, the appearance of radioactivity was lessened and not as great as it was with iv dosing. Very high radioactivity levels were seen in the liver and kidney at 5 minutes to 1 hour after iv dosing and at 20 minutes to 4 hours after i.g. dosing. The radioactivity in the liver was still high at 24 hours after i.g. dosing in mice and rats. Radioactivity was also seen in the intestinal contents at 1 to 4 hours after iv dosing, again indicating biliary excretion. At longer intervals after iv injection, 4 hours-4 days, radioactivity was detected in the bronchi of mice. While radioactivity was observed in the ovaries of gravid mice and some fetal tissues following dosing with [carbonyl-^{14}C]diethylhexyl adipate, none was detected in the ovaries of gravid mice after dosing with [2-ethylhexyl-1-^{14}C]diethylhexyl adipate, and very little radioactivity was seen in some fetal tissues.

The effect of vehicle on the absorption and biliary and urinary excretion of diethylhexyl adipate was also examined using rats in a gavage study with [^{14}C]diethylhexyl adipate. Radioactivity was measured every 30 minutes for 7.5 hours. The times and extent of absorption were different for all 4 preparations of [^{14}C]diethylhexyl adipate. Radioactivity levels in the blood increased faster and were greater with DMSO as the vehicle, as compared to corn oil. The highest blood radioactivity levels were found with [carbonyl-^{14}C]diethylhexyl adipate in DMSO. Biliary excretion of [^{14}C]diethylhexyl adipate was greatly affected by vehicle; with DMSO, 41% of the dose was detected in the bile, while only 10% of the dose was found with the corn oil vehicle. This difference was not seen with [carbonyl-^{14}C]diethylhexyl adipate. Finally, vehicle did not have much influence on urinary excretion. However, unlike the results reported by the previous researchers, little radioactivity was excreted in the urine. The researchers hypothesized that since the study duration was only 7.5 hours, urinary excretion may not have been complete.

The metabolism of diethylhexyl adipate was examined in vivo using male Wistar rats and compared to in vitro metabolism using hepatocytes.[94] In vivo, rats were dosed with 0.665 or 1.5 g/kg diethylhexyl adipate in corn oil by gavage for 5 days, and the controls were given vehicle only. Urine was collected daily. Diethylhexyl adipate was not recovered in the urine after 24 hours. Adipic acid was the main metabolite of diethylhexyl adipate. In vitro, the first hydrolysis of diethylhexyl adipate appears to be a rate-limiting step. In vivo, it was thought that this hydrolysis probably occurs in the gastrointestinal tract. Metabolic pathways (ω and ω-1oxidations, glucuronidation) seemed to prove that transformations of diethylhexyl adipate are localized mainly in the liver.

Oral administration of diethylhexyl adipate to cynomolgus monkeys results in rapid elimination, with 47% to 57% of the dose excreted in the urine.[17] Unchanged diethylhexyl adipate is absorbed from the gastrointestinal tract, and the glucuronide of MEHA and traces of unchanged diethylhexyl adipate were found in the urine. (Details were not provided).

In Vivo—Human

The pharmacokinetics of [$^{2}H_{10}$]diethylhexyl adipate, labeled on the ethyl side-chains, were examined using 6 male participants.[95] A dose of 46 mg [$^{2}H_{10}$]diethylhexyl adipate in corn oil, for a total volume of 0.5 cm^3, was administered orally in a gelatin capsule. Blood samples were taken for up to 31 hours after dosing, and urine samples were taken at intervals for up to 96 hours after dosing. In the plasma, unconjugated [$^{2}H_5$]2-ethylhexanoic acid was the only measurable diethylhexyl adipate-related compound. This compound appeared rapidly in the plasma, and the peak concentrations (1.6 $\pm$0.5 µg/cm^3) occurred between 1 and 2 hours. [$^{2}H_5$]2-Ethylhexanol was detected, but it was below the limit of quantification. The rate of metabolite formation was calculated, since there was no evidence of diethylhexyl adipate absorption, as 1.63 $\pm$ 1.19 hr^{-1}. The rate of elimination from the plasma was also rapid and estimated to be 0.42 $\pm$ 0.15/h, which corresponded to an elimination half-life of 1.65 hours. Although there were inter-individual differences in the rate and extent of [$^{2}H_5$]2-ethylhexanoic acid formation, it was below the limit of detection in all participants by 31 hours.

In the urine, [$^{2}H_5$]2-ethylhexanoic acid was again the principal metabolite, and it was probably eliminated as a

conjugated product. This conjugated form, most likely the glucuronide, accounted for up to 99% of the total [$^2H_5$]2 ethylhexanoic acid measured. Conjugation of the other urinary metabolites was minimal. Peak urinary elimination of the measured metabolites occurred within 8 hours of dosing, and no metabolites were detected in the urine after 36 hours. The rates of elimination were similar for all metabolites, with a mean elimination half-life of 1.5 hours. The measured urinary metabolites accounted for 12.1% of the dose, with the majority being eliminated in 24 hours. Fecal analysis determined that a minor portion of the dose was present as diethylhexyl adipate (0.43%) and [$^2H_5$]MEHA (0.27%). The researchers noted that recovery of the administered dose was incomplete and hypothesized that it was most probably due to further systemic metabolism.

Diethylhexyl sebacate. Diethylhexyl sebacate is not readily absorbed through the skin of guinea pigs (no further details were provided).[1] It was noted that the metabolism of diethylhexyl sebacate in rodents and humans may follow partially common pathways.

Toxicologic studies

Peroxisome Proliferation

Diethylhexyl adipate is a peroxisome proliferator requiring extensive phase I metabolism to produce the proximate peroxisome proliferator, which in both mice and rats, appears to be 2-ethylhexanoic acid.[96,97] Diethylhexyl adipate has been studied because it is structurally related to diethylhexyl phthalate, although diethylhexyl adipate is not as potent a proliferator as diethylhexyl phthalate.[98,99]

Peroxisome proliferation causes an increase in liver weights and can induce hepatocarcinogenicity in rats and mice. Peroxisome proliferation is not believed to pose the risk of inducing hepatocarcinogenesis in humans, as a species difference in response to peroxisome proliferators exists.[100] In vitro and in vivo studies examining the induction of peroxisome proliferation by diethylhexyl adipate and diethylhexyl sebacate are summarized in Table 12.[67,96-99,101,102] While proliferation was observed, these ingredients have much weaker activity than diethylhexyl phthalate and ciprofibrate, which are very effective peroxisome proliferators.

Humans do not react to peroxisome proliferators in the manner that rodents do.[100] There is no effect on organelle proliferation and induction of peroxisomal and microsomal fatty acid-oxidizing enzymes in species other than rats and mice, including humans. Consequently, these results have no relevance to humans.

DNA Binding/DNA Synthesis

Diethylhexyl adipate. The potential of diethylhexyl adipate to bind to liver DNA of female NMRI mice was evaluated by administering a solution of 119 mg diethylhexyl adipate/mL with 3.85 mCi/mL of [^{14}C]diethylhexyl adipate (labelled at C1 of the alcohol moiety) and 27.7 mCi/mL of [^{3}H]diethylhexyl adipate (tritiated at positions 2 and 3 of the alcohol moiety) in olive oil.[103] The animals were dosed by gavage, and the livers were excised 16 hours after dosing. Some animals were pretreated with 10 g/kg of unlabeled dietary diethylhexyl adipate for 4 weeks. Diethylhexyl adipate did not covalently bind to hepatic DNA in mice. Pretreatment with diethylhexyl adipate caused an increase in liver weight, but no increase in DNA binding. The researchers stated that tumorigenicity of diethylhexyl adipate must be due to an activity other than DNA binding.

The ability of diethylhexyl adipate to stimulate liver DNA synthesis in male F344 rats was investigated using radiolabeled thymidine.[104] Contrary to expected results, diethylhexyl adipate did stimulate DNA synthesis. The stimulation factor, which is indicated by the ratio of the thymidine incorporation in treated animals compared to controls, was 10.5 and the doubling dose, which is the dose that produced a doubling of the control level DNA synthesis, was 0.7 mmol/kg.

The effect of dosing with diethylhexyl adipate on 8-hydroxydeoxyguanosine (8-OH-dG) in liver and kidney DNA of rats was examined.[105] Groups of 10 male F344 rats were fed a diet containing 0 or 2.5 diethylhexyl adipate. Five animals per group were killed after 1 week, and the other 5 after 2 weeks of dosing. Relative liver to bws were statistically significantly increased after 1 and 2 weeks of dosing, and the relative kidney to bws were statistically significantly increased only after 2 weeks. A statistically significant increase in 8-OH-dG was observed in the liver DNA, but not the kidney DNA, at week 1 and 2.

The IARC remarked that the weight of evidence for diethylhexyl adipate, and other rodent peroxisome proliferators in general, demonstrated that rodent peroxisome proliferators do not act as direct DNA-damaging agents.[17]

Hepatic Lipid Metabolism

Diethylhexyl adipate. Dietary administration of diethylhexyl adipate affects hepatic lipid metabolism.[17] Hepatic fatty acid-binding protein and microsomal stearoyl-CoA desaturation were increased in Wistar rats fed 2% diethylhexyl adipate for 7 days.[106,107] When fed to rats for 14 days, an increase in hepatic phospholipid levels and a decrease in phosphatidylcholine:phosphatidylethanolamine ratio was reported.[108] In male NZB mice fed 2% diethylhexyl adipate for 5 days, an induction of fatty acid translocase, fatty acid transporter protein, and fatty acid binding protein in the liver was reported.[109]

Cellular Effects

Dibutyl adipate. Dibutyl adipate was tested for cytotoxicity in the metabolic inhibition test. A dilution series of dibutyl adipate was suspended in HeLa cells. Dibutyl adipate had no acute toxicity to the cells, which was attributed to its insolubility in water.[5]

Table 12. Induction of Peroxisome Proliferation—Esters of Dicarboxylic Acids

Test System/Procedure	Test Compound/Dose	Results/Observations	Reference
Diethylhexyl Adipate			
hepatocytes from male 5wiss mice and rats	diethylhexyl adipate (DEHA) 1° metabolites: MEHA; 2-ethylhexanol, 0.5 mmol/L	no peroxisome proliferation 5-fold induction of peroxisomal β-oxidation in mouse hepatocytes, as measured by cyanide-insensitive palmitoyl CoA oxidase (PCO) activity; 4-5 fold increase in rat hepatocytes	Cornu et al[96]
	2° metabolite: 2-ethylhexanoic acid, 1mM	25-fold induction of PCO activity in mouse hepatocytes;9-fold increase in rat hepatocytes; 2-ethylhexanoic acid was the proximate peroxisome proliferator	
	2° metabolite: 2-ethyl-5-hydroxy-1-oic acid, 2mM	S-fold stimulation of PCO	
cultured guinea pig hepatocytes	DEHA and metabolites, ≤2 mmol/L	did not stimulate PCO	Cornu et al[96]
cultured marmoset hepatocytes	DEHA and metabolites, ≤2 mmol/L	did not stimulate PCO	
male and female Wistar rats and Swiss mice, 5/gender/group; dosed orally by gavage for 14 days in corn oil	DEHA, 0-2.5 g/kg 2-ethylhexanol, 0-1.75 g/kg 2-ethylhexanoic acid, 0-1.0 g/kg	• relative liver to body weights increased dose-dependently • on a molar basis, DEHA was twice as potent as 2-ethylhexanol or 2-ethylhexanoic acid • peroxisomal β-oxidation was induced in a linear dose-response manner; • PCO was stimulated to the greatest effect in male mice • 2-ethylhexanoic acid was the primary proliferator	Keith et al[97]
male and female F344 rats or female B6C3F$_1$ mice, 5/gender/group; dosed orally by gavage for 14 days in corn oil	≤2.5 g/kg/day DEHA	• PCO activity was increased to the greatest extent, 15-fold, in male rats • dose-related peroxisome proliferation was statistically significantly increased in both rat and mice • relative liver weights were increased in a dose-dependent manner	Keith et al[97]
female F344 and B6C3F$_1$ mice, 5-8/group;dosed for 1, 4,or 13 weeks	0-4.0% DEHA in the diet (rats) 0%-2.5% DEHA in the diet (mice)	• PCO induction was markedly increased in rats and mice at all 3 time frames • microsomal cytochrome activity and stimulation of replicative DNA was significantly increased in mice, but not in rats	Lake et al[101]
male F344 rats and female B6C3F$_1$ mice, 5/group; 5 mL/kg for 14 days; route of administration not specified	0-2 g/kg DEHA	• PCO and catalase activity, but not glutathione activity, were statistically significantly increased • steady-state hydrogen peroxide activity increased 2-fold compared to controls	Tomaszewski et al[102]
F344 rats, 3-4/group; dietary administration, 30 days	0.25-2% DEHA 0.25-2% diethylhexyl phthalate 0.001-0.02% ciprofibrate (a very potent peroxisome proliferator)	• hepatomegalic potencies of diethylhexyl phthalate were 200 and of ciprofibrate were 1000—fold greater than DEHA • DEHA produced moderate peroxisome proliferation at 2%, but not at lower concentrations	Reddy et al[98]
rats, 2 males and 2 females/group; dietary administration, 21 days	≤2.5% DEHA	at 2.5%, peroxisome proliferation was markedly increased in males and moderately increased in females; overall, however, activity was weak	Barber et al[99]
Diethylhexyl Sebacate			
4 male F344 rats; dietary administration for 3 weeks	2% diethylhexyl sebacate	hepatic peroxisome proliferation was observed, evidenced by increased liver size, hepatic activities of peroxisome-associated enzymes, and hypolipidemia	Moody et al[67]

Single Dose (Acute) Toxicity

Acute toxicity data on esters of dicarboxylic acids esterase metabolites are presented in Table 13.

Repeated Dose Toxicity

Dibutyl Adipate

Oral. Male and female Crj:CD(SD) rats, number per group not specified, were dosed orally, by gavage, with 0, 20, 140, or 1000 mg/kg bw dibutyl adipate in olive oil daily for 28 days.[110] No clinical, hematological, or microscopic test-article related changes were observed.

Dermal. Groups of 10 rabbits were dosed dermally with 0.5 or 1.0 mL/kg/d of a 20% dispersion of dibutyl adipate, 5x/week for 6 weeks. A significant reduction in bw gain was seen for animals of the 1.0 mL/kg/d group, and renal lesions were seen in 1 animal of each group.[5]

No adverse effects were reported in a study using 4 dogs in which entire-body applications of an emulsion con×tain×ing 6.25% dibutyl adipate were made 2x/week for 3 mos.[5]

Diethylhexyl Adipate

Oral. In a 14-day dietary study, groups of 5 male rats and mice were given ≤50 000 ppm and groups of 5 female rats and mice were given ≤100,000 ppm diethylhexyl adipate. Male rats and mice fed 50 000 ppm and female rats and mice fed ≥25,000 ppm had decreased weight gains or weight loss. (It is not specified whether the results were statistically significant.) One female rat and all female mice of the 100 000 ppm group died.[2]

In a 14-day study in which 5 male and 5 female Wistar and F344 rats and Swiss and B6C3F$_1$ mice were dosed with 0 to 2.5 g/kg diethylhexyl adipate in corn oil for 14 days, diethylhexyl adipate was toxic to female B6C3F$_1$ mice, causing mortality, at a dose level of 2.5 g/kg.[97] The toxicity of 2 metabolites of diethylhexyl adipate, 2-ethylhexanol and 2-ethylhexanoic acid, was also examined using Wistar rats and Swiss mice. 2-Ethylhexanol was toxic to male and female rats, with mortality reported at doses >1.05 g/kg in male and female rats. 2-Ethylhexanoic acid was toxic to female rats, with mortality reported at doses ≥1.9 g/kg; mortality was not reported for male rats. These effects were not reported in mice.

In a 1 and 4-week dietary study in which groups of 5 to 8 rats and mice were fed diets containing 0% to 4.0% and 0% to 2.5% diethylhexyl adipate, respectively, feed consumption by rats was decreased in the 4.0% group at 1 week and in the 2.5 and 4.0% dose groups at 4 weeks.[101] Body weights were significantly decreased in these groups. Feed consumption by mice was not affected, but a significant decrease in bws was seen in the 1.2 and 2.5% dose groups at 4 weeks.

Toxicity was evaluated in a study in which groups of 10 female Crl:CD(SD) rats were dosed, by gavage, with 5 mL/kg of 0, 200, 1000, or 2000 mg/kg bw diethylhexyl adipate in corn oil for 2 or 4 weeks.[125] All animals survived until study termination. In the 2-week study, no statistically significant findings were observed for the animals dosed with 200 mg/kg bw, and the only statistically significant finding in the 1000 mg/kg bw dose group was an increase in relative liver to bw. In the 2000 mg/kg bw dose group, there was staining around the perineum, statistically significant increases in relative liver and kidney to bws, and a statistically significant decrease in the relative weight of the left ovary. Microscopically, abnormal findings were reported for both the ovary and kidney. In the ovary, an increase in atresia of the large follicle and a decrease in currently formed corpora lutea were seen in animals dosed with 1000 and 2000 mg/kg bw, and in the 2000 mg/kg bw group, an increase in follicular cysts was observed. In the kidney, an eosinophilic change of the proximal tubule was observed for the 2000 mg/kg bw dose group. The NOAEL was 200 mg/kg bw.

In the rats dosed for 4 weeks, similar observations were made. There was staining around the perineum of animals dosed with 1000 and 2000 mg/kg bw diethylhexyl adipate, and final bws of animals dosed with 2000 mg/kg bw were statistically significantly decreased. The relative kidney to bws were statistically significantly increased in animals at all dose levels, and liver weights were statistically significantly increased in animals of the 1000 and 2000 mg/kg bw dose groups. The mean estrous cycle length was statistically significantly decreased in the 200 mg/kg bw dose group, but this was not considered treatment-related since a dose-response was not seen. The same microscopic abnormalities reported in the 2 weeks study were seen in the ovaries and kidneys of the animals dosed with 1000 and 2000 mg/kg bw in the 4-week study. As in the 2-week study, the NOAEL for ovarian toxicity was 200 mg/kg bw.

In a 13-week dietary study, groups of 10 rats and 10 mice were fed ≤25 000 ppm diethylhexyl adipate. With the exception of decreased weight gain for some of the groups, no compound-related toxicologic effects were observed.[2]

In a 90-day dietary study, groups of 10 rats per group were fed 0 to 4740 mg/kg bw diethylhexyl adipate for 90 days.[113] Mortality occurred in the 4740 mg/kg bw group, but the number of deaths was not specified. Decreased growth and feed consumption was reported for animals fed 2920 mg/kg bw. Changes in kidney and liver weights were noted, but no details were given. The NOEL was 610 mg/kg bw, and the LOEL was 2920 mg/kg bw diethylhexyl adipate.

Groups of 15 male and 15 female Sprague Dawley rats were fed 0 or 2.5% diethylhexyl adipate for 90 days.[92] At study termination, all animals were killed for necropsy. Body weight gains were statistically significantly decreased for treated males and females, and relative kidney and liver to bws were statistically significantly increased for treated females, when compared to controls.

In a 13-week dietary study in which groups of 5 to 8 rats and mice were fed diets containing 0% to 4.0% and 0% to 2.5% diethylhexyl adipate, feed consumption by rats was decreased in the 2.5 and 4.0% dose groups, and bws were significantly reduced in these groups.[101] Feed consumption by mice was not affected, but a significant decrease in bws was seen in the 1.2 and 2.5% dose groups.

Table 13. Single Dose (Acute) Toxicity—Esters of Dicarboxylic Acids

Animals	No./Gender/Group	Dose	median lethal dose/ concentration, or result	Reference
ORAL				
Diethyl Malonate				
rats	not specified	not specified	15 000 mg/kg	39
Dimethyl Malonate				
rats	not specified	not specified	>2000 mg/kg	39
Diethyl Succinate				
rats	not specified	not specified	8530 mg/kg	111
Dibutyl Adipate				
rats	*not specified*	*20% dispersion*	*11 260-12 900 mg/kg*	5
rats	*not specified*	*undiluted*	*1520 mg/kg*	5
rats	not specified	not specified	1290 mg/kg	110
rats	not specified	undiluted	12 900 mg/kg	92
Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid				
Sprague-Dawley rats	5-10; males/females	2000-15 800 mg/kg, undiluted	greater than highest dose tested	92
Ditridecyl Adipate				
Sherman Wistar rats	5/gender	16 000 mg/kg	greater than highest dose tested	92
Wistar rats	5/gender	15 000 mg/kg	greater than highest dose tested	92
Diisopropyl Adipate				
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 1.08%*	*1 female died*	2
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 1.08%*	*no animals died*	2
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 5%*	*no animals died*	2
[2]rats	*5 males/f5 females*	*formulation containing 0.7%*	*>76 800 mg/kg*	2
rats	*not specified*	*formulation containing 20.75%*	*>15 000 mg/kg*	2
Diisobutyl Adipate				
NMRI mice	5 males	2000 mg/kg	greater than highest dose tested	112
Diethylhexyl Adipate				
mice	*5 males/5 females*	*≤20 000 mg/kg in corn oil*	*males: 15 000 mg/kg; females: 24 600 mg/kg*	2
rats	*5 males/5 females*	*≤20 000 mg/kg, undiluted*	*2 males of the 10 000 mg/kg group died; 1 male and 1 female of the 20 000 mg/kg group died*	2
albino rats	*5 males/5 females*	*7400 mg/kg*	*1 animal died*	2
rats	*not specified*	*not specified*	*single oral toxic dose—9.11 g/kg*	2
rats	*not specified*	*not specified*	*no-effect dose: 6000 mg/kg; central nervous system effects seen at higher concentrations*	2
Harlan-Wistar rats	*5 males/5 females*	*formulations containing 0.175%*	*>6500 mg/kg*	2
rats	not specified	not specified	9110 mg/kg	113
rats	5 males/females	7380 mg/kg, undiluted	>7300 mg/kg	92
rats	not specified	not specified	9.1 g/kg	92
Diisooctyl Adipate				
rats	5/group	2000-64 000 mg/kg, undiluted	greater than highest dose tested	92
guinea pigs	not specified	not specified	>5 mL/kg	92
Diisononyl Adipate				
rats	5/group	346-10 000 mg/kg, undiluted	greater than highest dose tested	92
Diisodecyl Adipate				
NMRI mice	5 male	2000 mg/kg	greater than highest dose tested	114
rats	not specified	undiluted	20 500 mg/kg	92
Dioctyldodecyl Adipate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	115
rats	not specified	not specified	NOAEL <4000 mg/kg	35
Diisocetyl Adipate				
NMRI mice	5 males	2000 mg/kg	greater than highest dose tested	116
Diisopropyl Sebacate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	117
Diethylhexyl Sebacate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	118
mice	not specified	undiluted	9.5 g/kg	92

(continued)

Table 13. (continued)

Animals	No./Gender/Group	Dose	median lethal dose/ concentration, or result	Reference
rats	not specified	undiluted	5.0 cc/kg	92
rats	not specified	undiluted	12.8 g/kg	92
rats	not specified	undiluted	17 g/kg	92
Dioctyldodecyl Dodecanedioate				
Wistar rats	5 male/5 female	5000 mg/kg	greater than highest dose tested	119
Diisocetyl Dodecanedioate				
Wistar rats	5 male/5 female	5000 mg/kg	greater than highest dose tested	120
Esterase Metabolites (summary information/results only provided)				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats			1516-7000 mg/kg	121
mice			2500-3768 mg/kg	121
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats			3131-4900 mg/kg	121
mice			103-1950 mg/kg	121
Butyloctanol (metabolite of dibutyloctyl sebacate)				
rats			12 900 mg/kg	111
Decyl Alcohol (metabolite of decyl succinate)				
rats			9800 mg/kg	111
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)				
rats		mixture of C7-9 branched alkyl alcohols	>2000 mg/kg	121
Nonyl Alcohol (metabolite of diisononyl adipate)				
rats		mixture of C8-10 branched alkyl alcohols	3000 mg/kg	121
Isodecyl Alcohol (metabolite of diisodecyl adipate)				
rats		mixture of C9-11 branched alkyl alcohols	4600 mg/kg	121
DERMAL				
Diethyl Malonate				
rabbits	not specified	not specified	16 700 mg/kg	39
Dibutyl Adipate				
rabbits	*not specified*	*96%*	*20 mL/kg*	5
rats	not specified	i.m.	NOAEL >8000 mg/kg	122
Ditridecyl Adipate				
rabbits	3	2000 mg/kg	greater than highest dose tested	92
rabbits	10	5000 m/kg to abraded skin; semi-occlusive	greater than highest dose tested	92
Diethylhexyl Adipate				
rabbits	8	*≤8700 m/kg to abraded skin; occlusive*	*mild irritation; no systemic toxic effects*	2
rabbits	1 male/1 female	≤8660 mg/kg for 24 hours, occluded, 1 intact and 1 abraded site	>8670 mg/kg	113
Diisononyl Adipate				
rabbits	4/group	50-3160 mg/kg to abraded skin	greater than highest dose tested	92
Diethylhexyl Sebacate				
guinea pigs	not specified	not specified	<10 000 mg/kg	1
Dioctyldodecyl Dodecanedioate				
NZW rabbits	5 male/5 female	2000 mg/kg, intact skin, 24 hours occlusive	>2000 mg/kg	123
Esterase Metabolites (summary information/results only provided)				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats			>3000 mg/kg	121
rabbits			1980-2600 mg/kg	121
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats			1500 mg/kg	121
rabbits			1500 - >500 mg/kg	121

(continued)

Table 13. (continued)

Animals	No./Gender/Group	Dose	median lethal dose/ concentration, or result	Reference
Butyloctanol (metabolite af dibutyloctyl sebacate)				
rabbits			3.36 mL/kg	111
Decyl Alcohal (metabolite of decyl succinate)				
rabbits			3.5 mL/kg	111
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)				
rats		mixture of C7-9 branched alkyl alcohols	>2600 mg/kg	121
Nonyl Alcohol (metabolite of diisononyl adipate)				
rats		mixture of C8-10 branched alkyl alcohols	3160 mg/kg	121
Isodecyl Alcohol (metabolite of diisodecyl adipate)				
rats		mixture of C9-11 branched alkyl alcohols	>2600 mg/kg	121
INHALATION				
Diethyl Malonate				
rats	not specified	concentrated vapors for 8 hours	no deaths	39
Diethyl Succinate				
rats	not specified	concentrated vapors for 8 hours	no deaths	111
Dibutyl Adipate				
albino rats	*6 male*	*flowing stream of saturated air, 8* hours	*no mortality*	2
Diethylhexyl Adipate				
rats	not specified	concentrated vapors for 8 hours	no deaths	111
Diethylhexyl Sebacate				
rats	not specified	250 mg/m^3 for 4 hours	no effect on lung or liver	1
rats	3	saturated vapor, 6 hours	no lung toxicity	1
rats	4	940 mg/m^3, 7 hours	3 rats died, may be attributable to thermal decomp products	1
guinea pigs	2	940 mg/m^3, 7 hours	no animals died	1
rabbits	4	940 mg/m^3, 7 hours	2 rabbits died, may be attributable to thermal decomp products	1
Esterase Metabolites (generally, summary information/results only provided)				
Ethylhexyl Alcohol (metabolite af diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats	3 males/3 females	vapor conc. of 0.89 mg/L or aerosol/ vapor conc of 5.3 mg/ L, 4 hours	0.89 mg/L: all animals survived; 5.3 mg/L: all animals died	121
mice, rats, and guinea pigs	10	227 ppm, 6 hours	all animals survived	121
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats		21 mg/L, 1 hours	greater than highest dose tested	121
Butyloctanol (metabolite af dibutyloctyl sebacate)				
rats		concentrated vapors for 8 hours	no deaths	111
Decyl Alcohal (metabolite af decyl succinate)				
rats		concentrated vapors for 8 hours	no deaths	111
PARENTERAL				
Dimethyl Adipate				
Sprague-Dawley rats	not specified	ip	1.8 mL/kg	124
Diethyl Adipate				
Sprague-Dawley rats	not specified	ip	2.5 mL/kg	124
Dipropyl Adipate				
Sprague-Dawley rats	not specified	ip	3.8 mL/kg	124
Dibutyl Adipate				
rats	not specified	ip	5.2 mL/kg	5
Diisopropyl Adipate				
rats	not specified	iv	640 mg/kg	2
Diethylhexyl Adipate				
rats	not specified	iv	900 mg/kg	2
rabbits	not specified	iv	540 mg/kg	2
Sprague-Dawley rats	not specified	ip	>50 mL/kg	124

Intragastric doses of ≤2.0 g/kg diethylhexyl adipate to rats (number not stated) for 6 mos produced no enzymatic changes, but levels of sulphydryl compounds in the blood were increased. Hepatic detoxification appeared depressed at the onset of the study, but it was accelerated after 6 mos. Administration of 0.1 g/kg for 10 mos decreased central nervous system excitability.[2]

Dermal

Diethylhexyl adipate, 410 or 2060 mg/kg bw, was applied to the shaved abdomen of male rabbits, 4 per group, 5 days per week for 2 weeks.[113] Mineral oil was applied in the same manner to a group of 4 rabbits as a negative control. A collar was used to restrict ingestion. One animal in the 410 mg/kg bw group died during week 2 of the study. All other animals in this group appeared normal. Slight to moderate erythema was observed at the test site. No animals of the 2060 mg/kg bw group died, but 3 of the 4 did not gain weight, and they had labored breathing and were lethargic during week 2. Moderate erythema was observed in this group. Microscopically, 1 animal of the 2060 mg/kg bw group had altered cytology of the liver parenchymal cells. No other microscopic lesions were noted.

Diisopropyl adipate. An immersion study was performed using guinea pigs in which a product containing 20.75% diisopropyl adipate was diluted, giving an actual adipate concentration of 0.10%. The animals were immersed 4 h/d for 3 days. There were no signs of systemic toxicity, and the degree of dermal irritation was considered minimal.[2]

Diethylhexyl sebacate. No deaths occurred when 4 rats, 2 guinea pigs, 2 rabbits and 1 cat were exposed to 400 mg diethylhexyl sebacate/m^3, 7 h/d, for 10 days.[1] Details were not provided.

Groups of 12 F344 rats, gender not specified, were exposed 4 hours/d, 5 days/week, to 25 or 250 mg/m^3 diethylhexyl sebacate for ≤13 weeks.[92] No adverse systemic or lung effects were observed.

Diethyl malonate. Groups of 10 to 16 male and female CD rats were fed diets containing either 0, 36 (males) or 41 mg/kg bw/d (females) diethyl malonate for 90 days.[39] No treatment related effects were observed.

Di-C7-9 branched and linear alkyl esters of adipic acid. Groups of 15 male and 15 female Sprague-Dawley rats were fed a diet containing 0, 0.1, 0.5, or 2.5% di-C7-9 branched and linear alkyl esters of adipic acid for 90 days, corresponding to approximately 1500 mg/kg bw/d for high-dose males and 1900 mg/kg bw/d for high-dose females.[92] All rats were killed for necropsy at study termination. No systemic toxicity was reported. Small, but significant, increases in absolute and relative kidney to bws reported for females of the 2.5% dose group were not considered treatment-related. The NOAELs for male and female rats were 1500 and 1950 mg/kg bw/d, respectively.

Diisononyl adipate. Groups of 10 male and 10 female rats were fed 0, 50, 150, or 500 mg/kg bw diisononyl adipate for 13 weeks.[92] A statistically significant increase in relative kidney to bws was reported for males and females given 500 mg/kg bw, but absolute kidney weights were not affected and no significant microscopic effects were seen. Microscopic changes in any of the organs, including the testes and epididymis of males and ovaries of females, were not observed. There were no significant toxicological findings, and the NOAEL was 500 mg/kg bw/d.

In another 13-week study, groups of 4 male and 4 female Beagle dogs were fed 0, 0.3, 1.0, or 3.0% diisononyl adipate; the high dose was increased to 6% during weeks 9 to 13.[92] No significant findings were reported for the 0.3 or 1.0% groups. In the high-dose group, decreased bws, testes weight, and feed consumption, increased liver weights, elevated enzyme levels, liver and kidney discoloration, and microscopic changes in the liver, testes, spleen, and kidneys were reported. The dietary NOAEL for diisononyl adipate was 1.0%.

Ditridecyl adipate. Ditridecyl adipate, 0, 800, or 2000 mg/kg bw, was applied to the backs of groups of 10 male and 10 female Sprague-Dawley rats, 5 days/week for 13 weeks.[92] The test sites were not occluded, but the animals wore Elizabethan collars. Slight erythema and flaking of the skin was observed in the treated groups, with hyperplasia of the sebaceous glands in the dermis, but otherwise no significant differences were observed between test and control animals. Differences in relative organ to bws were not statistically significant, and ditridecyl adipate did not appear to cause systemic toxicity.

Dibutyl sebacate. Groups of 5 male and 5 female Sprague-Dawley rats were fed a diet containing 0, 0.01, 0.05, 0.25, or 1.25% dibutyl sebacate for 1 year.[122] Necropsies were performed whenever rats exhibited significant weight losses or other evidences of severe concurrent infection. Dibutyl sebacate had no effect on growth or well-being.

The researches then fed groups of 16 male Sprague Dawley rats a diet containing 0.01, 0.05, 0.25, 1.25, or 6.25% dibutyl sebacate for 2 years.[122] Two control groups were given untreated feed. Necropsies were performed on 3 rats from each group after 1yr, and the experiment was terminated at the end of the 2-year feeding period. Interim, animals were killed whenever they became moribund. In such instances the rats usually had incapacitating tumors or severe intercurrent infections. Dibutyl sebacate did not adversely affect growth or survival, and it did not produce significant hematological changes in peripheral blood. As the rats increased in age, slight changes in distribution of leukocytes were found, but these trends occurred in both the control and treatment groups.

Ocular Irritation

Ocular irritation data on esters of dicarboxylic acids and esterase metabolites are presented in Table 14.

Table 14. Ocular Irritation—Esters of Dicarboxylic Acids[a]

Concentration	Animals/System	Procedure	Results	Reference
Diethyl Malonate				
undiluted	rabbits, no./gender not specified	0.1 mL	slight to moderate irritation	39
Dimethyl Malonate				
undiluted	rabbits, no./gender not specified	0.1 mL,unrinsed	slight to moderate irritation; cleared by day 8	39
Dibutyl Adipate				
undiluted	*rabbits, no. not specified*	*unrinsed*	*minimally irritating*	5
undiluted	*2 New Zealand rabbits*	*unrinsed*	*slight irritation*	5
0.1% in olive oil	*rabbits*	*unrinsed*	*nonirritating*	5
Diisopropyl Adipate				
undiluted	*6 albino rabbits*	*0.1 mL, unrinsed*	*negligible irritation*	2
undiluted	*6 albino rabbits*	*0.1 mL, unrinsed*	*nonirritating*	2
0.7% in formulation	*9 albino rabbits*	*0.1 mi, undiluted, rinsed*	*some corneal stippling*	2
5% in formulation	*6 albino rabbits*	*not specified*	*nonirritating*	2
20.75% in formulation	*6 albino rabbits*	*not specified*	*nonirritating*	2
undiluted	3 albino rabbits	0.1 mL, unrinsed	nonirritating	112
Diethylhexyl Adipate				
undiluted	*6 albino rabbits*	*0.1 mL, unrinsed*	*nonirritating*	2
0.01% in formulation	*6 albino rabbits*	*0.1 mL, unrinsed*	*nonirritating*	2
0.175% in formulation	*6 albino rabbits*	*0.1 mL, unrinsed*	*mild transient irritant*	2
Diisopropyl Sebacate				
	6 rabbits	0.1 mL, unrinsed	minimally irritating	126
Diethylhexyl Sebacate				
1.2% in formulation	EpiOcular MTT viability assay	undiluted	nonirritating	127
Dioctyldodecyl Dodeconedioate				
undiluted	6 rabbits	0.1 mL, unrinsed	MMTS = 0.0; nonirritating	129
Diisocetyl Dodecanedioate				
undiluted	6 rabbits	0.1 mL, unrinsed	MMTS = 0.0; nonirritating	128
Esterase Metabolites (generally, summary information/results only provided)				
Ethylhexyl Alcohol (metobolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
	rabbits	20 µg	moderately severe corneal irritation	121
Isopropyl Alcohol (metabolite of diisopropyl adipate and diisopropyl sebocate)				
	rabbits		severely irritating	130
Hexyl Alcohol (metabolite of dihexyl adipate)				
	rabbits		highly irritating	121

[a]Data from original safety assessments are in italics.

Diethyl malonate. The ocular irritation potential of diethyl malonate was evaluated using rabbits, number and gender not specified.[39] A volume of 0.1 mL was instilled into the conjunctival sac of 1 eye, which was not rinsed, and the contralateral eye was untreated and served as the negative control. Diethyl malonate produced slight to moderate irritation.

In a similar study, undiluted dimethyl malonate produced slight to moderate irritation in rabbit eyes.[39] All signs of irritation were cleared by day 8.

Dibutyl Adipate Nonhuman. Undiluted dibutyl adipate was minimally irritating to the eyes of rabbits, and 0.1% in olive oil was nonirritating.[5]

Human. Dibutyl adipate, 0.1% in paraffin oil, was not an ocular irritant in 2 participants.[5]

Diisopropyl Adipate. The ocular irritation potential of 2 lots of undiluted diisopropyl adipate was evaluated using rabbits. One caused negligible irritation, while the other was nonirritating. A formulation containing 0.7% diisopropyl adipate produced some corneal stippling in rabbit eyes, while a formulation containing 5.0% and 1 containing 20.75% were nonirritating to rabbit eyes.[2]

The ocular irritation of undiluted diisopropyl adipate was evaluated using 3 albino rabbits.[112] A volume of 0.1 mL was instilled into the conjunctival sac of 1 eye, which was not rinsed. The contralateral eye was untreated and served as the negative control. Diisopropyl adipate was not irritating.

Diethylhexyl adipate. Undiluted diethylhexyl adipate was nonirritating to rabbit eyes and a formulation containing 0.0175% was, at most, a mild transient irritant.[2]

Diisopropyl Sebacate. A primary ocular irritation study was performed using 6 New Zealand white rabbits to determine the ocular irritation potential of diisopropyl sebacate.[126] A volume of 0.1 mL was instilled into 1 eye of each animal, which was not rinsed, and the contralateral eye of each animal served as the control. The average Draize scores were 2.0 at 24 and 48 hours, 0.3 at 72 hours, and 0.0 at 4 days. Diisopropyl sebacate was a minimal ocular irritant.

Diethylhexyl sebacate. The ocular irritation of a cream containing 1.2% diethylhexyl sebacate was evaluated using the in vitro EpiOcular MTT viability assay.[127] The tissue samples were exposed to undiluted test material for 64 minutes, 256 minutes, or 1200 minutes. Following treatment, the viability of those tissues were calculated. The ET_{50} (time for tissue viability to be reduced by 50%) was 484.9 minutes, and diethylhexyl sebacate was considered to be nonirritating.

Dioctyldodecyl dodecanedioate. The primary eye irritation of dioctyldodecyl dodecanedioate was evaluated using 6 albino rabbits.[119] A volume of 0.1 mL was instilled into 1 eye of each animal, which was not rinsed. and the contralateral eye served as a negative control. They eyes were evaluated at 24, 48, and 72 hours. At 24 hours, the maximum mean total score (MMTS) was 0.00, and dioctyldodecyl dodecanedioate was considered not irritating.

Diisocetyl dodecanedioate. The primary eye irritation of diisocetyl dodecanedioate was evaluated using the procedure described above.[128] The MMTS was 0.00, and diisocetyl dodecanedioate was considered not irritating to the eyes of rabbits.

Comedogenicity

Dibutyl adipate. Dibutyl adipate, 10% to 100% (vehicle not stated), was not comedogenic in clinical testing.[5]

Dermal Irritation/Sensitization

Nonhuman dermal irritation and sensitization data on esters of dicarboxylic acids and esterase metabolites are presented in Table 15. Human dermal irritation and sensitization data on esters of dicarboxylic acids and esterase metabolites are presented in Table 16.

Dimethyl Malonate Nonhuman. Dimethyl malonate was applied undiluted to rabbit skin for 4 hours under a semi-occlusive patch.[39] Slight erythema was observed only at 30 to 60 minutes after patch removal, and dimethyl malonate was considered nonirritating to rabbit skin.

Dimethyl malonate was not a sensitizer in a Buehler guinea pig sensitization test according to OECD TG 406.[39]

Human. The sensitization potential of 8% dimethyl malonate in petrolatum was evaluated in a maximization test using 25 participants.[39] Dimethyl malonate was not a sensitizer.

Diethyl Malonate. The dermal irritation potential of diethyl malonate was evaluated using a 24 hours occlusive application.[39] Diethyl malonate was slightly irritating to rabbit skin.

Dibutyl Adipate Nonhuman. Application of undiluted butyl adipate to rabbit skin resulted in a primary irritation score of 2/8. Undiluted dibutyl adipate caused moderate erythema in rabbits following repeated dermal exposure. However, material impregnated with dibutyl adipate was not irritating to the skin of rabbits. Application of dibutyl adipate at 10% in acetone produced no observable adverse effect when applied to rabbit ears, and no dermal reaction was observed following twice daily application for 14 days to the backs of hairless mice. Two perfume formulations containing 1.1% diisopropyl adipate were not primary dermal irritants using rabbits[5]

Dibutyl adipate was not a dermal sensitizer in guinea pigs when tested at 25% in a maximization test.[5]

Human. Undiluted dibutyl adipate was not irritating in a 24-hr clinical patch test with 10 participants. Slight reactions (not defined) were reported for 4 of 18 participants in a 24-hour patch test with dibutyl adipate, 20% in alcohol.[5]

Diisopropyl Adipate Nonhuman. Draize tests of undiluted diisopropyl adipate resulted in, at most, mild irritation of rabbit skin. In Draize tests with formulations containing 5.0% or 20.75% diisopropyl adipate, minimal irritation was reported with both formulations.[2]

Human. The dermal irritation and sensitization of diisopropyl adipate was evaluated in a number of studies. Undiluted diisopropyl adipate produced no irritation in 4 hours patch tests, but was moderately irritating in a 21-day cumulative irritancy test. Formulations containing 0.26% to 20.75% diisopropyl adipate caused minimal to mild irritation, but no sensitization.[2]

Diethylhexyl Adipate Nonhuman. Undiluted diethylhexyl adipate was a very mild irritant when applied under occlusion to intact and abraded rabbit skin. A formulation containing 0.175% diethylhexyl adipate had an irritation index of 1.6/4.[2]

Diethylhexyl adipate, 0.1%, was not a sensitizer in a maximization study using guinea pigs.[2]

Human. The dermal irritation and sensitization of diethylhexyl adipate was evaluated in a number of studies with formulations containing 0.01% to 9% diethylhexyl adipate. Mild reactions were seen with a formulations containing 0.01%. Using a formulation containing 0.7%, on participant reacted strongly following the second challenge, with erythema and papules observed. Strong reactions were seen for 3 participants in a patch test of a formulation containing 9.0% diethylhexyl adipate.[2]

Diisodecyl Adipate, Dioctyldodecyl Adipate, Diisocetyl Adipate. The dermal irritation potential of diisodecyl adipate, dioctyldodecyl adipate and diisocetyl adipate was determined using

Table 15. Dermal Irritation and Sensitization—Esters of Dicarboxylic Acids[a]

Dose/Conc.	Animals	Procedure	Results	Reference
DERMAL IRRITATION				
Diethyl Malonate				
not specified	rabbits	occlusive application; 4 hours	slightly irritating	39
Dimethyl Malonate				
not specified	rabbits	semi-occlusive application; 4 hours	not irritating; slight erythema at 30-60 minutes after patch removal	39
Dibutyl Adipate				
undiluted	rabbits	applied to belly	PII of 2/8	5
undiluted	5 albino rabbits	0.1 mL, applied 8x in 4 hours	moderate erythema at 24 hours	
undiluted	3 rabbits	impregnated bands, 3 d application, 3 weeks	moderate erythema	5
undiluted	5 rabbits	impregnated bands, applied 2w/ week for 6 applications	no progressive skin damage	5
undiluted	3 rabbits	0.025 mL to intact and abraded skin, 3 applications at 3 hours intervals for 3 days	erythema and capillary injection during the study; desquamation was observed	5
10% in acetone	5 hairless mice	applied to ear, 1x/day, 10 days	no adverse effect	5
10% in acetone	mice	application to backs, 2x/day, 14 days	no adverse effect	5
Diisopropyl Adipate				
undiluted	9 albino rabbits	24 hours, 0.1 mL, occlusive	PII of 1.6/4; mild irritant	2
undiluted	9 albino rabbits	24 hours, 0.1 mL, occlusive	PII of 1.3/4; mild irritant	2
undiluted	9 albino rabbits	24 hours, 0.1 mL, occlusive	PII of 0.06/4; minimally irritating	2
5% in formulation	9 albino rabbits	24 hours, 0.1 mL, occlusive	PII of 0.33; minimally irritating	2
20.75% in formulation	9 albino rabbits	24 hours, 0.1 mL, occlusive	PII of 0.11; minimally irritating	2
undiluted	3 albino rabbits	semi-occlusive application; 4 hours, undiluted	nonirritating	112
Diethylhexyl Adipate				
undiluted	6 albino rabbits	intact and abraded skin, 0.5 mL, 24 hours, occlusive	very mild irritant	2
0.175% in farmulation	3 albino rabbits	4, 0.5 mL applications	irritation index of 1.6/4	2
Diisodecyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 hours, undiluted	nonirritating; scores of 0-1 for erythema and 0 for edema at 1-72 hours; reversible	114
Dioctyldodecyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 hours, undiluted	nonirritating; scores of 0-1 for erythema and 0 or 1 for edema at 24-72 hours; reversible	115
Diisocetyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 hours, undiluted	nonirritating; scores of 0-2 for erythema and 0 or 1 for edema at 1-72 hours; reversible	116
Diethyl Sebacate				
undiluted	8 rabbits	intact and abraded skin, occlusive, 0.3 mL	PII of 0.0	117
30% in ethanol	8 rabbits	intact and abraded skin, occlusive, 0.3 mL	PII of 0.3	117
Diisopropyl Sebacate				
undiluted	6 rabbits	intact and abraded skin, occlusive, 0.5 mL	PII of 2.88; not a primary irritant	126
undiluted	3 albino rabbits	semi-occlusive application, 4 hours, undiluted	nonirritating; scores of 1 for erythema, with a 2 at 24 hours, and 0 or 1 for edema at 1-72 hours; reversible	117

(continued)

Table 15. (continued)

Dose/Conc.	Animals	Procedure	Results	Reference
Diethylhexyl Sebacate				
undiluted	3 albino rabbits	semi-occlusive application, 4 hours, undiluted	nonirritating; scores of 1 for erythema and 0 for edema at 1-72 hours; reversible	118
undiluted	2-4 rabbits	occlusive application; 48 hourshours	not irritating	
Dioctyldodecyl Dodecanedioate				
undiluted	6 NZW rabbits	occlusive application, 24 hours, 0.5 mL	PII = 0; not a primary irritant	131
Diisocetyl Dodecanedioate				
undiluted	6 NZW rabbits	occlusive application, 24 hours, 0.5 mL	PII = 0; not a primary irritant	132
Esterase Metabolites				
Ethylhexyl Alcohol (metabolite of diethylhexyl adipate and diethylhexyl sebacate)				
	3 male rabbits	occlusion, 4 hours	irritating	121
	rabbits	occlusive, 0.5 mL	highly irritating; not reversible	121
Caprylic Alcohol (metabolite of dicapryl succinate, dicapryl adipate, and dicaprylyl/capryl sebacate)				
undiluted	rabbits		mild irritation	133
SENSITIZATION				
Dimethyl Malonate				
not specified	guinea pigs	Buehler method	not sensitizing	39
Dibutyl Adipate				
25%	*5 guinea pigs*	*maximization test*	*not sensitizing*	5
Diethylhexyl Adipate				
0.1% in olive oil	*10 male guinea pigs*	*induction: 10 injections; 2 weeks nontreatment pd; challenge: 0.05 mL injection*	*not sensitizing*	2
Diethylhexyl Sebacate				
undiluted	rabbits	occlusive patches, details not provided	no reactions	1
Dioctyldodecyl Dodecanedioate				
0.1 mL for intraderm induction; 0.5 mL top. induction /challenge	10 female guinea pigs	maximization test	not sensitizing; slight erythema at induction	134
Esterase Metabolites				
Hexyl Alcohol (metabolite of dihexyl adipate)				
1% in petrolatum	guinea pigs	maximization test	not sensitizing	121

[a]Data from original safety assessments are in italics.

3 albino rabbits.[114-116] Undiluted test material was applied to the skin for 4 hours under a semi-occlusive patch. The erythema scores for each of the 3 materials were 0 to 1 during 1 to 72 hours, and the edema scores were 0. Diisodecyl adipate, dioctyldodecyl adipate and diisocetyl adipate were considered nonirritating to rabbit skin.

Diisostearyl Adipate. A human repeat insult patch test (HRIPT) using 50 participants was used to evaluate the irritation and sensitization potential of diisostearyl adipate.[135] Two-tenths mL was applied neat to the back of each participant under an occlusive patch for 24 hours, after which time the participant removed the patch. This procedure was performed 3 times per week for 3 weeks, for a total of 9 induction patches. Following a 10 to 14 day nontreatment period, a 24-hour challenge patch was applied to a previously untreated site, and reactions were scored at 24 and 48 hours. No adverse reactions were observed, and diisostearyl adipate was not a primary irritant or a sensitizer.

Diethyl Sebacate Nonhuman. Undiluted diethyl sebacate and 30% diethyl sebacate in ethanol were tested on 8 male Japanese White strain rabbits (gender not specified).[117] The flank of the animals was clipped free of hair 1 day prior to application of test substance. The skin of 4 animals was abraded. The test substance, 0.3 mL, was applied occlusively to the back of all animals for 24 hours. The skin reactions were evaluated at 24 hours and 72 hours. The primary irritation score was 0.0 (none to weak irritant) in undiluted diethyl sebacate and 0.3 (none to weak irritant) in 30% diethyl sebacate. These results suggest that 100% diethyl sebacate has no primary skin irritation under these test conditions.

Table 16. Clinical Dermal Irritation and Sensitization—Esters of Dicarboxylic Acids[a]

Test Material	No. of Participants	Procedure	Results	Reference
Dimethyl malonate				
8% in petrolatum	25	Maximization test	not a sensitizer	39
Dibutyl Adipate				
undiluted	10	24 hours patch test	no irritation at 24 or 48 hours	5
20% in alcohol	10	24 hours occlusive patch test	slight reactions in 4 participants	5
Diisopropyl Adipate				
undiluted	19	24 hours occlusive patch, 0.1 mL	no irritation	2
undiluted	19	24 hours occlusive patch, 0.1 mL	no irritotion	2
undiluted	15	24 hours occlusive patch, 0.1 mL	no irritotion	2
undiluted	15	24 hours occlusive patch, 0.1 mL	no irritotion	2
undiluted	16	cumulotive irritancy test	moderately irritating; score of 395/945; irritation in 14/16 participants on doy 6	2
0.7% in formulation	13	cumulative irritancy test	nonirritating; score of 2/630	2
1.1% in formulation	17	cumulative irritancy test	low potential for hazard to consumer; score of 0.29/84	2
1.1% in formulation	17	cumulotive irritancy test	low potential for hazard to consumer; score of 0.24/84	2
20.75% in a bath oil	7	cumulative irritancy test	score of 8/84	2
20.75% in formulation diluted to 1.25%	19	24 hours occlusive patch, 0.1 mL	minimal irritation	2
5.0% in formulation	19	24 hours occlusive patch, 0.1 mL	no irritation	2
1.08% in formulation	235	HRIPT	no sensitization; slight hyperpigmentation	2
3.0% in formulation	50	HRIPT	no irritation or sensitization	2
5.0% in formulation	108	HRIPT	no irritation or sensitization	2
5.0% aqueous dispersion of a product containing 20.75%	116	HRIPT	minimol, faint erythema produced throughout the study	2
0.7% in formulation	25	maximization test	no contact sensitization potentiol	2
Diethylhexyl Adipate				
0.175% in formulation	11	cumulative irritancy test	slightly irritating; score of 72/630	2
0.01% in formulotion	100	Schwartz-Peck prophetic patch test	not an irritont or a sensitizer	2
0.01% in formulation	49	Shelanski and Shelanski HRIPT	weak reactions in up to 4 porticipants and strong reactions in 1 participant	2
9.0% in formulation	209	modified Draize-Shelanski patch test	3 strong reactions and 1 faint reaction at 2nd challenge	2
9.0% in formulation	151	modified Draize-Shelanski patch test	irritant reactions in 2 porticipants; no sensitization	2
product containing 0.7% of a 25% solution	not given	Shelanski-Jordan RIPT	1-2 participants had reoctions during the study	2
Diisostearyl Adipate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	135
1.5% in formulation	20	SIOPT	not irritating	136
1.5% in formulation	25	maximization test	no contact sensitization potential	137
Diisopropyl Sebacate				
1.8% in formulation	20	SIOPT	not irritating	138
undiluted	105	patch test	no irritation or sensitization	139
2.2% in formulation	27	maximization test	no irritation or sensitization	140
1% in formulation	110	modified HRIPT, semi-occlusive	not an irritant or a sensitizer	141
1% in formulation	110	modified HRIPT, semi-occlusive	not an irritant or a sensitizer	141
7.2% in formulation	51	HRIPT, semi-occlusive	no skin reactivity observed	142
Diethyl Sebacate				
1.5% in formulation	20	SIOPT	nonirritating; PII of 0.00	136
1.5% in formulation	25	maximization test	not sensitizing	138

(continued)

Table 16. (continued)

Test Material	No. of Participants	Procedure	Results	Reference
Diethylhexyl Sebacate				
undiluted	15-30	occlusive patches	no reactions	1
Dioctyldodecyl Dodecanedioate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	135
Diisocetyl Dodecanedioate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	135
Esterase Metobolites				
Methanol (metabolite of dimethyl succinate, dimethyl glutarate, and dimethyl adipate				
			primary irritation of the skin	143
3.2%	274	provocative occupational study	positive results	143
5%		closed patch test	slight positive reaction (+)	143
7 and 70%		closed patch test	+++ reactions	143
Propyl Alcohol				
undiluted	20	24 hours patch test	no reactions	144
undiluted	116	48 hours patch test	no reactions	145
undiluted	16	24 hours patch test	no reactions	146
undiluted	42	48 hours patch test	no reactions	147
undiluted	16	24 hours patch test	no reactions	148
undiluted	7	24 hours patch test	no reactions	90
Isopropyl Alcohol (metabolite of diisopropyl alcohol and diisopropyl sebacate)				
80.74% spray concentration	9		no sensitization potential	149
2.85% in formulation	109	HRIPT	no sensitization	150
undiluted	12	24 hours patch test	no reactions	151
Cetyl Alcahol (metabolite of dicetyl succinate and dicetyl adipate)				
11.5% in formulation	80	topical tolerance study	reaction in 1 participant	152
6.0% in formulation	12	cumulative irritancy test	mild cumulative irritation	152
8.4% in formulation	110	HRIPT	not a primary irritant or sensitizer	152
3.0% in formulation	25	HRIPT	not a sensitizer	152
Myristyl Alcohol (metabolite of dimyristyl adipate)				
0.80% in formulation	53	4 weeks application	no irritation	152
0.25% in formulation	51	4 weeks application	1 reaction by 1 participant	152
0.25% in formulation	229	10 - 24 hours occlusive patch	not an irritant or an allergen	152
Stearyl Alcohol ((metabolite of distearyl succinate)				
undiluted		5IOPT	mild irritation	153
Isostearyl Alcohol (metabolite of diisostearyl glutarate, diisostearyl adipate, or diisostearyl sebacate)				
25% in petrolatum	19		no irritation	152
25.0% in formulation			no irritation	152
27.0% in formulation			no irritation	152
28.0% in formulation			no irritation	152
25% in 95% isopropyl alcohol	12	HRIPT	3 participant slight erythema at induction; no sensitization	152
5% in formulation	148	HRIPT, with rechallenge for reactors; add'l challenge with 5% in ethanol	12 participants had possible sensitization reactions at 1st challenge; 6 reacted at rechallenge; all 6 had positive reactions to 5% in alcohol	152
5% in formulation	60	HRIPT, rechallenge of 5% in ethanol for reactors	5 participants reacted at 1 challenge1/5 rechallenged reacted	152
Caprylic Alcohol (metobolite of dicopryl succinate, dicapryl adipate, and dicaprylyl/capryl sebacate)				
2% in petrolatum	25	48 hours closed patch	no irritation	133
Decyl Alcohol (metabolite of decyl succinate and didecyl sebacate)				
3% in petrolatum	25	48 hours closed patch	no irritation	133

[a]Data from original safety assessments are in italics.

Human. A single insult occlusive patch test (SIOPT) was performed using 20 participants to determine the irritation potential of a body cream containing 1.5% diethyl sebacate.[136] The test patch was applied for 24 hours. The PII was 0.00, and the body cream containing 1.5% diethyl sebacate was nonirritating.

The sensitization potential of a body cream containing 1.5% diethyl sebacate was evaluated in a maximization study.[137] During induction, 0.05 mL of 0.25% aqueous sodium lauryl sulfate (SLS) was applied under an occlusive patch for 24 hours. At that time, the patch was removed and 0.05 mL of the test material was applied to the same site under an occlusive patch for 48 to 72 hours. If no irritation was present at the test site upon patch removal, an occlusive patch with 0.25% aqueous SLS was applied for 24 hours, followed by a patch of the test material. This sequence was used for 5 induction patches. If irritation developed during induction, the SLS patch was eliminated. After a 10-day nontreatment period, a challenge was performed at a previously untreated site. The challenge site was pretreated with 0.05 mL of 5.0% aqueous SLS under an occlusive patch for 1 hour, followed by an occlusive patch of the test material for 48 hours. Twenty-five participants completed the study. No reactions were seen at challenge, and a body cream containing 1.5% diethyl sebacate did not have contact-sensitizing potential.

A number of investigators have reported cases of allergic contact dermatitis in response to diethyl sebacate-containing products, and have demonstrated diethyl sebacate to be the substance, or 1 of several substances in the products, eliciting the dermatitis.[31,154-158]

Diisopropyl Sebacate Nonhuman. A primary dermal irritation study on diisopropyl sebacate was performed using 6 New Zealand white rabbits.[126] A dermal application of 0.5 mL of undiluted test material was applied to an abraded and an intact site on each animal. The test sites were occluded for 24 hours and observed individually for erythema, edema, and other effects 24 and 72 hours after application. Mean scores from the 24 and 72 hours reading were averaged to give a primary irritation index (PII) of 2.88. Diisopropyl sebacate was not considered a primary dermal irritant.

The dermal irritation potential of diisopropyl sebacate was determined using 3 albino rabbits.[117] Undiluted test material was applied to the skin for 4 hours under a semi-occlusive patch. The erythema scores were 1 during 1 to 72 hours, and the edema scores were 0 to 1. Diisopropyl sebacate was considered nonirritating to rabbit skin.

Human. An SIOPT was performed using 20 participants to determine the irritation potential of a foundation containing 1.8% diisopropyl sebacate.[139] The patch was applied for 24 hour. The foundation containing 1.8% diisopropyl sebacate was not irritating.

The irritation and sensitization potential of diisopropyl sebacate was evaluated in a patch test that consisted of four 24-hour applications of diisopropyl sebacate as supplied (approximately 100%) during weeks 1, 2, 3, and 6 on a 2 cm x2 cm area of skin on the right upper arm of each participant.[139] Examinations were performed immediately after patch removal. The induction phase was performed during weeks 1 to 4 using 107 participants. No clinically significant effects were detected on any of the participants during this phase. During week 6, the challenge phase was conducted on 105 participants. No clinically significant effects were noted in any of the participants during this phase. Diisopropyl sebacate was not observed to have any significant skin-irritating or sensitizing activity under the conditions of this study.

A maximization assay was performed, using a modified protocol of the maximization assay procedure described earlier, to determine the contact-sensitization potential of a foundation containing 2.2% diisopropyl sebacate.[140] In this study, the test material was allowed to volatilize for 30 minutes before the occlusive patch was applied. Twenty-five participants completed the study. No reactions were seen at challenge, and a foundation containing 2.2% diisopropyl sebacate did not have contact-sensitizing potential.

Two heat protection hair spray products containing 1% diisopropyl sebacate were tested using a modified Draize HRIPT procedure to determine the potential of those products to induce irritation and contact sensitization.[141] The products were tested neat and allowed to volatilize prior to patch application. Samples were patched under semi-occlusive conditions. Approximately 0.2ml was used in each patch. One hundred ten participants completed the study. Generally transient, barely perceptible (0.5-level) to mild (1-level) patch test responses on 22 test participants for 1 formulation and only barely perceptible (0.5-level) patch test response on 15 test participants with the other formulation during the induction and/or challenge phases of the study were reported. Both products were considered to be nonirritating and nonsensitizing.

A heat protection hair spray product containing 7.2% diisopropyl sebacate was tested using an HRIPT to determine the potential of this product to induce irritation and contact sensitization.[142] The product was tested neat under semi-occlusive conditions. Approximately 0.2 mL sample was used in each patch. Fifty-one participants completed the study. No skin reactivity was observed in any of the test participants during the course of the study.

Two case studies were reported of allergic reactions to lotion containing diisopropyl sebacate.[159,160]

Diethylhexyl Sebacate Nonhuman. The dermal irritation potential of diethylhexyl sebacate was evaluated using the same procedure.[118] The erythema scores were 1 during 1 to 72 hours, and the edema scores were 0. Diethylhexyl sebacate was considered nonirritating to rabbit skin.

Patch tests with diethylhexyl sebacate (neat; 48-hr occluded) did not irritate the skin of 2 to 4 rabbits.[1] It was also reported that diethylhexyl sebacate was nonirritating to the skin of guinea pigs. No further study details were provided.

A limited attempt was made to sensitize a group of 2 to 4 rabbits by applying diethylhexyl sebacate using occlusive patches.[1] No reactions were seen in an occlusive challenge

with the undiluted test article 2 weeks later. Details were not provided.

Human. Diethylhexyl sebacate was applied neat using occlusive patches to the skin of 15 to 30 participants (sex not specified) for 48-hour.[1] No local reactions were observed in the challenge phase (48-hour covered contact with neat liquid) that was carried out 2 weeks later, presumably due to limited induction.

In a case study where 1 patient was sensitized to other sebacate esters, a patch test with diethylhexyl sebacate did not elicit a response.[159]

Dioctyldodecyl Dodecanedioate Nonhuman. A maximization test was performed to evaluate the sensitization potential of dioctyldodecyl dodecanedioate.[134] Ten female guinea pigs were used. The dose used at intradermal injection was 0.1 mL, and 0.5 mL was used for the topical challenge. Slight erythema was observed at induction, but a sensitization reaction was not observed.

Human

Dioctyldodecyl Dodecanedioate. An HRIPT with 50 participants was performed to evaluate the irritation and sensitization potential of dioctyldodecyl dodecanedioate.[135] Two-tenths milliliter of the test material, neat, was applied to the back of each participant under an occlusive patch for 24 hours, after which time the participant removed the patch. This procedure was performed 3 times per week for 3 weeks, for a total of 9 induction patches. Following a 10- to 14-day nontreatment period, a 24 hours challenge patch was applied to a previously untreated site, and reactions were scored at 24 and 48 hours. No adverse reactions were observed, and dioctyldodecyl dodecanedioate was not a primary irritant or a sensitizer.

Diisocetyl dodecanedioate. An HRIPT with 50 participants was performed as described above to evaluate the irritation and sensitization potential of diisocetyl dodecanedioate.[135] No adverse reactions were observed, and diisocetyl dodecanedioate was not a primary irritant or a sensitizer.

Phototoxicity

Dibutyl adipate. Dibutyl adipate, as a 10% dilution in liquid paraffin, was not phototoxic in a clinical phototoxicity study using 30 participants.[5]

Diethylhexyl adipate. In a photopatch test on 9.0% diethylhexyl adipate using 25 participants, no phototoxic or photoallergic reactions were observed.[2]

Diisopropyl Adipate

Nonhuman. Two perfume formulations containing 1.1% diisopropyl adipate were not phototoxic in rabbits.[2]

Human. In photopatch test studies using 49 to 98 participants, formulations containing 0.7% to 17.0% diisopropyl adipate were not phototoxic, primary irritants, or sensitizers.[2]

Mucous Membrane Irritation

Diethylhexyl adipate. A product containing 0.175% diethylhexyl adipate did not produce irritation of the genital mucosa in rabbits.[2]

Reproductive and Developmental Toxicity

Dimethyl Malonate. Groups of 10 male and 10 female Wistar rats were dosed with 0, 100, 300, or 1000 mg/kg bw dimethyl malonate orally, by gavage.[39] Males were dosed for 2 weeks prior to mating, during mating, and 2 weeks after mating, for a total of 39 doses. Females were dosed 2 weeks prior to mating, during mating, and through day 4 of lactation. A recovery group of 5 male and 5 female high-dose animals were observed for 14 days after the termination of dosing. Microscopically, the incidence of treatment-related hepatocellular hypertrophy of the liver was observed for males and females given 1000 mg/kg bw dimethyl malonate. This effect was not observed in the recovery animals or in the other test groups. No other significant toxicological effects were observed. Performance in a functional observation battery was similar for test and control animals. There was no effect on fertility. In the 100 mg/kg bw group, a statistically significant decrease in the number of live pups was due to an increase in post-implantation loss. This effect was not considered treatment related, and no developmental toxicity was reported. The NOAEL was 300 mg/kg bw for repeated doses and maternal toxicity and 1000 mg/kg bw for fertility and developmental toxicity.

Dimethyl Adipate. Groups of 5 gravid Sprague Dawley rats were dosed ip with 0.0603 to 0.6028 mL/kg dimethyl adipate (1/30, 1/10, 1/5, and 1/3 of the ip LD_{50} value) on days 5, 10, and 15 of gestation.[124] A pooled volume control consisted of animals dosed with 10 mL/kg distilled water, saline, or cottonseed oil. A positive control group was not used. All animals were killed and examined on day 20 of gestation. The mean fetal weights and the numbers of live fetuses were not statistically significantly different between treated and blunt-needle control groups. Resorptions in animals dosed with 0.1809 mL/kg were statistically significantly increased when compared to the pooled controls, but not the blunt-needle controls. Gross and skeletal abnormalities, but not visceral, were statistically significantly increased in fetuses of the 0.3617 and 0.6028 mL/kg groups. The NOEL was 0.0603 mL/kg dimethyl adipate.

Diethyl Adipate. Following the same procedure described above, rats were dosed ip with 0.0837 to 0.8373 mL/kg diethyl adipate.[124] The mean fetal weight and the number of live fetuses were not statistically significantly different between treated and blunt-needle control groups, and the number of resorptions was similar between treated animals and both the blunt needle and pooled controls. There were no differences in

the incidences of gross, skeletal, or visceral abnormalities in fetuses of the treated groups compared to pooled controls.

Dipropyl Adipate. Following the same procedure described above, rats were dosed ip with 0.1262 to 1.2619 mL/kg dipropyl adipate.[124] The numbers of live and dead fetuses were not statistically significantly different between treated and blunt-needle control groups, but there was a statistically significant decrease in the mean fetal weight of the 0.7572 mL/kg group. Resorptions in animals dosed with 1.2619 mL/kg were statistically significantly increased when compared to the pooled controls, but not the blunt-needle controls. Gross abnormalities, but not skeletal or visceral, were statistically significantly increased in fetuses of the 1.2619 mL/kg group. The NOEL was 0.1262 mL/kg dipropyl adipate.

Dibutyl Adipate. Groups of 5 gravid Sprague Dawley rats were dosed ip with 0.1748 to 1.7480 mL/kg dibutyl adipate on days 5, 10, and 15 of gestation. The incidence of gross abnormalities was only statistically significantly increased in the high-dose group when compared to pooled controls.[5]

Dibutyl adipate was evaluated in a study Sprague-Dawley rats.[110] Groups of 13 male and 13 female rats were dosed with 0, 100, 300, or 1000 mg/kg bw dibutyl adipate orally, by gavage, for 14 days prior to mating through parturition; males were dosed for a total of 42 days and female dams were dosed until day 3 of lactation. The test article had no effect on fertility. Body weight gains of males of the 1000 mg/kg bw group were slightly decreased. Kidney weights of the high-dose males and females sere increased compared to controls. No gross or microscopic effects were noted at necropsy, and the internal genitalia were normal. Dosing with dibutyl adipate did not produce any reproductive effects. The only effect on the offspring was a decrease in pup weight on post-natal days 0 and 4 and in viability on post-natal day 4 in the 1000 mg/kg group. The NOEL for parental and offspring toxicity was 300 mg/kg bw/d. The reproductive NOEL was 1000 mg/kg bw/d.

Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid. Groups of 24 gravid Sprague Dawley rats were dosed orally by gavage with 0, 1000, 4000, or 7000 mg/kg bw/d di-C7-9 branched and linear alkyl esters of adipic acid on days 6 to 19 of gestation, and all animals were killed and examined on day 20.[92] All dams survived until study termination. Body weights were significantly decreased for dams of the 7000 mg/kg bw group. Weights of male and female fetuses of the 7000 mg/kg bw group were slightly, but not statistically significantly, decreased compared to the other groups. A greater incidence of rudimentary structures was observed for high-dose fetuses as compared to the other groups in this study, but the incidence was within the range of historical controls. There was no evidence of developmental toxicity at any dose tested.

Ditridecyl Adipate. Groups of 15 mated female Sprague-Dawley rats were given doses of 0, 800, and 2000 mg/kg bw of ditridecyl adipate applied dermally without occlusion on days 0 to 19 of gestation, and the dams were killed on day 20.[92]. Mild skin irritation consisting of erythema and flaking were observed at the test sites of the treated animals. No maternal mortality was reported. Weight gains were statistically significantly decreased for the 2000 mg/kg bw group during days 0 to 3 and 16 to 20 of gestation. Weight gains were statistically significantly decreased in the 800 mg/kg bw group during days 0 to 3 of gestation. No differences in skeletal anomalies were observed, but there were some differences in visceral anomalies, including increased incidence of levocardia at 2000 mg/kg bw. These anomalies were not considered treatment-related. The NOAEL for maternal toxicity was 2000 mg/kg bw/d, and for developmental and reproductive effects it was 800 mg/kg bw/d.

Groups of 25 mated female rats were dosed dermally with 0 and 2000 mg/kg bw ditridecyl adipate following the same study protocol as above. Again, there were no signs of maternal toxicity. No developmental toxicity was reported, and there were no visceral anomalies or levocardia.

Tridecyl adipate, 2000 mg/kg bw, was applied, unoccluded, to groups of 10 male Sprague-Dawley rats, 5 days/week for 13 weeks, and the effect on sperm morphology was evaluated.[92] (The 'Subchronic Dermal Toxicity' study was described earlier.) No differences in sperm morphology were observed between control and test animals.

Diisobutyl Adipate. Diisobutyl adipate was evaluated following the procedure described earlier in the ip study.[124] These rats were dosed ip with 0.1983 to 1.9833 mL/kg diisobutyl adipate. The numbers of live and dead fetuses were not statistically significantly different between treated and blunt-needle control groups, but there was a statistically significant decrease in the mean fetal weight of the 1.1900 and 0.9833 mL/kg dose groups. The number of resorptions was similar between treated animals and both the blunt needle and pooled controls. Gross abnormalities, but not skeletal or visceral, were statistically significantly increased in fetuses of the 0.5950 and 1.9833 mL/kg groups.

Diethylhexyl Adipate. Groups of 10 male Swiss mice were dosed ip with $\leq$9.3 g/kg diethylhexyl adipate and then mated with undosed females. A reduction in the number of gravid females was considered an anti-fertility effect, and the dominant lethal mutation was determined directly from the dose-dependent increase in the number of early fetal deaths and indirectly from the dose- and time-dependent decrease in implantations. There were no test article-related changes in the incidence of late fetal deaths. It was noted that the experimental design and interpretation have been questioned by some. Diethylhexyl adipate, $\leq$9.3 g/kg, was administered by ip injection to groups of 5 gravid Sprague Dawley rats on day 5, 10, and 15 of gestation. Resorption rates were similar to controls. A decrease in the mean fetal bw and a significant increase in gross fetal abnormalities at the high dose were observed when compared to pooled control values. However teratogenic effects were not observed when compared to concurrent controls. It was stated that the lack of historical and positive controls affected the validity of the results.[2]

Groups of 15 male and 30 female Wistar rats were fed a diet containing 0, 0.03, 0.18, or 1.2% diethylhexyl adipate (calculated as 28, 170, or 1080 mg/kg bw/d) for 10 weeks prior to mating.[92] Dosing was terminated, and the animals were mated. (A different source indicated that dosing continued throughout the study).[161] A reduction in bw gain was reported during gestation for the dams of the 1.2% group. No test article-related effects on fertility were observed. Fetal weight, total litter weight, and litter size were reduced in the 1.2% group, but the number of pups born live, or their survival, was not affected. The NOAEL was 170 mg/kg bw/d and the LOAEL was 1080 mg/kg bw/d.

In another study in which gravid females were fed the same doses as above on days 1 to 22 of gestation, maternal bw and feed consumption were statistically decreased in the 1.2% group. No significant effects on fetal weight or litter size were reported. Animals of the 0.18 and 1.2% groups had slightly increased incidences of minor skeletal abnormalities; this increase was attributed to fetotoxicity. The NOEL for maternal toxicity was 170 mg/kg bw/d. The NOAELs for developmental toxicity and fetotoxicity were 170 and 28 mg/kg bw/d, respectively. The LOAEL was 1080 mg/kg bw day.

A dose-range finding study was performed using groups of 8 gravid Wistar rats that were dosed by gavage with 2 mL/kg of 0, 800, or 1200 mg/d diethylhexyl adipate, in peanut oil, from day 7 of gestation until day 17 after parturition.[162] No signs of toxicity were reported in any of the groups. In the 800 mg/kg bw group, the only statistically significant observation made was decreased bws of male and female pups on day 3. In the 1200 mg/kg bw group, statistically significant effects were observed for a number of parameters, including decreased maternal weight gain during days 7 to 21 of gestation, increased length of gestation (by 1 day), decreased pup bws at birth and day 3, and an increase in perinatal loss per litter. (Perinatal loss was 42% in the 1200 mg/kg bw groups, as compared to 4.6% in controls.)

Based on the results of the dose-range finding study, groups of 20 gravid Wistar rats were dosed with 2 mL/kg of 0, 200, 400, or 800 mg/kg bw diethylhexyl adipate, in peanut oil, from day 7 of gestation until post-natal day 17. At postnatal day 21, all dams and pups were killed, with the exception that 1 male and 1 female pup per litter was kept for further evaluation. No signs of toxicity were reported in any of the groups. No significant effects were observed in the 200 mg/kg bw group. In the 400 mg/kg bw dose groups, the number of postnatal deaths per number of pups was statistically significant increased. In the 800 mg/kg bw group, statistically significant effects were observed for a number of parameters, including increased length of gestation (by 1 day), decreased pup bws at birth and days 3 and 13, increased mean number of postnatal deaths, and an increase in postnatal death per number of pups. The percentage of perinatal loss per litter was twice as high in the 400 and 800 mg/kg bw groups (23%) as compared to controls (11%), but the change was not statistically significant. Testicular testosterone levels were unaffected in any of the pups that were killed on postnatal day 21 or the adult male offspring, and serum luteinizing hormone and prolactin levels were similar to controls. None of the sperm parameters that were evaluated were affected by dosing. The only statistically significant effects, noted in the 800 mg/kg bw group, were increased relative liver to bws in male pups on day 21 and increased bws and decreased adrenal weights in adult male offspring. Diethylhexyl adipate did not produce any antiandrogenic effects in the study. Fetal steroidogenesis was not evaluated. NOAEL was 200 mg/kg bw.

Groups of 10 female Crl:CD(SD) rats were dosed with 5 mL/kg, by gavage, of 0, 200, 1000, or 2000 mg/kg bw diethylhexyl adipate in corn oil for 2 weeks prior to mating with undosed males, throughout mating, and until day 7 of gestation.[125] The dams were killed on day 14 of gestation. All animals survived until study termination. Body weights and body weight gains were significantly decreased in the 2000 mg/kg bw dose group prior to mating. Staining around the perineum was observed in the 1000 and 2000 mg/kg bw dose groups. No statistically significant differences were observed for the 200 mg/kg bw group compared to controls. The mean estrous cycle length was statistically significantly increased in the 1000 and 2000 mg/kg bw groups, and the post-implantation loss rate was also statistically significantly increased in these groups. Additionally, in the 2000 mg/kg bw group, there was a significant decrease in implantation rate, and the number of live embryos was statistically significantly decreased and the pre-implantation loss rate statistically significantly increased. The researchers stated that the effects observed in this fertility study, in conjunction with the ovarian effects described earlier in the repeated dose study, suggest that diethylhexyl adipate disturbed ovulation. This correlated with the effect on estrous cycle length.

The testicular toxicity of diethylhexyl adipate was examined using male F344 rats.[163] Groups of 6 rats were fed a diet containing 6000 or 25 000 ppm diethylhexyl adipate for 4 weeks, and the controls were given untreated feed. Some groups were dosed ip with 200 mg/kg bw thioacetamide, 3x/week for 4 weeks, and prior to dosing with diethylhexyl adipate to evaluate whether liver disease enhanced testicular effects. (There was a 1-week rest period prior to dosing with diethylhexyl adipate.) The final bws of animals given 25 000 ppm diethylhexyl adipate, with and without prior administration of thioacetamide, were statistically significantly decreased compared to their respective controls. The relative liver to bws of these animals were statistically significantly increased. No significant effect on the relative weights of the testes or epididymis was seen for any of the test groups. Diethylhexyl adipate did not have any testicular toxic effects, with or without the induction of hepatic damage.

Diisononyl Adipate. In a subchronic dietary study described earlier, groups of male and female Beagle dogs were fed 0, 0.3, 1.0, or 3.0% (weeks 1-8) and 6.0% (weeks 9-13) diisononyl adipate for 13 weeks.[92] Reproductive tissues were evaluated. No significant findings were reported for the 0.3% and 1.0% groups. In the high-dose group, testes weight was decreased. At

microscopic examination, it was found that the epididymal ducts were devoid of spermatozoa, the seminiferous tubules were composed of Sertoli cells and spermatogonia, spermatocytes and spermatids were not evident, and there was almost total aspermatogenesis. Ovaries were not weighed at necropsy. There were no gross or microscopic changes in the ovaries of the high-dose group compared to controls.

Dibutyl Sebacate. A test group of 20 male and 20 female Sprague-Dawley rats was fed a diet containing 6.25% dibutyl sebacate for 10 weeks, while a control group of 12 male and 12 female rats were fed the basal diet, and then animals of each group were then mated.[122] The dams were allowed to deliver their litters, and at weaning, 24 male and 24 female offspring were randomly chosen, fed the test diet for 21 days, and then killed for necropsy. The study results indicated that ingestion of a diet containing 6.25% dibutyl sebacate had no adverse effect on fertility, litter size, or survival of offspring. Growth was decreased during the pre-weaning and post-weaning periods. However, no gross pathological changes were found among young rats killed at the end of the 21-day post-weaning period.

Diethylhexyl Sebacate. Reproduction, suckling and growth were normal in a 4-generation study of rats fed a diet containing 200 ppm diethylhexyl sebacate (~10 mg/kg bw/d).[1] No reproductive or developmental toxicity was observed.

Dimethyl Glutarate/Dimethyl Succinate/Dimethyl Adipate Mixture. The developmental toxicity produced by the inhalation of dibasic esters (mixture of 65.1% dimethyl glutarate, 17.8% dimethyl succinate, and 16.8% dimethyl adipate) was evaluated in rats.[164] Groups of 24 gravid Crl:CD rats were exposed for 6 hours/d to 0, 0.16, 0.4, or 1.0 mg/L dibasic esters, by whole body inhalation, on days 7 to 16 of gestation. The aerosol particle size in the 1.0 mg/L chamber was 5.3 to 5.4 μm, with 72% to 74% of the aerosol <10 μm. The animals were killed on day 21 of gestation. All animals survived until study termination. Body weight gains were statistically significantly decreased in the 0.4 and 1.0 mg/L groups. Feed consumption by these groups was reduced during the first 6 exposures; statistical significance was not given. Statistically significant differences in absolute and relative liver to bws were not observed, but there was a significant trend of decreased absolute, but not relative, liver weights. The only significant clinical signs observed were perinasal staining and wet fur of rats in the 1.0 mg/L group. Reproductive and developmental effects were not observed, and the dibasic esters mixture was not a developmental toxicant in rats following inhalation of ≤1.0 mg/L.

Groups of 20 Crl:CD(SD)BR rats/gender were exposed for 6 hours/d, 5 days/week, to 0, 0.16, 0.40, or 1.0 mg/L dibasic esters by whole body inhalation for 14 weeks prior to mating, and then 7 days/week for 8 weeks of mating, gestation, and lactation.[165] The mean aerosol particle size in the 1.0 mg/L chamber was 6.2 μm, with 69% of the aerosol <10 μm. Exposure was discontinued from day 19 of gestation through day 3 post-partum. All parental rats and 10 pups/gender were killed and necropsied on day 21 post-partum. The remaining pups were not necropsied. Maternal bws in the 0.40 mg/L group were decreased during the last week of the study, while bws of male and female rats of the 1.0 mg/L group were slightly decreased from week 7 on. Relative liver to bws were slightly, but not significantly, decreased in the 0.4 and 1.0 mg/L groups. Other differences in organ weights were not considered dose-related. With the exception of a statistically significant decrease in pup bws at birth and day 21, no reproductive or developmental effects were observed. The only microscopic findings were squamous metaplasia in the olfactory epithelium of all treated parental rats. This effect was minimal in the 0.16 mg/L group and mild to moderate in the 0.4 and 1.0 mg/mL groups. The NOEL for reproductive parameters was 1.0 mg/L.

Endocrine Disruption

Diethylhexyl adipate. A 28-day repeated-dose toxicity study was performed based on the Enhanced OEDC Test Guideline no. 407 (enhanced TG 407) to determine whether diethylhexyl adipate has endocrine-mediated activities.[166] Groups of 10 male and 10 female Crj:CD (SD) rats were dosed orally by gavage with 0, 40, 200, or 1000 mg/kg bw diethylhexyl adipate in corn oil, at a volume of 10 mL/kg, for a minimum of 28 days. In addition to clinical observations, a functional observation battery was performed during week 4, estrous cycling was assessed from day 22, hormone analysis was measured at the end of the test period, and sperm morphology and sperm count were examined. Male animals were killed and necropsied on day 29, while females were killed and necropsied on days 30 to 34 when in diestrous. Signs of toxicity were not observed, and no clinical chemistry or hematological findings were recorded. Hormonal and spermatological analyses were normal. Statistically significant increases were seen in relative kidney to bws in males of the 200 and 1000 mg/kg bw groups, relative liver to bws of males in the 1000 mg/kg bw group, and in relative liver, kidney, and adrenal to bws in females of the 1000 mg/kg bw group. Microscopically, increased eosinophilic bodies and hyaline droplets were seen in the kidneys of male rats of the 1000 mg/kg bw group. Ovarian follicle atresia was observed in 4 females of the 1000 mg/kg bw group, accompanied by a prolonged estrous cycle in 2 of these rats. A change in the estrous cycle is an important endpoint for determination of endocrine-mediated effects in the enhanced TG 407 assay. The researchers stated that this effect, in conjunction with the microscopic findings, appears to be related to endocrine-mediated effects of diethylhexyl adipate. However, it was also stated that these findings may be attributable to the disturbance of ovarian function according to the hypothalamic-pituitary-gonad axis. The changes in relative kidney to bws and liver to bws, and accompanying histopathological changes, were considered toxic effects, and these findings indicated that the NOEL was 40 mg/kg bw/d.

The effect diethylhexyl adipate. at concentrations of 1×10^{-10} to 5×10^{-5} mol/L, on estrogen receptor and thyroid hormone (TH) functions was also examined.[167] The TH-like

activity was assessed using the rat pituitary tumor cell line GH3 expressing intracellular TH and estrogen receptors and responding to physiological concentration of TH by proliferation. At "low potency", diethylhexyl adipate stimulated the TH- dependent rat pituitary GH3 cell proliferation in a concentration-dependent manner. (The lowest tested concentration at which a statistically significant effect was detected was 10^{-5} mol/L.) Cotreatment of GH3 cells with diethylhexyl adipate potentiated the L-3,5,3′-triiodothyronine (T3)-EC_{50} potentiated the T3-induced GH3 cell proliferation.

Genotoxicity

Details of the genotoxicity studies on esters of dicarboxylic acids and esterase metabolites are described in Table 17.

Diethyl Malonate. Diethyl malonate was not mutagenic in an Ames test or a cytogenetic assay using human peripheral lymphocytes at concentrations ≤5000 μg/plate.[39]

Dimethyl Malonate. Dimethyl malonate was not mutagenic in an Ames test at concentrations ≤5000 μg/plate.[39]

Dimethyl Succinate. Dimethyl succinate was not mutagenic in an Ames tests with concentrations of ≤20 000 μg/plate[168] or in a preincubation assay with concentrations of ≤10 000 μg/plate.[169]

Dimethyl Glutarate. Dimethyl glutarate was not mutagenic in a preincubation assay with concentrations of ≤10 000 μg/plate.[170]

Dimethyl Adipate. Dimethyl adipate was not mutagenic in a preincubation assay with concentrations of ≤10 000 μg/plate.[171]

Dibutyl Adipate. Dibutyl adipate was mutagenic in an Ames test at concentrations of ≤5000 μg/plate. It was not genotoxic in an in vivo mouse micronucleus assay in which the animals were dosed with ≤2000 mg/kg bw.[5]

Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid. Di-C7-9 branched and linear alkyl esters of adipic acid were not mutagenic in an Ames test at concentrations of ≤10.0 μl/plate.[92]

Ditridecyl Adipate. Ditridecyl adipate was not mutagenic in an Ames test at concentrations of 0 to 10 μL/plate, and it was not clastogenic in an in vivo micronucleus assay using rats dosed dermally with 0, 800, or 2000 mg/kg bw ditridecyl adipate.[92]

Diethylhexyl Adipate. Diethylhexyl adipate was not mutagenic in an Ames (concentrations tested were not provided) test.[2]

Diethylhexyl adipate was not mutagenic in a number of genotoxicity studies. In vitro, negative results were reported in Ames tests at concentrations ranging from ≤150 to 10 000 μg/plate,[92,173-175] in an NTP preincubation assay,[176] in a liquid suspension assay,[177] and in a forward mutation assay using L5178Y cells at concentrations ≤1000 μg/mL.[180] In an assay for sister chromatid exchanges and chromosomal aberrations using concentrations of ≤200 μg/plate, results were negative,[179] while in another assay with ≤400 μl/plate, results were negative without, but equivocal with, metabolic activation in the sister chromatid exchange assay and there was some evidence of genotoxicity without, but none with, metabolic activation in the chromosomal aberration assay.[178] In a ^{3}H-thymidine assay, there was a dose-dependent inhibition of ^{3}H-thymidine incorporation into replicating DNA, with a dose-dependent increase in the ratio of acid-soluble DNA-incorporated ^{3}H-thymidine.[174] In vivo, results were negative in micronucleus tests[92,190] and chromosomal aberration assays.[191,192]

An Ames test was performed on urine of rats dosed with diethylhexyl adipate to assess whether mutagenic substances occur in the urine following diethylhexyl adipate adminstration.[181] Groups of ≥6 male Sprague-Dawley rats were dosed orally by gavage with 0 or 2000 mg/kg bw diethylhexyl adipate in corn oil for 15 days. Urine was collected daily. The urine was not mutagenic in the Ames test, indicating that diethylhexyl adipate is not converted to mutagenic urinary metabolites. The urine of rats dosed with 1000 mg/kg bw 2-ethylhexanol by gavage for 15 days was also tested in an Ames assay. The urine of these rats also was not mutagenic. Urine from rats that were dosed with a known mutagen gave a positive response in an Ames test.

Diisononyl Adipate. Diisononyl adipate was not mutagenic in an Ames assay at ≤1000 μg/plate, and it was not genotoxic in a mouse lymphoma assay, a transformation assay, or a BALB/3t3 assay at concentrations of ≤100, 1000, or 1.3 μg/mL, respectively.[182]

Diethyl Sebacate. Diethyl sebacate was nonmutagenic in an *Escherichia coli* Sd-4-73 reversion (streptomycin dependence to independence) assay.[183]

Dibutyl Sebacate. Dibutyl sebacate, ≤10 000 μg/plate, was not mutagenic in the Ames assay.[184,185]

Diethylhexyl Sebacate. Diethylhexyl sebacate was not mutagenic in an Ames assay at concentrations of ≤10 000 μg/plate.[175,186] In the rat liver foci test, diethylhexyl sebacate demonstrated no evidence of promotion activity when administered orally at 500 mg/kg bw 3x/week for 11 weeks, following a single oral treatment with a known carcinogen.[193]

Carcinogenicity

Diethylhexyl Adipate. In an NTP carcinogenicity study, administration of ≤25 000 ppm diethylhexyl adipate to rats in the diet for 103 weeks did not produce carcinogenic effects. However, mice fed the same amount for 103 weeks had dose-related bw reductions and a higher incidence of hepatocellular adenoma and carcinoma than the controls. In another study in which rats were fed ≤2.5% diethylhexyl adipate for 2 years,

Table 17. Genotoxicity Studies—Esters of Dicarboxylic Acids[a]

Concentration	Vehicle	Procedure	Test System	Results	Reference
In Vitro					
Diethyl Malonate					
≤5000 µg/plate	not specified	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA98, TA100	negative	39
≤5000 µg/plate	not specified	cytogenetic assay, ± metabolic activation	human peripheral lymphocytes	negative; cytotoxic at 5000 µg/plate	39
Dimethyl Malonate					
≤5000 µg/plate	not specified	Ames test, ± metabolic activation	S *typhimurium* TA153S, TA1537, TA98, TA100	negative; cytotoxic at ≥1000 µg/plate	39
Dimethyl Succinate					
20 000 µg/plate	DMSO	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA98, TA100	negative	168
≤10 000 µg/plate	water	NTP preincubation assay, ± metabolic activation	S *typhimurium TA100, TA1535, TA97, TA98*	negative	169
Dimethyl Glutarate					
≤10 000 µg/plate	DMSO	NTP preincubation assay, ± metabolic activation	S *typhimurium TA100, TA1535, TA97, TA98*	negative	170
Dimethyl Adipate					
≤10 000 µg/plate	DMSO	NTP preincubation assay, ± metabolic activation	S *typhimurium TA100, TA1535, TA97, TA98*	negative	171
Dibutyl Adipate					
≤5000 µg/plate		*Ames test, ± metabolic activation*	S *typhimurium TA98, TA100, TA1535, TA1537, TA1538*	*negative*	5
Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid					
≤10.0 µl/plate	not specified	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA98, TA100	negative	92
Ditridecyl Adipate					
≤10 µl/plate	DMSO	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	92
Diisobutyl Adipate					
≤10 000 µg/plate	DMSO	Ames test, ± metabolic activation	S *typhimurium* TA98, TA100, TA102, TA97, TA98, *E coli* wp2	negative	172
Diethylhexyl Adipate					
≤5 mg/plate	*not specified*	*Ames test, ± metabolic activation*	S *typhimurium TA1535, TA1537, TA1538, TA98, TA100*	*negative*	2
5000 µg/plate	not specified	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	173
≤0.01 mol/L	not specified	Ames test, ± metabolic activation	S *typhimurium* TA98, TA100	negative	174
10 000 µg/plate	DMSO	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537,. TA98, TA100	negative	175
10 000 µg/plate	95% ethanol	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537,. TA98, TA100	negative	175
10 000 µg/plate	acetone	NTP preincubation assay, ± metabolic activation	S *typhimurium TA100, TA1535, TA97, TA98*	negative	176
≤150 µg/plate	not specified	Ames test, ± metabolic activation	S *typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	92
not specified	DMSO	liquid suspension assay	S *typhimurium TA100*	negative	177

(continued)

Table 17. (continued)

Concentration	Vehicle	Procedure	Test System	Results	Reference
≤400 µg/mL	not specified	sister chromatid exchange assay, ± metabolic activation	Chinese hamster ovary cells	negative w/out activation; equivocal w/activation	178
≤200µg/plate, 3 or 51 hours	DMSO	sister chromatid exchange assay	female F344 rat hepatocytes	negative	179
≤400 µg/mL	not specified	chromosomal aberration assay, ± metabolic activation	Chinese hamster ovary cells	some evidence w/out activation; negative w/ activation	178
≤200µg/plate, 3 or 51 hours	DMSO	chromosomal aberration assay	female F344 rat hepatocytes	negative	179
≤0.01 mol/L	not specified	^{3}H-thymidine assay, ± metabolic activation	splenic lymphoid cells	dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, w/a dose-dependent increase in the ratio of acid-soluble to DNA-incorporated ^{3}H-thymidine	174
≤1000 µg/plate		forward mutation assay, ± metabolic act.	L5178Y cells	negative	180
urine of rats dosed with 2000 mg/kg diethylhexyl adipate					
	corn oil	Ames test		negative	181
Diisononyl Adipate					
≤1000 µg/plate		Ames test, ± metabolic activation	*S typhimurium* TA98, TA100, TA1535, TA1537, TA1538	negative	182
≤100 µg/mL		lymphoma assay, ± metabolic activation	mouse lymphoma L5178Y cells	negative	
≤1000 µg/mL		transformation assay	Syrian hamster embryo cells	negative	182
≤1.3 µ/mL		BALB/3T3 assay		negative	182
Diethyl Sebacate					
		reversion assay	*E coli* Sd-4-73	negative	183
Dibutyl Sebacate					
not specified	not specified	Ames test	*S typhimurium* TA98, TA100, TA1535, TA1537, TA1538	negative	184
≤10 000 µg/plate	DMSO & Tween 80	Ames test, ± metabolic activation	*S typhimurium* TA98, TA100, TA1535, TA1537; *E coli wp2 uvrA*	negative	185
Diethylhexyl Sebacate					
≤10 000 µg/plate	DMSO	Ames test, ± metabolic activation	*S typhimurium TA98, TA100, TA1535, TA1537*	negative	175
≤5000 µg/plate	DMSO	Ames test, ± metabolic activation	*S typhimurium* TA98, TA100, TA1535, TA1537; *E coli wp2 uvrA*	negative	186
Esterase Metabolites					
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)					
10 000 µg/plate		Ames test, ± metabolic activation		negative	175
		Ames test		negative	182
≤0.01 mol/L	not specified	Ames test, ± metabolic activation	*S typhimurium* TA98, TA100	negative	174
≤5000 µg/plate	not specified	Ames test, ± metabolic activation		negative	121
0-1.5 mmol/L	DMSO	liquid suspension assay	*S typhimurium* TA100	negative	177
not specified	not specified	mouse lymphoma assay		negative	182
not specified	not specified	unscheduled DNA synthesis		negative	182

(continued)

Table 17. (continued)

Concentration	Vehicle	Procedure	Test System	Results	Reference
≤0.01 mol/L	not specified	^{3}H-thymidine assay, ± metabolic activation	splenic lymphoid cells	dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, w/a dose-dependent increase in the ratio of acid-soluble to DNA-incorporated ^{3}H-thymidine	174
1000 mg/kg	corn oil	Ames test performed on urine from rats dosed orally for 15 days		negative	181
MEHA (metabolite of diethylhexyl adipate)					
10 000 µg/plate		Ames test, ± metabolic activation		negative	175
≤1000 µg/plate		Ames test		negative	91
Mono-(2-Ethyl-5-Hydroxyhexyl)Adipate (metabolite of diethylhexyl adipate)					
≤1000 µg/plate		Ames test		negative	91
Mono-(2-Ethyl-5-Oxohexyl)Adipate (metabolite of diethylhexyl adipate)					
≤1000 µg/plate		Ames test		negative	91
Propyl and Isopropyl Alcohol (metabolite of dipropyl adipate, diisopropyl adipate, and diisopropyl sebacate)					
		bacterial and mammalian cell assays		negative	130
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)					
C7-9 branched alkyl alcohols		bacterial and mammalian cell assays		negative	121
In Vivo					
Dimethyl Succinate					
≥1250 mg/kg	corn oil	micronucleus test, ip	male F344 rats	negative	187
Dimethyl Glutarate					
≥1250 mg/kg	corn oil	micronucleus test, ip	male F344 rats	negative	188
Dibutyl Adipate					
≤2000 mg/kg	olive oil	mouse micronucleus test	mice	negative	5
≥724 mg/kg	corn oil	micronucleus test, ip	male F344 rats	negative	189
Ditridecyl Adipate					
≤2000 mg/kg	none	micronucleus test; dosed dermally for 13 weeks	groups of 10 male and 10 female Sprague Dawley rats	negative	92
Diethylhexyl Adipate					
2000 mg/kg	corn oil	micronucleus test; dosed ip for 3 days	5 male B3C3F$_1$ mice	negative	190
≤5000 mg/kg	corn oil	chromosomal aberration assay	8 male B3C3F$_1$ mice	negative	191
not specified	corn oil	chromosomal aberration assay	8 B6C3F$_1$ mice	negative	192
5000 mg/kg	corn oil	micronucleus test single ip dose	6 male/6 female B3C3F1 mice	negative	92
Dibutyl Sebacate					
943-2829 mg/kg	olive oil	micronucleus test, ip	micronucleus test	negative	184
Diethylhexyl Sebacate					
500 mg/kg	not specified	rat liver foci test	single dose of known carcinogen, the dosing 3x/week for 11 weeks	no activity	193
Esterase Metabolites					
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)					
not specified	not specified	micronucleus test	mice	negative	182
not specified	not specified	transformation assay	BALB/3T3	negative	182
Propyl and Isopropyl Alcohol (metabolite of dipropyl adipate, diisopropyl adipate, and diisapropyl sebacate)					
C7-9 branched alkyl alcohols		micronucleus test		negative	130

[a]Data from original safety assessments are in italics.

tumor incidence for the test animals was similar to that of controls. The same researchers found no tumors in dogs fed up to 0.2% diethylhexyl adipate for 1 year. A single 10 mg dose of diethylhexyl adipate given by sc injection was not carcinogenic in mice. In a lifetime study, diethylhexyl adipate caused no skin tumors when 10 mg was applied weekly to the back skin of mice.[2]

Research has shown that other compounds with a 2-ethylhexyl group that have been evaluated for carcinogenicity had some evidence of hepatocarcinogenicity, ranging from very strong to equivocal, in rodents.[194]

In an evaluation of the carcinogenic risk of diethylhexyl adipate, the IARC stated that there was limited evidence in experimental animals for the carcinogenicity of diethylhexyl adipate.[17] Therefore, the overall evaluation of diethylhexyl adipate was *not classifiable as to its carcinogenicity to humans (Group 3).*

Diethylhexyl Sebacate. No evidence of carcinogenicity was observed in an unspecified number of rats fed a diet providing about 10 mg diethylhexyl sebacate/kg/d for up to 19 months.[1] No further study details were provided.

Tumor Promotion

Diethylhexyl adipate. A group of 14 male F344 rats were used to assess the carcinogenic potential of diethylhexyl adipate in a medium-term liver bioassay.[195] The rats were given a single ip dose of diethylnitrosamine, and 2 weeks later they were given 20 000 ppm diethylhexyl adipate in the diet. At week 3, a partial hepatectomy was performed. Positive results for carcinogenic potential were indicated by a significant increase in GST-P positive foci. Diethylhexyl adipate did not have an enhancing effect on the development of GST-P-positive foci.

Risk Assessment

Diethylhexyl adipate. According to the Integrated Risk Information System of the EPA, the weight-of-evidence classification for diethylhexyl adipate was "possible human carcinogen".[161] The classification was based on an absence of human data and increased liver tumors in female mice. The only genotoxic effect was a positive dominant lethal assay. It was noted that diethylhexyl adipate exhibits structural relationships to other nongenotoxic compounds that are classified as probable and possible carcinogens.

Summary

This safety assessment includes sebacic acid and other alkyl α,ω-dicarboxylic acids, salts, monoesters and diesters, for a total of 56 ingredients. The dicarboxylic acids are terminally functionalized straight alkyl chains characterized by a separation between the acid functional groups of 1 to 10 carbons. The simple alkyl di-esters are the result of the condensation of alkyl dicarboxylic acids and 2 equivalents of alkyl alcohols. These ingredients can be metabolized via hydrolysis back to the parent alcohol, the mono-ester, and the parent dicarboxylic acid. The simple alkyl esters (mono- and di-) of these dicarboxylic acids have straight or branched side chains ranging in length from 1 to 18 carbons. This safety assessment is divided into 2 parts—(1) 12 dicarboxylic acids and their salts and (2) 44 esters of dicarboxylic acids.

A safety assessment of diethylhexyl adipate (called dioctyl adipate at the time of that assessment) and diisopropyl adipate was published in 1984 with the conclusion that these ingredients are safe as used in cosmetics. This conclusion was reaffirmed in 2006. Additionally, dibutyl adipate was previously reviewed in 1996 and the available data were found insufficient to support the safety of dibutyl adipate in cosmetic formulations. When re-reviewed in 2006, additional data were made available to address the needs identified by the CIR Expert Panel, and an amended conclusion was issued stating that dibutyl adipate is safe for use in cosmetic formulations.

While many of the alkyl dicarboxylic acids occur in natural products, commercial production of these acids has historically occurred via alkali pyrolysis of lipids.

A relationship exists between the molecular weight and the log octanol—water partioning coefficient. Physical properties change as chain length increases, and the water solubility of these acids is inversely proportional to their chain length. Odd versus even chain length also plays a role. The alternating effects are believed to be the result of the inability of odd carbon number compounds to assume an in-plane orientation of both carboxyl groups with respect to the hydrocarbon chain. The diesters, in contrast, are much more lipid soluble and more difficult to dissolve in water. The short-chain alkyl mono- and diesters are more soluble in water, less lipophilic, and relatively more volatile than the corresponding longer chain alkyl esters.

The ingredients included in this review would not be expected to have any meaningful UV absorption.

The ingredients in this report function in cosmetics as pH-adjusters, fragrance ingredients, plasticizers, skin-conditioning agents and/or solvents and corrosion inhibitors. The majority of the dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients. Six of the 12 dicarboxylic acids and their salts and 24 of the 44 esters included in this safety assessment are reported to be used in cosmetic formulations. For the dicarboxylic acids and their salts, disodium succinate has the greatest number of reported uses, with a total of 45. The acid with the greatest concentration of use is succinic acid, 26%; use at this concentration is in a bath product that will be diluted during use. The greatest leave-on concentration is 0.4%, disodium succinate, with dermal contact exposure. For the esters, diisopropyl adipate has the greatest number of uses, with 70 reported. The concentration of use is greatest for dimethyl glutarate, 15% in a dermal rinse-off product. The ingredients with the greatest leave-on use concentrations, which are all dermal contact exposures, are diethylhexyl adipate, 14%, diisostearyl adipate, 10%, and diisopropyl sebacate, 10%.

Dicarboxylic Acids and Their Salts

Dicarboxylic acids are endogenous metabolic products of the ω-oxidation of monocarboxylic acids when the β-oxidation of

free fatty acids is impaired. Under normal physiological conditions, dicarboxylic acids are rapidly β-oxidized, resulting in very low cellular concentrations and practically nondetectable concentrations in the plasma. Oxidation of odd- and even-numbered chains proceeds to different end points; even chains are completely, while odd-number chains are not completely, oxidized.

Unchanged dicarboxylic acid was found in the urine of rats. With oral dosing, approximately 53% to 67% adipic acid, 40% azelaic acid, and 50% dodecanedioate was recovered with the respective acid. With iv dosing, 59% to 71% adipic acid and 35% sebacate was recovered. In humans, 6.76 to 61 adipic acid, and 61% azelaic acid were found in the urine after dosing with the respective acid. With azelaic acid and dodecanedioic acid, radioactivity was found in all tissues, and it decreased after 24 hours in all tissues except adipose tissue. Radioactivity was found in expired carbon dioxide at 24 hours after dosing adipic acid (70%), azelaic acid (14.5%), and disodium sebacate (25%). For rats dosed orally with azelaic, sebacic, undecanedioic, and dodecanedioic acid, 2.5, 2.1, 1.8, and 1.6% of the respective acid was found in the urine unchanged. The amount recovered decreased with increasing chain length. After oral dosing, 60, 17, 5, and 0.1% of azelaic, sebacic, decanedioic, and undecanedioic acids, respectively, were recovered unchanged in the urine. In the plasma of both animals and humans, dicarboxylic acid catabolites that were 2-, 4-, or 6- carbons shorter than the corresponding dicarboxylic acid were found.

Adipic acid did not induce peroxisome proliferation. Dicarboxylic acids did have some cellular effects and inhibited mitochondrial oxidoreductases, reversibly inhibited microsomal NADPH and cytochrome P450 reductase, and competitively inhibited tyrosinase in vitro.

The oral LD_{50} values of the dicarboxylic acids had a wide range; for example, adipic acid had values in rats ranging from 0.94 g/kg to greater than the highest dose tested (11 g/kg). Most reported values for the acids were >2 g/kg. The reported dermal LD_{50} values ranged from >6 g/kg dodecanedioic acid to >10 g/kg glutaric acid.

In short-term oral toxicity studies, ≤3000 mg/kg bw/d adipic acid did not produce significant toxicological effects in rats. Signs of toxicity were seen at >3600 mg/kg bw/d. No toxicity was observed with guinea pigs fed 400 to 600 mg/d adipic acid. Short-term inhalation exposure to 126 mg/m^3 adipic acid to rats did not produce signs of toxicity, but exposure of mice to 460 mg/m^3 resulted in decreased weight gain and produced effects in the upper respiratory tract, liver, kidneys, and central nervous system.

In a subchronic oral study, 10 male and 10 female rats exposed to 10% sodium succinate in the drinking water died, but no compound-related lesions were found. Body weights were decreased in rats given ≥2.5% sodium succinate for 13 weeks, but toxicological treatment-related changes were not observed. Glutaric acid had a low degree of toxicity to rats (at 2%) and dogs (concentration not specified) when given in the feed. Dietary administration of ≤3400 mg/kg bw/d adipic acid for 19 weeks produced slight effects in the liver of male rats; the NOAEL was 3333 mg/kg bw. A mixture of adipic, glutaric, and succinic acids had a low degree of toxicity in rats when tested at 3% for 90-days. Signs of toxicity were reported in a subchronic inhalation study in which mice were exposed to 13 or 120 mg/m^3 adipic acid.

Slight effects were seen in the livers of rats fed ≤3200 mg/kg bw/d adipic acid for 33 weeks, and the NOAEL for rats fed a diet containing adipic acid for 2 years was 1%; no significant toxicological effects were seen at concentrations of ≤5%. No significant toxicological effects were observed for mice fed ≤280 mg/kg bw or rabbits fed ≤400 mg/kg bw azelaic acid for 180 days. Disodium sebacate was not toxic to rats or rabbits fed up to 1000 mg/kg bw for 6 mos.

For the dicarboxylic acids, the severity of ocular irritation seems to decrease with increasing carbon number. Succinic acid was a severe ocular irritant, glutaric acid was moderately irritating, and dodecanedioic acid was a slight irritant. Ocular irritation produced by adipic acid was dose-dependent. Slight to mild dermal irritation was observed in rabbits for succinic, glutaric, and adipic acid, while dodecanedioic acid was not an irritant in rabbits. Using guinea pigs, adipic acid, dodecanedioic acid, and a mixture of succinic, glutaric, and adipic acids are not sensitizers.

Reproductive and developmental effects were not seen upon oral dosing with the dicarboxylic acids or disodium sebacate. Malonic acid, at 0.1% in vitro, has a spermicidal effect on human spermatozoa. Glutaric acid was tested at doses of ≤1300 mg/kg bw in rats and 500 mg/kg bw in rabbits, adipic acid at doses of ≤263 mg/kg bw in mice, 288 mg/kg bw in rats, 205 mg/kg bw in hamsters, or 250 mg/kg bw in rabbits, azelaic acid at doses of ≤140 mg/kg bw in rats and 200 mg/kg bw in rabbits, disodium sebacate at 500 mg/kg bw in rats and 1000 mg/kg bw in rabbits, and dodecanedioic acid was tested at ≤1000 mg/kg bw using rats. Embryotoxic effects were reported in a reproductive study of 2500 mg/kg bw/d azelaic acid using rats and in reproductive studies with ≤500 mg/kg bw/d azelaic acid using rabbits and monkey. In vitro, sodium salts of some dicarboxylic acid had a specific inhibitory effect on muscle activity of the uterine horn, and this effect progressively increased with chain length.

The dicarboxylic acids are not genotoxic, and consistently were not mutagenic in Ames tests. Positive results were seen in a transformation assay on glutaric acid using Balb/c-3T3 cells, both with and without metabolic activation. The results of a mouse lymphoma assay, with and without metabolic activation, on glutaric acid were negative in a neutral pH range. Equivocal results were obtained in an in vitro chromosomal aberration assay of ≤15 mg/mL disodium succinate using Chinese hamster fibroblast cells. The dicarboxylic acids were not genotoxic in in vivo assays.

Carcinogenicity was not seen in rats given up to 2% sodium succinate in the drinking water or 5% adipic acid in feed for 2 years. An increase in the incidence of C-cell adenoma/carcinoma of the thyroid in females given 2% sodium succinate, and a positive trend in the occurrence of this tumor, was considered a function of experimental variability and not related to dosing.

Adipic acid was not carcinogenic when given orally to rats at up to 5% in the diet.

In a cumulative irritancy test, the cumulative irritation of a 15% azelaic acid gel increased with successive patching. It is not known if the vehicle played a role in the irritation scores. Daily application of a 20% azelaic cream causes erythema and irritation.

Esters of Dicarboxylic Acids

The metabolism of diesters in animals is expected to occur, initially, via enzymatic hydrolysis, leading to the corresponding dicarboxylic acids and the corresponding linear or branched alcohol. These dicarboxylic acids and alcohols can be further metabolized or conjugated to polar products that are excreted in urine, or, the enzymatic hydrolysis may be incomplete and result, at least for some diesters, in the production of monoesters.

In in vitro absorption studies using pig skin, 8.8% and 3% of undiluted diethyl malonate were found in the skin and receptor fluid, respectively, after 50 hours. Absorption was enhanced when diethyl malonate was diluted with ethanol and reduced when diluted in acetone. Using human skin, 16% of the applied diethyl malonate penetrated in 24 hours. In vivo, absorption of diethyl malonate, estimated from urinary and fecal recovery, was 15% in nude mice, 4% in human skin grafted to nude mice, 6% in pig skin grafted to nude mice, 2.5% in pigs, and 4% in dogs.

Approximately 11% of ditridecyl adipate was absorbed through the skin of rats; 5.5% to 7.4% of the applied dose was found in the tissues, 3.5% to 4.7%was found in the urine, and 0.4% to 0.7% was found in the feces after 4 days. Prior dosing with ditridecyl adipate did not significantly affect absorption.

In vitro, diethylhexyl adipate was readily hydrolyzed to mono-(2-ethylhexyl) adipate (MEHA) or adipic acid in rat liver, pancreas, and small intestine tissue preparations. In rats, diethylhexyl adipate is hydrolyzed to adipic acid and 2-ethylhexanol or MEHA. 2-Ethylhexanol is converted to 2-ethylhexanoic acid, which may form a glucuronide conjugate or may be subjected to ω- and (ω-1)-oxidation and further metabolism. More than 98% of diethylhexyl adipate administered orally to rats was excreted in 48 hours; 21% to 45% of the radioactivity was expired in carbon dioxide and 34% to 52% was excreted in the urine. Diethylhexyl adipate and MEHA are not found in the blood or urine; diethylhexyl adipate or the metabolites are recovered in the tissues. Metabolism studies have shown that excretion in the urine is not as unchanged diethylhexyl adipate; mostly adipic acid is found. In humans, peak urinary elimination of all metabolites occurred within 8 hours of dosing.

Diethylhexyl sebacate is not readily absorbed through the skin of guinea pigs. Metabolism in rodents and humans may follow partially common pathways, producing 2-ethylhexanol as an intermediary metabolite.

Diethylhexyl adipate is a peroxisome proliferator requiring extensive phase I metabolism to produce the proximate peroxisome proliferator, which in both mice and rats appears to be 2-ethylhexanoic acid. Diethylhexyl adipate is not as potent a proliferator as diethylhexyl phthalate. Peroxisome proliferation causes an increase in liver weights and can induce hepatocarcinogenicity in rats and mice. Peroxisome proliferation is not believed to pose the risk of inducing hepatocarcinogenesis in humans, as a species difference in response to peroxisome proliferators exists.

Diethylhexyl adipate did not bind covalently to hepatic DNA in mice. It did stimulate DNA synthesis in livers of rats. In another study, a statistically significant increase in 8-OH-dG occurred in the liver DNA, but not the kidney DNA, at week 1 and 2. The IARC remarked that the weight of evidence for diethylhexyl adipate demonstrated that rodent peroxisome proliferators do not act as direct DNA-damaging agents.

The oral and dermal LD_{50} values are greater than 2 g/kg. No mortality occurred in rats exposed to concentrated vapors of diethyl malonate diethyl succinate, dibutyl adipate, or diethylhexyl adipate for 8 hours. Some deaths, possibly due to thermal decomposition were seen in rats and rabbits exposed to 940 mg/m3 for 7 hours. In a 4-hr inhalation toxicity study, a mixture of dimethyl glutarate, dimethyl succinate, and methyl adipate, the anterior and posterior nasal passageways were affected.

Oral administration of $\leq$1000 mg/kg bw dibutyl adipate for 28 days did not produce toxic effects in rats. In short-term oral dosing with diethylhexyl adipate, decreased weight gain was reported for rats and mice. The NOELs for rats and mice were 2 and 0.63%, respectively, in feed; 5/5 female mice fed 10% dibutyl adipate in feed died. In 2- and 4-week studies of diethylhexyl adipate, the oral NOAEL for ovarian toxicity was 200 mg/kg bw in rats; an increase in atresia of the large follicle and a decrease in currently formed corpora lutea were seen in females dosed with 1000 and 2000 mg/kg bw diethylhexyl adipate.

In a short-term dermal study in which 10 rabbits were dosed dermally with 0.5 or 1.0 mL/kg of a 20% dispersion of dibutyl adipate for 6 weeks, there was a significant decrease in bws in the high-dose group, and renal lesions in 1 animal of each group. There were no signs of toxicity in guinea pigs in an immersion study with 20.75% diisopropyl adipate, diluted to an actual concentration of 0.10% adipate. Dermal administration of diethylhexyl adipate to rabbits for 2 weeks resulted in slight to moderate erythema at the test site, but toxic effects were not reported for most of the animals.

In a 90-day oral toxicity study in which rats were fed 36 to 41 mg/kg bw diethyl malonate, no treatment-related effects were observed. Dietary administration of $\leq$2.5% di-C7-9 branched and linear alkyl esters of adipic (approx. 1500 and 1900 mg/kg bw/d for males and females, respectively) for 90 days did not result in systemic toxicity. The NOAELS for male and female rats were 1500 and 1950 mg/kg bw/d, respectively. Subchronic oral administration of diethylhexyl adipate to rats caused significant decreases in bw gains and increases in liver and kidney weights. The dietary NOEL for rats in a 90-day study was 610 mg/kg bw. A decrease in bws was seen in mice fed a diet with 1.2 and 2.5% diethylhexyl adipate. For diisononyl adipate, dietary administration of up to 500 mg/kg bw to

rats for 13 weeks resulted in a statistically significant increase in relative kidney weights, but there were no toxicological findings. With dogs, 3.0% dietary diisononyl adipate resulted in a decrease in bws, testes weight, and feed consumption, increased liver weight, elevated enzyme levels, liver and kidney discoloration, and microscopic changes in the liver, testes, spleen, and kidneys.

No adverse effects were reported with whole-body application of a 6.25% emulsion of dibutyl adipate to dogs 2x/week for 3 mos. Unoccluded dermal application of up to 2000 mg/kg bw ditridecyl adipate for 13 weeks to rats produced slight erythema, but no systemic toxicity.

In a 6-month study in which rats were dosed intragastrically with diethylhexyl adipate, hepatic detoxification appeared depressed at the beginning of the study, while in a 10-mos study, a decrease in central nervous system excitability was noted. Dietary administration of $\leq$1.25% dibutyl sebacate for 1 year or $\leq$6.25% for 2 years did not have an effect on growth

Ocular irritation appeared to lessen in severity as chain length of the dicarboxylic acid esters increased. Undiluted diethyl malonate was slightly to moderately irritating to rabbit eyes. Dibutyl, diisopropyl, and diethylhexyl adipate, at concentrations ranging from 0.1% to 100%, were non- or minimal ocular irritants. Diisopropyl sebacate was minimally irritating. Diethylhexyl sebacate was nonirritating in an MTT viability assay. Undiluted dioctyldodecyl and diisocetyl dodecanedioate were not irritating to rabbit eyes.

The esters of dicarboxylic acids were mostly non- or mildly irritating to rabbit skin. Some minimal irritation was seen with diisopropyl adipate, undiluted or at 5 to 20; 75% in formulation, and moderate erythema was reported with undiluted dibutyl adipate. Dimethyl malonate, dibutyl and diethylhexyl adipate, diethylhexyl sebacate, and dioctyldodecyl dodecanedioate were not sensitizers in guinea pigs or rabbits. Perfume formulations containing 1.1% diisopropyl adipate were not phototoxic in rabbits.

Oral administration of up to 1000 mg/kg bw dimethyl malonate to Wistar rats did not have an effect on fertility, and no developmental toxicity was reported. The NOAEL was 300 mg/kg bw for repeated dose and maternal toxicity and 1000 mg/kg bw for fertility and developmental toxicity. Oral administration of up to 100 mg/kg bw dibutyl adipate to Sprague-Dawley rats did not cause any reproductive effects, and the NOEL for parental and offspring toxicity was 300 mg/kg bw/d and for reproductive toxicity was 100 mg/kg bw/d. Oral administration of $\leq$7000 mg/kg bw di-C7-9 branched and linear alkyl esters of adipic acid to Sprague-Dawley rats did not result in developmental toxicity. Dietary administration of up to 1.2% diethylhexyl adipate did not affect fertility when fed to rats prior to mating. Fetal weight, total litter weight, and litter size were reduced with 1.2% diethylhexyl adipate. In a study in which gravid rats were fed the same doses during gestation, no significant effects on fetal weight or litter size were reported. An increased incidence of minor skeletal abnormalities was attributed to fetotoxicity. In a study in which diethylhexyl adipate was given orally to rats from day 7 of gestation until postnatal day 17, antiandrogenic effects were not observed, although some increase in post-natal death was observed. Administration of up to 2000 mg/kg bw diethylhexyl adipate prior to dosing and through day 7 of gestation did have an effect on the mean estrous cycle length at a dose of 1000 and 2000 mg/kg bw, and did appear to disturb ovulation. Significant decreases were also seen in implantation rate and number of live embryos, as well as an increase in pre-implantation loss. Diethylhexyl adipate did not produce testicular toxic effects in male F344 rats when fed at up to 25 000 ppm in the diet for 4 weeks. Dietary administration of 6.25% dibutyl sebacate to male and female Sprague-Dawley rats for 10 weeks prior to mating had no adverse effects on fertility, litter size, or survival of offspring. Diethylhexyl sebacate, 200 ppm in the diet, did not produce reproductive or developmental effects in rats.

Dermal applications of 2000 mg/kg bw ditridecyl adipate did not have an effect on sperm morphology. Some visceral anomalies were reported. The NOAELs for maternal toxicity and developmental and reproductive effects were 2000 and 800 mg/kg bw/d, respectively.

Dimethyl, diethyl, dipropyl, dibutyl, diisobutyl, and diethylhexyl adipate were evaluated for fetotoxic and teratogenic effects in rats when administered ip at 1/3 to 1/30 of the ip LD_{50} values. Some effect on resorptions and abnormalities were seen with all but diethyl adipate.

Inhalation by rats of $\leq$1.0 mg/L of a mixture of dimethyl glutarate, dimethyl succinate, and dimethyl adipate on days 7 to 16 of gestation or for 14 days prior to mating, during mating and gestation, and lactation, no adverse developmental or reproductive effects were observed. The only exception was a statistically significant decrease in pup weight at birth and day 21.

Diethylhexyl adipate appeared to have endocrine-mediated effects in Crj:CD (SD) rats in a 28-day oral study; however, it was stated that the findings may be attributable to the disturbance in ovarian function according to the hypothalamic-pituitary-gonad axis. Diethylhexyl adipate simulated thyroid hormone-dependent rat pituitary GH3 cell proliferation in a concentration-dependent manner.

The esters of dicarboxylic acids were not mutagenic or genotoxic in a battery of in vitro and in vivo tests. The only non-negative results reported were equivocal results in a sister chromatid exchange assay with $\leq$400 µg/mL diethylhexyl adipate in the presence of metabolic activation and a dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, with a dose-dependent increase in the ratio of acid-incorporated ^{3}H-thymidine with $\leq$0.01 mol/L diethylhexyl adipate. (The same effect was seen in the ^{3}H-thymidine assay with 2-ethylhexanol.)

In an NTP 2-year dietary study, $\leq$25 000 ppm diethylhexyl adipate did not produce tumors in male or female rats, but it did increase the incidence of hepatocellular adenoma and carcinoma in male and female mice. Diethylhexyl adipate did not cause skin tumors with weekly application of 10 mg to the back of mice in a lifetime study. Other compounds with a 2-ethylhexyl group that have been evaluated for carcinogenicity

had some evidence of hepatocarcinogenicity, ranging from very strong to equivocal, in rodents. Feeding of diethylhexyl sebacate to rats for 19 mos did not result in carcinogenic effects.

In a number of clinical irritation and sensitization studies, the diesters of dicarboxylic acids are not irritants or sensitizers. The only exception noted was that undiluted diisopropyl adipate was moderately irritating in 1 cumulative irritancy test, and some slight irritation was seen with formulations containing diethylhexyl adipate. A 10% dilution of dibutyl adipate tested on 30 participants and formulations containing 0.7% to 17% diisopropyl adipate, tested on 49 to 98 participants, and 9% diethylhexyl adipate, tested on 25 participants, were not phototoxic.

Cases of allergic contact dermatitis in response to diethyl sebacate-containing products have been reported, and it has been demonstrated that diethyl sebacate was the substance, or one of several substances in the products, eliciting the dermatitis. Two case studies were reported of allergic reactions to lotion containing diisopropyl sebacate.

According to the Integrated Risk Information System of the EPA, the weight-of-evidence classification for diethylhexyl adipate was "possible human carcinogen". The classification was based on an absence of human data and increased liver tumors in female mice. The IARC has stated that diethylhexyl adipate is not classifiable as to its carcinogenicity in humans.

Discussion

The Expert Panel reviewed the available data on dicarboxylic acids and their salts, and the data on the esters of dicarboxylic acids, and determined that these ingredients are safe as used in cosmetics. In reaching this conclusion, the Expert Panel considered a number of issues.

The Expert Panel noted gaps in the available safety data for some of the dicarboxylic acid and salts and esters of dicarboxylic acids in this safety assessment. The available data on many of the ingredients are sufficient, and similar structural activity relationships, biologic functions, and cosmetic product usage, suggest that the available data may be extrapolated to support the safety of the entire group. For example, a concern was expressed regarding the extent of dermal absorption for certain long-chain branched diesters. The Panel inferred that since dermal penetration of long chain alcohols is likely to be low and the dermal penetration for the diesters is likely to be even lower, toxicity characteristics from ingredients where toxicity data were available was appropriate.

The CIR Expert Panel considered the dangers inherent in using animal-derived ingredients, namely the transmission of infectious agents. While tallow may be used in the manufacture of some ingredients in this safety assessment and is clearly animal-derived, the Expert Panel notes that tallow is highly processed and tallow derivatives even more so. The Panel agrees with determinations by the U.S. FDA that tallow derivatives are not risk materials for transmission of infectious agents.

The Panel noted that the only significant toxic effect of the dicarboxylic acids was irritation to the skin and eyes, which would be expected for acids. Dicarboxylic acids reviewed in this safety assessment are not expected to be appreciably absorbed from cosmetic formulations, exhibit low single-dose or repeated-dose toxicity in animal studies, and are not genotoxic or carcinogenic in animal studies. Since a use of these acids in cosmetics is as a pH adjuster, the irritating property of these acids would be lost. The highest use of an acid in leave-on formulations is 0.3% azelaic acid, of a salt is 0.4% disodium succinate, and of an ester is 14% diethylhexyl adipate. Although bath products can contain higher concentrations of these acids, salts, or esters, contact time is short and the product will be diluted as it is being rinsed.

Case studies have reported reactions to products containing diethyl sebacate. Follow-up patch tests performed with $\geq$5% diethyl sebacate, which is greater than the reported use concentration, had positive results. Diethyl sebacate is reported to be used in cosmetics at 1.5%, and no irritation or sensitization was reported in clinical studies of a formulation containing 1.5% diethyl sebacate.

The Expert Panel also noted that esters of dicarboxylic acids, in particular diethylhexyl adipate, have the potential to induce peroxisome proliferation. This effect has been examined because ethylhexyl adipate is structurally related to a notable peroxisome proliferator, diethylhexyl phthalate. Diethylhexyl adipate is not as potent a peroxisome proliferator as diethylhexyl phthalate, and, while peroxisome proliferation is toxicologically well-studied, this is an effect observed only in rodents and is not relevant to humans. Accordingly, the hepatocarcinogenic effects observed in rodents are related to this effect and not believed to pose the risk of inducing hepatocarcinogenesis in humans.

The reproductive and developmental toxicity of the dicarboxylic acids and their esters were generally well studied. The results of these studies did not cause any concern for the Panel.

The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration and the duration of the exposure and their site of deposition within the respiratory system. In practice, aerosols should have at least 99% of their particle diameters in the 10 to 110 μm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 μm. Particles with an aerodynamic diameter of $\leq$ 10μm are respirable. In the absences of inhalation toxicity data, the panel determined that dicarboxylic acids and their salts and the esters of dicarboxylic acids can be used safely in hair sprays, because the product size is not respirable.

Conclusion

The CIR Expert Panel concluded that dicarboxylic acids and their salts, and the esters of dicarboxylic acids, as listed below, are safe in the present practices of use and concentration. Were ingredients in these groups not in current use (as indicated by *) to be used in the future, the expectation is that they would be

used in product categories and at concentrations comparable to others in these groups.

Acids and salts:

- malonic acid*
- succinic acid
- sodium succinate
- disodium succinate
- glutaric acid*
- adipic acid
- azelaic acid
- dipotassium azelate*
- disodium azelate*
- sebacic acid
- disodium sebacate*
- dodecanedioic acid*

Esters:

- diethyl malonate
- decyl succinate*
- dimethyl succinate
- diethyl succinate*
- dicapryl succinate
- dicetearyl succinate*
- diisobutyl succinate*
- diethylhexyl succinate
- dimethyl glutarate
- diisobutyl glutarate*
- diisostearyl glutarate
- dimethyl adipate
- diethyl adipate*
- dipropyl adipate*
- dibutyl adipate
- dihexyl adipate
- dicapryl adipate
- di-C12-15 alkyl adipate*
- ditridecyl adipate*
- dicetyl adipate*
- diisopropyl adipate
- diisobutyl adipate
- diethylhexyl adipate
- diisooctyl adipate*
- diisononyl adipate*
- diisodecyl adipate
- dihexyldecyl adipate*
- diheptylundecyl adipate
- dioctyldodecyl adipate
- diisocetyl adipate*
- diisostearyl adipate
- isostearyl sebacate
- diethyl sebacate
- dibutyl sebacate*
- dicaprylyl/capryl sebacate*
- diisopropyl sebacate
- diethylhexyl sebacate
- dibutyloctyl sebacate*
- diisooctyl sebacate
- dihexyldecyl sebacate*
- dioctyldodecyl sebacate
- diisostearyl sebacate*
- dioctyldodecyl dodecanedioate
- diisocetyl dodecanedioate

Author's Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th St, Suite 412, Washington, DC 20036, USA.

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. BIBRA Working Group.Diethylhexyl sebacate toxicity profile. 1996:6.
2. Elder RL (ed). Final report on the safety assessment of dioctyl adipate and diisopropyl adipate. *J Am Coll Toxicol.* 1984;3(3): 101-129.
3. Andersen FA (ed). Annual review of cosmetic ingredient safey assessments - 2002/2003. *Int J Toxicol.* 2005;24(suppl 1): 1-102.
4. Andersen FA (ed). Annual review of cosmetic ingredient safety assessments - 2004/2005. *Int J Toxicol.* 2006;25(suppl 1):1-89.
5. Andersen FA (ed). Amended final report of the safety assessment of dibutyl adipate as used in cosmetics. *Int J Toxicol.* 2006; 25(suppl 1):129-134.
6. Vasihtha AK, Trivedi RK, Das G. Sebacic acid and 2-Octanol from castor oil. *J Am Oil Chem Soc.* 1990;67(5):333-337.
7. Kroschwitz J., *ed. Kirk-Othmer Concise Encyclopedia of Chemical Technology.* 4th ed. 2001. New York, NY: Wiley; 1999.
8. Nghiem NP, Davison BH, Thompson JE. Effect of biotin on the production of succinic acid by anaerobiospirillum succinicproducents. Gov't Reports Announcements & Index. *Appl Biochem Biotechnol.* 1996;57-58(1):633-639.
9. Kadesch, Richard G. Dibasic Acids. *J Am Oil Chem Soc.* 1954; 31(11):568-573.
10. Breathnach AS. Azelaic acid - biological activities and therapeutic applications. *Drug Today.* 1989;25(7):463-472.
11. Gupta AK, Gover MD. Azelaic acid (15% gel) in the treatment of acne rosacea. *Int J Dermatol.* 2007;46(5):533-538.
12. Kadesch, Richard G. Dibasic Acids. *J Am Oil Chem Soc.* 1954; 31(11):568-573.
13. Ayorinde FO, Powers FT, Streete LD, Shepard RL, Tabi TN. Synthesis of dodecanedioic acid from *Vernonia galamensis* oil. *J Am Oil Chem Soc.* 1989;66(5):690-692.

14. The Merck Index. http://themerckindex.cambridgesoft.com/TheMerckIndex/index.asp. Accessed October 20, 2009.
15. US Environmental Protection Agency. EPI Suite (for Windows). Washington DC. 2009.
16. Kirk-Othmer. *Concise Encyclopedia of Chemical Technology*. 4th ed. New York, NY: Wiley; 1999.
17. International Agency for Research on Cancer (IARC). Di(2-ethylhexyl) adipate. *IARC Monogr Eval Carcinog Risk Chem Hum*. 2000;77:149-175.
18. Kvatadze MK. The gas-chromatographic detection of sebacic acid in the air. *Gig Tr Prof Zabol*. 1989;9:45-46.
19. Gottschalck TE, Bailey J (eds). *International Cosmetic Ingredient Dictionary and Handbook*. 13th ed. Washington DC: Personal Care Products Council; 2010.
20. Food and Drug Administration. Frequency of use of cosmetic ingredients. FDA Database. Washington DC.
21. Personal Care Products Council. *Concentration of Use Information: Sebacic Acid and Related Ingredients*. June 11, 2009. Unpublished data provided by the Personal Care Products Council.
22. Personal Care Products Council. *Concentration of Use - Diisobutyl Succinate and Diisobutyl Glutarate*. October 13, 2010. Unpublished data provided by the Personal Care Products Council.
23. Personal Care Products Council. *Concentration of Use of Dicarboxylic Acids and Their Salts and Esters*. February 25, 2010. Unpublished data provided by the Personal Care Products Council.
24. European Commission. Cosing database on cosmetic ingredient and substances. http://ec.europa.eu/consumers/cosmetics/cosing/. Accessed July 27, 2010.
25. Dekker. Ingredients. The Chemistry and Manufacture of Cosmetics. 1999. 1II:(2).
26. Food and Drug Administration. GRAS status sof succinic acid. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=339. Accessed July 28, 2010.
27. Food and Drug Administration. GRAS status of adipic acid. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=9. Accessed June 20, 2010.
28. Food and Drug Administration. Azelaic Acid 20% Cream—Drug Approval Label. http://www.accessdata.fda.gov/drugsatfda_docs/label/2003/20428slr016_azelex_lbl.pdf. Accessed June 20, 2010.
29. Food and Drug Administration. Azelaic Acid 15% Gel—Drug Approval Label. http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/021470s003lbl.pdf. Accessed June 20, 2010.
30. Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. Priming in systemic plant immunity. *Science*. 2009;324(5923):89-91.
31. Schneider KW. Contact dermatitis due to diethyl sebacate. *Cont Derm*. 1980;6:506-507.
32. Shikani AH, Eisele DW, Domb AJ. Polymer delivery of chemotherapy for squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg*. 1994;120(11):1242-1247.
33. Kroha K. Industrial biotechnology provides opportunities for commercial production of new lon-chain dibasic acids. *Inform*. 2004;15(9):568-571.
34. Bergman K, Albanus L. Di-(2-ethylhexyl) adipate: absorption, autoradiographic distribution and elimination in mice and rats. *Food Chem Toxicol*. 1987;25(4):309-316.
35. Mochida K, Gomyoda M, Fujita T. Acetyl tributyl citrate and dibutyl sebacate inhibit the growth of cultured mammalian cells. *Bull Environ Contamin Toxicol*. 1996;56(4):635-637.
36. Greco AV, Mingrone G. Dicarboxylic acids, an alternate fuel substrate in parental nutrition: an update. *Clin Nutr*. 1995;14(3):143-148.
37. Bertuzzi A, Gandolfi A, Salinari S, Mingrone G, Greco AV. Pharmacokinetics of dicarboxylic acids in man. *Eng Med Biol*. 1994;13:472-478.
38. Nguyen QH, Bui TP. Azelaic acid: pharmacokinetic and pharmacodynamic properties and its therapeutic role in hyperpigmentary disorders and acne. *Int J Dermatol*. 1995;34(2):75-84.
39. Organisation for Economic Co-Operation and Development. SIDS Initial Assessment for SIAM 22 -Malonic Acid Diesters, Dimethylmalonate, 108-59-8, and Diethylmalonate, 105-53-3. http://www.chem.unep.ch/irptc/sids/OECDSIDS/Malonates.pdf. Accessed July 29, 2010.
40. Organisation for Economic Co-Operation and Development. IUCLID data set for adipic acid. http://www.chem.unep.ch/irptc/sids/OECDSIDS/124049.pdf. Accessed July 29, 2010.
41. Kennedy GL. Toxicity of adipic acid. *Drug Chem Toxicol*. 2002;25(2):191-202.
42. Passi S, Nazzaro-Porro M, Picardo M, Mingrone G, Fasella P. Metabolism of straight saturated medium chain length (C9 to C12) dicarboxylic acids. *J Lipid Res*. 1983;24(9):1140-1147.
43. Akhavan A, Bershad S. Topical acne drugs: review of clinical properties, systemic exposure, and safety. *Am J Clin Dermatol*. 2003;4(7):473-492.
44. Allergan Inc. [Package Insert] AZELEX—Azelaic Acid cream (20%); 2004.
45. Passi S. Pharmacology and pharmacokinetics of azelaic acid. (Secondary reference in Breathnach 1999). *Rev Comtemp Pharmacother*. 1993;4:441-447.
46. Tataranni PA, Mingrone G, DeGaetano A, Raguso C, Greco AV. Tracer study of metabolism and tissue distributon of sebacic acid in rats. *Ann Nutr Metab*. 1992;36(5-6):296-303.
47. Favuzzi AM, Mingrone G, Bertuzzi A, et al. Pharmacokinetics of sebacic acid in rats. *Eur Rev Med Pharmacol Sci*. 1999;3(3):119-125.
48. Mingrone G, Greco AV, Castegneto M, et al. Tissue uptake and oxdation of disodium sebacate in man. *J Parenter Enteral Nutr*. 1991;15(4):454-459.
49. Greco AV, Mingrone G, Raguso C, et al. Metabolic effects and disposition of sebacate, an latemate dicarboxylic fuel substrate. *Ann Nutr Metab*. 1992;36(1):1-11.
50. Mingrone G, Tacchino RM, Castagneto M, Finotti E, Greco AV. Use of even-numbered carbon atom dicarboxylic salts in parenteral nutrition as fuel substrate. *JPEN J Parenter Enteral Nutr*. 1992;16(1):32-38.
51. Mingrone G, Greco AV, Tataranni PA, DeGaetano A, Castagneto M. Pharmacokinetic profile of dodecanedioic acid, a proposed alternative fuel substrate. *J Parenter Enteral Nutr*. 1994;18(3):225-230.
52. Del Rosso JQ, Lehman PA, Raney SG. Impact of order of application of moisturizers on percutaneous absorption kinetics: evaluation of sequential application of moisturizer lotions and azelaic

acid gel 15% using a human skin model. *Cutis*. 2009;83(3): 119-124.
53. Täuber U, Weiss C, Matthes H. Percutaneous absorption of azelaic acid in humans. *Exp Dermatol*. 1992;1(4):176-179.
54. Greco AV, Mingrone G, Arcieri Mastromattei E, Finotti E, Castegneto M. Toxicity of disodium sebacate. *Drug Exp.Clin. Res*. 1990;16(10):531-536.
55. Environmental Protection Agency. Robust summary for dicarboxylic acid category. http://www.epa.gov/HPV/pubs/summaries/dicarbx/c13108.pdf. Accessed July 19, 2010.
56. Organisation for Economic Co-Operation and Development. SIDS report on Dodecanedioic Acid, CAS No. 693-23-2. http://www.chem.unep.ch/irptc/sids/OECDSIDS/693232.pdf. Accessed July 29, 2010.
57. Organisation for Economic Co-Operation and Development. SIDS Initial Report for SIAM 18 - Adipic Acid, CAS No. 124-04-9. http://www.chem.unep.ch/irptc/sids/OECDSIDS/124049.pdf. Accessed July 29, 2010.
58. Maekawa A, Todate A, Onodera H, et al. Lack of toxicity/carcinogenicity of monosodium succinate in F344 rats. *Food Chem Toxicol*. 1990;28(4):235-241.
59. Mingrone G, Greco AV, Navarro-Porro M, Passi S. Toxicity of azelaic acid. *Drugs Exp Clin Res*. 1983;9:447-455.
60. Organisation for Economic Co-Operation and Development. SIDS Initial Assessment for SIAM 16 - Disodium Succinate, CAS No. 150-90-3. http://www.chem.unep.ch/irptc/sids/OECDSIDS/150903.pdf. Accessed July 29, 2010.
61. Lee KP, Valentine R, Bogdanffy MS. Nasal lesion development and reversibility in rats exposed to aerosols of dibasic esters. *Toxicol Pathol*. 1992;20(3 part 1):376-393.
62. Robins EJ, Breathnach AS, Ward BJ, et al. Effect of dicarboxylic acids on Harding-Passey and Cloudman S91 melanoma cells in tissue culture. *J Invest Dermatol*. 1985;85(3):216-221.
63. Passi S, Picardo M, Mingrone G, Breathnach AS, Navarro-Porro M. Azelaic Acid - biochemistry and metabolism. *Acta Derm Venereol Suppl (Stockh)*. 1989;143:8-13.
64. Detmar M, Mayer-da-Silva A, Stadler R, Orfanos CE. Effects of azelaic acid on proliferation and ultrastructure of mouse keratinocytes in vitro. *J Invest Dermatol*. 1989;93(1):70-74.
65. Nazzaro-Porro M, Passi S, Zina G, Bernengo A, Breathnach A. Effect of azelaic acid on human malignant melanoma. *Lancet*. 1980;1(8178):1109-1111.
66. Breathnach AS. Azelaic acid: potential as a general antitumoural agent. *Med Hypotheses*. 1999;52(3):221-226.
67. Moody DE, Reddy JK. Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds. *Toxicol Appl Pharmacol*. 1978;45(2):497-504.
68. Mingrone G, Manicelli R, Metro D. Influence of sodium salts of saturated medium chain length C6, C9, C10 and C12 dicarboxylic acids on the uterine horn of rat in vitro. *Q J Exp Physiol*. 1988; 73(2):153-162.
69. Szybalski W. Special microbiological systems - observations on chemical mutagenesis in microorganisms. *Ann NY Acad Sci*. 1958;76(3):475-489.
70. National Toxicology Program. Salmonella assayon malonic acid. Study ID: 228078. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=228078&cas%5Fno=141%2D82%2D2&activetab=detail. Accessed July 26, 2010.
71. Ishidate M Jr, Sofuni T, Yoshikawa K, et al. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol*. 1984;22(8):623-636.
72. TOXNET. CCRIS database - diodium succinate. Accessed July 26, 2010.
73. National Toxicology Program. Salmonella assay on glutaric acid. Study ID: 994718. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=994718&cas%5Fno=110%2D94%2D1&activetab=detail. Accessed July 26, 2010.
74. National Toxicology Program. Salmonella assay on adipic acid. Study ID: A86767. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=A86767&cas%5Fno=124%2D04%2D9&activetab=detail. Accessed July 26, 2010.
75. TOXNET. CCRIS database - Adipic acid. CAS No. 124-04-9. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~vDEUtx:1. Accessed June 10, 2010.
76. TOXNET. CCRIS database - Dodecanedioic Acid. CAS No 111-20-6. http://toxnet.nlm.nih.gov/cgi-bin/sis/search. Accessed July 26, 2010.
77. TOXNET. CCRIS database - Sodium Succinate. CAS No. 2292-54-5. Accessed July 26, 2010.
78. Bradford JC, Brown GL, Caldwell JA, Drobeck HP. Teratology and mutagenicity studies with glutaric acid. *Teratol*. 1984;29: 19A.
79. Otoshi T, Iwata H, Yamamoto S, et al. Severity of promotion by sodium salts of succinic acid in rat urinary bladder carcinogenesis correlates with sodium ion concentration under conditions of equal urinary pH. *Carcinogenesis*. 1993;14(11):2277-2281.
80. Gattermann T. Wieland. *Praxis des Organischen Chemikers*. 40th ed. 1961.
81. Cognis Deutchsland GmbH & Co. Data Profile: Cetiol® B. 2002: 4.
82. Hokoku Corporation. Impurity data on diisopropyl sebacate. Unpublished data submitted by the Personal Care Products Council. 2010:1.
83. James AC, Stahlhofen W, Rudolf G, Annexe D, et al. Deposition of inhaled particles. *Ann ICRP*. 1994;24(1-3):231-232.
84. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113(7):823-839.
85. Andersen FA (ed). Final report on the safety assessment of Dibutyl Adipate. *J Am Coll Toxicol*. 1996;15(4):295-300.
86. American Chemistry Council Aliphatic Esters Diesters Task Group. High Production Volume (HPV) chemical challenge program test plan for th diesters category of the aliphatic esters chemicals. June 2, 2010. http://www.epa.gov/oppt/chemrtk/pubs/summaries/alipestr/c13466rt8.pdf. Accessed July 8, 0010.
87. American Chemistry Council Aliphatic Esters Diesters Task Group. High Production Volume (HPV) chemical challenge program test plan for th diesters category of the aliphatic esters chemicals. Dated June 2, 2010. http://www.epa.gov/oppt/

chemrtk/pubs/summaries/alipestr/c13466rt8.pdf. Accessed July 8, 0010.

88. Bower D. Unpublished information on hair spray particle sizes provided at the September 9, 1999 CIR Expert Panel meeting. 1999. Washington, D.C.
89. Johnson MA. The influence of particle size. *Spray Technol Market*. 2004:24-27.
90. Agner T, Serup J. Seasonal variation ofskin resistance to irritants. *Br J Dermatol*. 2010;121(3):323-328.
91. Dirven HA, Theuws JL, Jongeneelen FJ, Bos RP. Nonmutagenicity of 4 metabolites of di(2-ethylhexyl)phthalate (DEHP) and 3 structurally related derivatives of di(2-ethylhexyl)adipate (DEHA) in the Salmonella mutagenicity assay. *Mutat Res*. 1991;260(1):121-130.
92. Environmental Protection Agency. Robust summaries for substances in the HPV test plan for the diesters category of the aliphatic esters chemicals. http://www.epa.gov/oppt/chemrtk/pubs/summaries/alipestr/c13466rr8.pdf. Accessed july 8, 2010.
93. Takahashi T, Tanaka A, Yamaha T. Elimination, distribution, and metabolsim of di-(2-ethylhexyl)adipate (DEHA) in rats. *Toxicology*. 1981;22(3):223-233.
94. Cornu MC, Keith Y, Elcombe CR, Lhuguenot JC. In vivo and in vitro metabolism of di-(2-ethylhexyl) adipate a peroxisome proliferator, in the rat. *Arch Toxicol*. 1988;(suppl 12):265-268.
95. Loftus NJ, Laird WJ, Steel GT, Wilks MF, Woollen BH. Metabolism and pharmacokinetics of deuterium-labelled di-2-(ethylhexyl) adipate (DEHA) in humans. *Food Chem Toxicol*. 1993; 31(9):609-614.
96. Cornu MC, Lhuguenot JC, Brady AM, Moore R, Elcombe CR. Identification of the proximate proliferator(s) derived from di (2-ethylhexyl) adipate and species differences in response. *Biochem Pharmacol*. 1992;43(10):2129-2134.
97. Keith Y, Cornu MC, Canning PM, Foster J, Lhuguenot JC, Elcombe CR. Peroxisome proliferation due to di (2-ethylhexyl) adipate, 2-ethylhexanol and 2-ethylhexanoic acid. *Arch Toxicol*. 1992;66(5):321-326.
98. Reddy JK, Reddy MK, Usman MI, Lalwani ND, Rao MS. Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl) phthalate, and di(2-ethylhexyl) adipate with a hypolipidemic drug. *Environ Health Perspect*. 1986;65:317-327.
99. Barber ED, Astill BD, Moran EJ, et al. Peroxisome induction studies on seven phthalate esters. *Toxicol Ind Health*. 1987;3(2):7-22.
100. Lake BG. Mechanisms of hepatocarcinogenicity of peroxisome-proliferating drugs and chemicals. *Annu Rev Pharmacol Toxicol*. 1995;35:483-507.
101. Lake BG, Price RJ, Cunninghame ME, Walters DG. Comparison of the effects of di-(2-ethylhexyl)adipate on hepatic peroxisome proliferation and cell replication in the rat and mouse. *Toxicology*. 1997;123(3):217-226.
102. Tomaszewski KE, Agarwal DK, Melnick RL. Invitro steady-state levels of hydrogen eroxide after exposure of male F344 rats and female B6C3F1 mice to hepatic peroxisome proliferators. *Carcinogenesis*. 1986;7(11):1871-1876.
103. von Däniken A, Lutz WK, Jäckh R, Schlatter C. Investigation of the potential for binding of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate to liver DNA *in vivo*. *Toxicol Appl Pharmacol*. 1984;73(3):373-387.
104. Büsser MT, Lutz WK. Stimulation of DNA synthesis in rat and mouse liver by various tumor promoters. *Carcinogenesis*. 1987; 8(10):1433-1437.
105. Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y. Significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats following short-term exposure to the peroxisome proliferators di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate. *Jpn J Cancer Res*. 1990;81(3):213-215.
106. Kawashima Y, Hanioka N, Matsumura M, Kozuka H. Induction of microsomal stearoyl-CoA desaturation by the administration of various peroxisome proliferators. (Secondary reference in IARC 2000). *Biochim Biophys Acta*. 1983; 752(2):259-264.
107. Kawashima Y, Nakagawa S, Tachibana Y, Kozuka H. Effects of peroxisome proliferators on fatty acid-binding protein in rat liver. (Secondary reference in IARC 2000). *Biochim Biophys Acta*. 1983;754:21-27.
108. Yanagita T, Satoh M, Nomura H, Enomoto N, Sugano M. Alteration of hepatic phospholipids in rats and mice by feeding di-(2-ethylhexyl)adipate and di-(2-ethylhexyl)phthalate. (Secondary reference in IARC 2000.). *Lipids*. 1987; 22(8):572-577.
109. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. (Secondary reference in IARC 2000). *J Biol Chem*. 1998;273(27): 16710-16714.
110. Organisation for Economic Co-Operation and Development. SIDS Initial Report for Siam 4 - Dibutyl Adipate CAS No. 105-99-7. http://www.chem.unep.ch/irptc/sids/OECDSIDS/105997.pdf. Accessed July 29, 2010.
111. Smyth HF, Carpenter CP, Weil CS. Range-finding toxicity data: List IV. *AMA Arch Ind Hyg Occup Med*. 1949;4(2):119-122.
112. EviC-CEBA. Study of acute toxicity and local tolerance (acute oral, acute dermal, and ocular irritation) on diisopropyl adipate. Study report: Te 297/98-1589 and Te 298/98-1589. Unpublished data submitted by the Council, May 27. 1998:4.
113. Organisation for Economic Co-Operation and Development. SIDS Initial Report for SIAM 10 - Bis(2-ethylhexyl) Adipate (DEHA), CAS No. 103-23-1. http://www.chem.unep.ch/irptc/sids/OECDSIDS/103231.pdf. Accessed July 29, 2010.
114. EviC-CEBA. Attestation of biological test (acute oral toxicity and dermal irritation) on diisodecyl adipate. Study ref. T 207/4076. Unpublished data submitted by the Council, May 9, 1994:2.
115. EviC-CEBA. Attestation of biological significance (acute oral toxicity and dermal irritation) on dioctyldodecyl adipate. Study ref. T 207/4071. Unpublished data submitted by the Council, May 9, 1994:2.
116. EviC-CEBA. Attestation of biological significance (acute oral toxicity and dermal irritation) on diisocetyl adipate. Study ref. T 207/4073. Unpublished data submitted by the Council, April 7, 1994:2.

117. Personal Care Products Council. Summary of safety data on diethyl sebacate (primary skin irritation, cumulative skin irritation, contact allergenicity). 1989:8.
118. EviC-CEBA. Attestation of biological significance (acute oral toxicity and dermal irritation) on diethylhexyl sebacate. Study ref. T 207/4072. Unpublished data submitted by the Council, April 7, 1994:2.
119. AMA Laboratories. Primary eye irritation of liquiwax DIADD (Dioctyldodecyl Dodecanedioate). AMA Lab No. WPAT90-59/PE5982.II. Unpublished data submitted by theCouncil, December 3, 1990:5.
120. AMA Laboratories. Acute oral toxicity of Liquiwax DlCDD (Diisocetyl Dodecanedioate). AMA Ref No: WPAT90-59/OT6098.Il. Unpublished data submitted by the Council, January 3, 1991:3.
121. ExxonMobil Chemical Company. High Production Volume (HPV) Chemical Challenge Program. Test plan for the alkyl alcohols C6-C13 Category. http://www.epa.gov/HPV/pubs/summaries/alkal613/c13590rs.pdf. Accessed 2010.
122. Smith CC. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate, and methoxyethyl oleate. *AMA Arch Ind Hyg Occup Med.* 1953;7(4):310-318.
123. AMA Laboratories. Acute dermal toxicity - limit test - Liquiiwax DlADD (Dioctyldedecyl Dodecanediaote). AMA Ref No. WPAT93-155/AD1076.BII. Unpublished data submitted by Council, June 14, 1993:4.
124. Singh AR, Lawrence WH, Autian J. Embryonic-fetal toxicity and teratogenic effects of adipic acid esters in rats. *J Pharm Sci.* 1973;62(10):1596-1600.
125. Wato E, Asahiyama M, Suzuki A, Funyu S, Amano Y. Collaborative work on evaluation of ovarian toxicity. 9) Effects of 2- or 4-week repeated dose studies and fertility study of di(2-ethylhexyl)adipate (DEHA) in female rats. *J Toxicol Sci.* 2009; 34(special issue 1):SP101-SP109.
126. Consumer Product Testing. Primary dermal irritation in rabbits, primary ocular irritation in rabbits of Schermcemol DlS (Diisopropyl Sebacate). Experiment Reference Number: 91055. (Material tested as supplied - approx. 100%). 1991:3.
127. MB Research Laboratories. EpiOcular MTT viability assay of a cream containing 1.2% Diethylhexyl Sebacate. 2003:10.
128. AMA Laboratories. Primary eye irritation of liquiwax DICDD (Diisocetyl Dodecanedioate). AMA Ref No: WPAT90-59/PE6098.Il. Unpublished data submitted by the Council, December 22, 1990:3.
129. AMA Laboratories. Acute oral toxicity of Liquiwax DIADD (Dioctyldodecyl Dodecanedioate). AMA Ref No. WPAT90-59/OT5982.lI. Unpublished data submitted by the Council, December 13, 1990:3.
130. Heldreth BA. Final report on methyl acetate, simple alkyl acetate estes and related alcohols (Draft). Report in progress. Available from the Cosmetic Ingredient Review. 2010.
131. AMA Laboratories. Primary skin irritation of liquiwax DlADD (Dioctyldodecyl Dodecanediaote.) AMA Ref No. WPAT90-59/PS5982.ll. Unpublished data submitted by the Council December 3, 1990:4.
132. AMA Laboratories. Primary skin irritation of liquiwax DICDD (Diisocetyl Dodecanedioate). Ref: WPAT90-59/PS6098.II. Unpublished data submitted by the Council, December 22, 1990:3.
133. Opdyke DLJ. Fragrance raw materials monographs - supplement to earlier monographs on fragrance materials. 1973:1079.
134. AMA Laboratories. Guinea pig maximization test (Kligman) on liquiwax DlADD (Dioctyldodecyl Dodecanedioate). AMA Ref No: WPAT93-GPMB1076.Bll. Unpublished data submitted by the Council, June 14, 1993:6.
135. AMA Laboratories. 50 Human subject repeat insult patch test skin irritation/sensitization evaluation on Liquiwax DlSA (Diisostearyl Adipate), DlCDD (Diisocetyl Dodecanedioate), and DIADD (Dioctyldodecyl Dodecanedioate). AMA Ref No: WP96-BERN1-18/RIPT4618.BIl. Unpublished data submitted by the Council, April 11, 1996.
136. Anonymous. Clinical evaluation report: human patch test of a body cream containing 1.5% diethyl sebacate. Unpublished data submittd by the Personal Care Products Council. 2003. Accessed August 18, 2009
137. KGL Inc. An evaluation of the contact-sensitization potential of a topical coded product (body cream containing 1.5% diethyl sebacate) in human skin by means of the maximization assay. 2003:10.
138. Anonymous. Clinical evaluation report: human patch test of a foundation containing 1.8% diisopropyl sebacate. Unpublished data submitted by the Council, August 18, 209. 2000.
139. Product Investigations Inc. Determination of the irritating and sensitizing propensities of Schercemol DlS (Diisopropyl Sebacate) on human skin. Report: Pll No. 19745. (Material tested as supplied - approx. 100%). 2005:12.
140. KGL Inc. An evaluation of the contact-sensitization potential of a topical coded product (foundation containing 2.2% diisopropyl sebacate) in human skin by means of the maximization assay. 2006:10.
141. Reliance Clinical Testing Services. Summary of HRIPT of 2 heat protection hair sprays containing 1% Diisopropyl Sebacate. 2007:1.
142. Essex Testing Clinic Inc. Summary of HRIPT of a heat protection hair spray containing 7.2% Diisopropyl Sebacate. 2007.
143. Andersen FA (ed). Final report on the safety assessment of Methyl Alcohol. *Injt J Toxicol.* 2001;20(suppl 1):57-85.
144. Stillman MA, Maiback HI, Shalita AR. Relative irritancy of free fatty acids of different chain length. *Contact Derm.* 1975;1(2): 65-69.
145. Wahlberg JE, MAibach HI. Nonanoic acid irrigation: a positive control at routine patch testing? *Contact Dermatitis.* 1980;6(2): 128-130.
146. Agner T, Serup J. Nonanoic acid irrigation: a positive contorl at routine patch testing? *Contact Dermatitis.* 1987;17(4):20-211.
147. Willis CM, Stephens JM, Wilkinson JD. Experimentally-induced irritant contact dermatitis. Determination of optimum irritant concentrations. *Contact Dermatitis.* 2010;18(1):20-24.
148. Agner T, Serup J. Contact thermography for assessment of skin damage due to experimental irritants. *Acta Derm Venereol.* 1988;68(2):192-195.

149. Damato JM, Martin DM, Fehn PA. Allergic contact sensitization test of a spray concentrate containining 80.74% isopropyl alcohol. 1979.
150. Anonymous. Final report repeated insult patch test of a hair dye base (3373) containing 2.85% isopropyl alcohol and 1.95% isopropyl acetate. Unpublished data submitted by teh Council. 2010.
151. Suihko C, Serup J. Fluorescence confocal laser scanning microscopy for *in vivo* imaging of epidermal reactions to two experimental irritants. *Skin Res Technol*. 2008;14(4):498-503.
152. Elder RL (ed). Final report on the safety assessment of cetearyl alcohol, cetyl alcohol, lsostearyl alcohol, myristyl alcohol, and behenyl alcohol. *J Am Coll Toxicol*. 1988;7(3):359-413.
153. Elder RL (ed). Final report on the safety assessment of stearyl alcohol, oleyl alcohol, and octyl dodecanol. *J Am Coll Toxicol*. 1985;4(5):1-29.
154. Kimura M, Kawada A. Contact dermatitis due to diethyl sebacate. *Contact Dermatitis*. 1999;40(1):48-49.
155. Narita T, Oiso N, Ota T, Kawara S, Kawada A. Allergic contact dermatitis due to diethyl sebacate in a hand cream. *Contact Dermatitis*. 2006;55(2):117.
156. Sasaki E, Hata M, Aramaki J, Honda M. Allergic contact dermatitis due to diethyl sebacate. *Contact Dermatitis*. 1997;36(3): 172.
157. Soga F, Katoh N, Kishimoto S. Contact dermatitis due to lanoconazole, cetyl alcohol and diethyl sebacate in lanoconazole cream. *Contact Dermatitis*. 2004;50(1):49-50.
158. Tanaka M, Kobayashi S, Murata T, Tanikawa A, Nishikawa T. Allergic contact dermatitis from diethyl sebacate in lanoconazole cream. *Contact Dermatitis*. 2000;43(4):233-234.
159. De Groot AC, Conemans JM, Schutte T. Contact allergy to diisopropyl sebacate in zineryt lotion. *Contact Dermatitis*. 1991;25(4):260-261.
160. Dooms-Goossens A, De Veyhlder H, De Boulle K, Maertens M. Allergic contact dermatitis due to diisopropyl sebacate. *Contact Dermatitis*. 1986;15(3):192.
161. Environmental Protection Agency. Integrated Risk Information System. Di(2-ethylhexyl)adipate. (CASRN 103-23-1). N:\C1R\DicarboxylicAcids\DatabaseSearchResults\EPA\IR1S-searched7-8-10_updatred3-16-10.mht. Integrated Risk Information System. Accessed July 8, 2010.
162. Dalgaard M, Hass U, Vinggaard AM, et al. Di(2-ethylhexyl) adipate (DEHA) induced developmental toxicity but not antiandrogenic effects in pre- and postnatally exposed Wistar rats. *Reprod Toxicol*. 2003;17(2):163-170.
163. Kang JS, Morimura K, Toda C, et al. Testicular toxicity of DEHP, but not DEHA, is elevated under conditions of thioacetamide-induced liver damage. *Reprod Toxicol*. 2006;21(3):253-259.
164. Alvarez L, Driscoll C, Kelly DP, Staples RE, Chromey NC, Kennedy GL Jr. Developmental toxicity of dibasic esters by inhalation in the rat. *Drug Chem Toxicol*. 1995;189(4):295-314.
165. Kelly DP, Kennedy GL KEENAN CMJr, . Reproduction study with dibasic esters following inhalation in the rat. *Drug Chem Toxicol*. 1998;21(3):253-267.
166. Miyata K, Shiraishi K, Houshuyama S, et al. Subacute oral toxicity study of di(2-ethylhexyl)adipate based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". *Arch Toxicol*. 2006;80(4):181-186.
167. Ghisari M, Bonefeld-Jorgensen EC. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicol Lett*. 2009;189(1):67-77.
168. Andersen PH, Jensen NJ. Mutagenic investigation of flavourings: dimethyl succinate, ethyl pyruvate and aconitic acid are negative in the Salmonella/mammalian-microsome test. *Food Addit Contam*. 1984;1(3):283-288.
169. National Toxicology Program. Salmonella assay on dimethyl succinate. Study 1D: 947738. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=947738&cas%5Fno=106%2D65%2D0&activetab=detail. Accessed July 26, 2010.
170. National Toxicology Program. Salmonella assay on dimethyl glutarate. Study ID A20348. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=A20348&cas%5Fno=1119%2D40%2D0&activetab=detail. Accessed July 26, 2010.
171. National Toxicology Program. Salmonella assay on dimethyl adipate. Study 1D: A45330. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=A45330&cas%5Fno=627%2D93%2D0&activetab=detail. Accessed July 26, 2010.
172. TOXNET. CCRIS database - Diisobutyl Adipate. CAS No. 141-04-8. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~p2E3uo:6. Accessed July 26, 2010.
173. Simmon VF, Kauhanen K, Tardiff RG. Mutagenic activity of chemicals identified in drinking water. *Dev Toxicol Environ Sci*. 1977;2:249-258.
174. Warren JR, Lalwani ND, Reddy JK. Phthalate esters as peroxisome proliferator carcinogens. *Environ Health Perspect*. 1982; 45:35-40.
175. Zeiger E, Haworth E, Mortelmans S, et al. Mutagenicity testing of di(2-ethylhexyl) phthalate and related chemicals in Salmonella. *Environ Mutagen*. 1985;7(2):213-232.
176. National Toxicology Program. Salmonella assay eith diethylhexyl adipate. Study 1D: 963935. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=963935&cas%5Fno=103%2D23%2D1&activetab=detail. Accessed July 26, 2010.
177. Seed JL. Mutagenic activity of phthalate estes in bacterial seed liquid suspension assays. *Environ Health Perspect*. 1982;45: 111-114.
178. Galloway SM, Armstrong MJ, Rueben C, et al. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluation of 108 chemicals. *Environ Mol Mutagen*. 1987;10(suppl 10):1-175.
179. Reisenbichler H, Eckl PM. Genotoxic effects of selected peroxisome proliferators. (Secondary reference in 1ARC 2000). *Mutat Res*. 1993;286:135-144.
180. McGregor DB, Brown A, Cattanach P, et al. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: 111. 72 coded chemicals. (Secondary reference in 1ARC 2000). *Environ Mol Mutagen*. 1988;12(1):85-154.

181. DiVincenzo GD, Hamilton ML, Mueller KR, Donish WH, Barber ED. Bacterial mutagenicity testing of urine from rats dosed with 2-ethylhexanol derived plasticizers. *Toxicology*. 1985; 34(3):247-259.
182. McKee RH, Lington AW, Traul KA. An evaluation of the genotoxic potential of di-isononyl adipate. *Environ Mutagen*. 1986; 8(6):817-827.
183. Wild D, King MT, Gocke E, Eckhardt K. Study of artificial flavoring substances for mutagenicity in the salmonella/microsome, basc and micronucleus tests. *Food Chem Toxicol*. 1983; 21(6):707-719.
184. TOXNET. CCRIS database - Dibutyl Sebacate. CAS No. 109-43-3. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~LfAk2H:2. Accessed July 26, 2010.
185. TOXNET. CCRIS database - Diethylhexyl Sebacate. CAS NO. 122-62-3. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~J98xpD:1. Accessed July 26, 2010.
186. National Toxicology Program. Micronucleus test on dimethyl succinate. Study ID: A71006. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=micronucleus.micronucleusData&endpointlist=MN&current%5Fstrain%5Fid=F344&cas%5Fno=106%2D65%2D0&study%5Fno=A71006&activetab=detail. Accessed July 26, 2010.
187. National Toxicology Program. Micronucleus test on dimetyl glutarate. Study ID A36863. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=micronucleus.micronucleusData&cas_no=1119%2D40%2D0&endpointlist=MN. Accessed July 26, 2010.
188. National Toxicology Program. Micronucleus test on dimethyl adipate. Study ID: A86146. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=micronucleus.micronucleusData&current%5Fstrain%5Fid=F344&endpointlist=MN&cas%5Fno=627%2D93%2D0&study%5Fno=A86146&activetab=detail. Accessed July 26, 2010.
189. Shelby MD, Erexson GL, Hook GH, Tice RR. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ Mol Mutagen*. 1993;21(2): 160-179.
190. National Toxicology Program. Chromosome aberration assay of diethylhexyl adipate. Study ID: 959525. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invivoca.casummary&study_no=959525&cas_no=103%2D23%2D1&endpointlist=CA. Accessed July 26, 2010.
191. Shelby MD, Witt KL. Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ Mol Mutagen*. 1995;25(4):302-313.
192. Osterle D, Deml E. Promoting activity of di(2-ethylhexyl)phthalate in rat liver foci bioassay. *J Cancer Res Clin Oncol*. 1988; 114(2):133-136.
193. Kluwe WM. Carcinogenic potential of phthalic acid esters and related compounds: structure-activity relationships. *Environ Health Perspect*. 1986;65:271-278.
194. Hasegawa R, Ito N. Liver medium-term bioassay in rats for screening of carcinogenesis and modifying factors in hepatocarcinogenesis. *Food Chem Toxicol*. 1992;30(11):979-992.
195. Ziel K, Yelverton CB, Balkrishnan R, Feldman SR. Cumulative irritation potential of metronidazole gel compared to azelaic acid gel after repeated applications to healthy skin. *J Drugs Dermatol*. 2005;4(6):727-731.
196. Feldman S, Careccia RE, Barham KL, Hancox J. Diagnosis and treatment of acne. *Am Fam Physician*. 2004;69(9):2123-2130.
197. Fukui S, Shimoyama T, Tamura K, Yamamura M, Satomi M. Mucosal blood flow and generation of superoxide in rat experimental colitis induced by succinic acid. *J Gastroenterol*. 1997; 32(4):464-471.
198. Brown-Woodman PD, Post EJ, Chow PY, White IG. Effects of malonic, citric and caffeic acids on the motility of human sperm and penetration of cervical mucous. *Int J Fertil*. 1985;30(3): 38-44.
199. EviC-CEBA. Attestation of biological significance (acute oral toxicity and dermal irritation) on diisopropyl sebacate. Study ref. T 207/4078. Unpublished data submitted by the Council, April 7, 1998:2.

2017 FDA VCRP RAW DATA

01C - Other Baby Products	MALIC ACID	8
02A - Bath Oils, Tablets, and Salts	MALIC ACID	2
03B - Eyeliner	MALIC ACID	3
03E - Eye Makeup Remover	MALIC ACID	1
04E - Other Fragrance Preparation	MALIC ACID	1
05A - Hair Conditioner	MALIC ACID	45
05B - Hair Spray (aerosol fixatives)	MALIC ACID	2
05E - Rinses (non-coloring)	MALIC ACID	1
05F - Shampoos (non-coloring)	MALIC ACID	35
05G - Tonics, Dressings, and Other Hair Grooming Aids	MALIC ACID	5
05I - Other Hair Preparations	MALIC ACID	12
06C - Hair Rinses (coloring)	MALIC ACID	9
06D - Hair Shampoos (coloring)	MALIC ACID	4
07E - Lipstick	MALIC ACID	4
07I - Other Makeup Preparations	MALIC ACID	1
08A - Basecoats and Undercoats	MALIC ACID	1
08B - Cuticle Softeners	MALIC ACID	2
08E - Nail Polish and Enamel	MALIC ACID	9
08G - Other Manicuring Preparations	MALIC ACID	3
10A - Bath Soaps and Detergents	MALIC ACID	5
10E - Other Personal Cleanliness Products	MALIC ACID	8
12A - Cleansing	MALIC ACID	13
12C - Face and Neck (exc shave)	MALIC ACID	13
12D - Body and Hand (exc shave)	MALIC ACID	9
12F - Moisturizing	MALIC ACID	17
12G - Night	MALIC ACID	4
12H - Paste Masks (mud packs)	MALIC ACID	5
12J - Other Skin Care Preps	MALIC ACID	16
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SODIUM MALATE	1
06G - Hair Bleaches	SODIUM MALATE	1
12A - Cleansing	SODIUM MALATE	1
12F - Moisturizing	SODIUM MALATE	1
12J - Other Skin Care Preps	SODIUM MALATE	1

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 19, 2011

SUBJECT: Summaries of Studies of Products Containing Malic Acid

Please note that the two studies summarized under Human Irritation and Human Sensitization are the same two studies.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

HUMAN IRRITATION								
Test Material	**Test Material Concentration**	**pH of test material**	**Active Malic Acid Amount**	**Test Pop.**	**Procedure**	**Study Dates**	**Results**	**Testing Facility**
Malic acid (50% solution) in hair styler (2.2725% malic acid	1%	3.6	0.022725%	101 Subjects	Modified Human Repeat Insult Patch Test. A three phase regimen was used. Induction phase of 21 days, a rest period of no treatment for 10-24 days, and then a challenge phase of 4 days. Semi-occlusive patches were used	01/17/2011 – 02/25/2011	Not predicted to be significant skin irritant. Possibly mild test agent. Standardized cumulative irritation= 1181.5, Negative control (undosed patch) = 45.6, Positive control (1% SLS) = 2052.8.	Stephens & Associates Colorado Springs, CO 80915
Malic acid (50% solution) in hair shampoo (0.75% malic acid)	0.5%	3.0	0.00375%	98 Subjects	Modified Human Repeat Insult Patch Test. A three phase regimen was used. Induction phase of 21 days, a rest period of no treatment for 10-24 days, and then a challenge phase of 4 days. Occlusive patches were used	05/27/2003 – 07/28/2003	Predicted to be a moderate skin irritant. Standardized cumulative irritation= 965.1, Negative control (distilled water) = 33, Positive control (0.1% SLS) = 1307.76.	RCTS, Inc. Irving, TX 75062

HUMAN SENSITIZATION								
Test Material	**Test Material Concentration**	**pH of test material**	**Active Malic Acid Amount**	**Test Pop.**	**Procedure**	**Study Dates**	**Results**	**Testing Facility**
Malic acid (50% solution) in hair styler (2.2725% malic acid	1%	3.6	0.022725%	101 Subjects	Modified Human Repeat Insult Patch Test. A three phase regimen was used. Induction phase of 21 days, a rest period of no treatment for 10-24 days, and then a challenge phase of 4 days. Semi-occlusive patches were used	01/17/2011 – 02/25/2011	Solution did not induce allergic contact dermatitis in any subject completing the study	Stephens & Associates Colorado Springs, CO 80915
Malic acid (50% solution) in hair shampoo (0.75% malic acid)	0.5%	3.0	0.00375%	98 Subjects	Modified Human Repeat Insult Patch Test. A three phase regimen was used. Induction phase of 21 days, a rest period of no treatment for 10-24 days, and then a challenge phase of 4 days. Occlusive patches were used	05/27/2003 – 07/28/2003	Solution did not induce allergic contact dermatitis in any subject completing the study	RCTS, Inc. Irving, TX 75062

In Vitro Ocular Irritation								
Test Material	**pH of test material**	**Test**	**Study Dates**	**Active Malic Acid Amount**	**CAMVA Results RC50 (%)/ CI (95%)**	**BCOP Results *In Vitro* Score/ Opacity Score/ Permeability Score**	**Prediction**	**Testing Facility**
Malic acid (50% solution) in hair styler (2.2725% malic acid)	3.6	Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability Tests (BCOP)	01/10/2011 – 03/15/2011	2.2725%	>50%/NA	74.3/69.9/0.295	Predicted to be a severe ocular irritant	Institute for In Vitro Sciences Gaithersburg, MD 20878
Malic acid (50% solution) in hair shampoo (0.75% malic acid)	3.0	Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability Tests (BCOP)	06/16/2003 – 08/13/2003	2.2725%	1.6/ (0.62-3.9)	9.09/5.4/0.246	Predicted to be an ocular irritant	MB Research, Spinnerstown, PA 18968

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 8, 2016

SUBJECT: Concentration of Use by FDA Product Category: Malic Acid and Sodium Malate

Concentration of Use by FDA Product Category – Malic Acid and Sodium Malate

Ingredient	Product Category	Maximum Concentration of Use
Malic Acid	Bath oils, tablets and salts	0.006-50%
Malic Acid	Eyeliners	0.000012%
Malic Acid	Hair conditioners	0.00025-4%
Malic Acid	Hair sprays Pump spray	 2.1%
Malic Acid	Permanent waves	0.0003%
Malic Acid	Rinses (noncoloring)	2.5%
Malic Acid	Shampoos (noncoloring)	0.00013-0.5%
Malic Acid	Tonics, dressings and other hair grooming aids	0.00013-1.9%
Malic Acid	Other hair preparations (noncoloring)	2%
Malic Acid	Hair dyes and colors	0.05%
Malic Acid	Hair rinses (coloring)	0.00015-0.01%
Malic Acid	Foundations	0.0007%
Malic Acid	Lipstick	0.0006-0.06%
Malic Acid	Nail creams and lotions	0.3%
Malic Acid	Dentifrices	0.029%
Malic Acid	Mouth washes and breath fresheners	0.55%
Malic Acid	Bath soaps and detergents	0.0085-0.95%
Malic Acid	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.005-0.15%
Malic Acid	Depilatories	1%
Malic Acid	Face and neck products Not spray	 0.001-1%
Malic Acid	Body and hand products Not spray Spray	 0.0004-0.8% 0.0011%
Malic Acid	Paste masks and mud packs	0.002-2%
Malic Acid	Skin fresheners	0.5%
Malic Acid	Other skin care preparations	0.0002-0.03%
Malic Acid	Suntan products Not spray	 0.0033-1%
Sodium Malate	Other skin care preparations	0.02%

Information collected 2015-2016

Table prepared February 17, 2016