Amended Safety Assessment of Salicylates as Used in Cosmetics

Status: Re-Review for Panel Review
Release Date: May 23, 2018
Panel Date: June 4-5, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, 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 Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 23, 2018
Subject: Re-Review Document on Salicylates

A CIR Final Report on Salicylic Acid and 16 salicylates (See Re-Review Document for complete list of ingredients) was published in 2003. The conclusion in that safety assessment states that these ingredients are safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

In accordance with its Procedures, the CIR evaluates the conclusions of previously-issued reports every 15 years; therefore a re-review document on Salicylates (*salicy062018rep*) has been prepared and is attached for the Panel's review. Also, according to the CIR Procedures, if the Panel concludes that a re-review is warranted, they may consider adding ingredients during the re-review process. Furthermore, if the Panel concludes that the data in the original Final Report substantially address the safety of the expanded list of ingredients, a Tentative Amended Report shall be issued that includes a summary of the data in the original Final Report plus all available new published and unpublished data for the expanded list of ingredients. Additionally, it should be noted that 2018 use concentration data (*salicy062018data1 and salicy062018data2*) on the salicylates were received from the Council, and that these data have been added to the re-review document and are attached for the Panel's review.

Also included in this package for your review are the CIR report history (*salicyp062018hist*), flow chart (*salicy062018flow*), literature search strategy (*salicy062018strat*), ingredient data profile (*salicy062018prof*), 2018 FDA VCRP data (*salicy062018FDA*), published CIR Final Report on Salicylates (*salicy062018prev*), and minutes from the 1999 and 2000 CIR Expert Panel meetings (*salicy062018min*).

The Panel is being asked to review the safety test data summarized in the re-review document to determine whether or not there are any new safety concerns that warrant re-opening the published Final Report to issue a revised conclusion. Please note that an overall summary of the available safety test data is not included and that the data summarized are not tabulated in this version of the report. The next report version will be organized in this manner.

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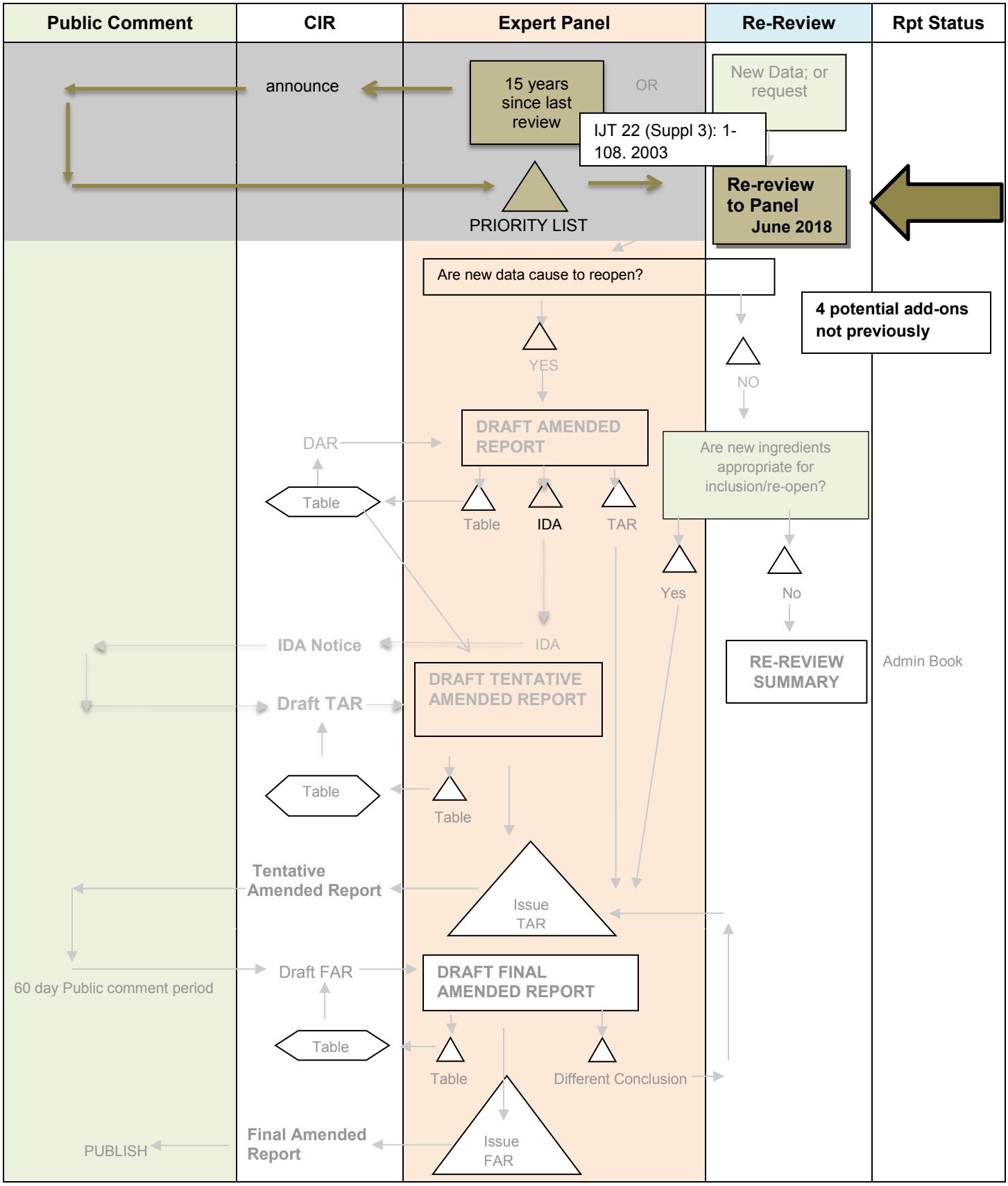
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Salicylates

MEETING June 2018



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

CIR History of:

Salicylates

Salicylic Acid, Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, TEA-Salicylate, Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Octyl Salicylate,Tridecyl Salicylate, Butyloctyl Salicylate, and Hexyldodecyl Salicylate

Draft Report, Teams/Panel: September 9-10, 1999

The combined list of data requests (both Teams) is as follows:
(1) A risk assessment for developmental/reproductive toxicity of concentrations delivered by cosmetic products alone and in combination with salicylic acid from other common sources (e.g., acne medications, aspirin, etc.)
(2) Additional uses intended by industry, i.e., exfoliant use
(3) Dermal irritation data using pH vs. concentration (like in the AHA report)
(4) Studies similar to those requested for the AHA report examining the effect of use and sun exposure, i.e., sunburn cell or pyrimidine dimer studies

Draft Report, Teams/Panel: February 14-15, 2000

The Panel voted unanimously in favor of issuing a Tentative Report with the conclusion that Salicylic Acid and its salts and esters are safe as used when formulated to avoid irritation, and when formulated to avoid increased sun sensitivity. It was also concluded that if enhanced sun sensitivity is expected, then directions for use including the daily use of sun protection should be provided.

Draft Final Report, Teams/Panel: September 11-12, 2000

The Panel voted unanimously in favor of issuing a Final Report on this group of ingredients with the following conclusion: Based on the available information, the CIR Expert Panel concluded that Salicylic Acid, the salts Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate, the esters Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate, and Tridecyl Salicylate, and the compounds Butyloctyl Salicylate and Hexyldodecyl Salicylate are safe as used when formulated to avoid irritation and when formulated to avoid increasing sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

Rereview, Teams/Panel: June 4-5, 2018

Data Profile on Salicylates for June 4th-5th, 2018 Panel – Wilbur Johnson

	Dermal Penetration			Nail Penetration	Penetration Enhancement	ADME					Acute Toxicity			Short-Term Toxicity	Sub-Chronic Toxicity	Chronic Toxicity	DART		Genotoxicity	Carcinogenicity	Other Relevant Studies		Dermal Irritation*	Dermal Sensitization /Photosensitization		Ocular Irritation *		Clinical Studies	Case Reports/ Clinical Reports (other)		Epidemiology Studies
	In Vivo -Animal	In Vitro	In Vivo-Human	In Vitro-Human	In Vitro-Animal	In Vitro	Animal-Dermal	Animal-Oral	Animal-Inhalation	Human-Oral	Animal-Dermal	Animal-Oral	Animal-Injection	Animal	Animal	Animal	In Vitro	In Vivo	In Vitro/In Vivo	In Vivo	In Vitro	In Vivo-Animal/Human	Animal/Human/In vitro	Animal/In Vitro/In Silico	Human	In Vitro	Animal	Human-MulticenterStudy	Human-Dermal	Human-Inhalation	Human
Butyloctyl Salicylate								X			X			X					X		X		X	X			X				
Calcium Salicylate																															
C12-15 Alkyl Salicylate																															
Capryloyl Salicylic Acid																													X		
Ethylhexyl Salicylate		X	X				X	X			X	X							X		X	X	X	X	X		X		X		
Hexyldodecyl Salicylate																															
Isocetyl Salicylate																															
Isodecyl Salicylate								X							X				X				X				X				
Magnesium Salicylate								X		X																					
Methyl Salicylate	X	X	X				X	X	X	X	X	X		X	X	X		X	X	X		X	X	X	X		X		X		
Myristyl Salicylate																															
Potassium Salicylate																															
Salicylic Acid	X	X	X		X			X		X	X			X			X	X	X	X		X	X	X	X			X	X		
Sodium Salicylate	X	X						X		X				X	X		X	X	X	X		X			X	X		X		X		
TEA-Salicylate			X																				X								
Tridecyl Salicylate								X			X								X				X	X			X				
Add - Ons																															
Amyl Salicylate														X	X								X	X				X	X		
Hexyl Salicylate								X			X	X											X	X	X			X			
Isotridecyl Salicylate																															
Silver Salicylate																															

X = data

[Salicylates–4/6/2018]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECET-OC	Web
Butyloctyl Salicylate	190085-41-7	1	116/0	1/1	1/1		No	1996 data	No									
Calcium Salicylate	824-35-1	1	12/0	5/0	4/2		Yes	No	No									
C12-15 Alkyl Salicylate		1	1/1	1/1	1/1		No	No	No									
Capryloyl Salicylic Acid	78418-01-6	1	156/4	6/4	1/1		No	No	No									
Ethylhexyl Salicylate	118-60-5	1	52/10	41/3	19/1		Yes	1990 data	No									
Hexyldodecyl Salicylate	220778-06-3	1	8/0	1/1	1/1		No	No	No									
Isocetyl Salicylate	138208-68-1	1	15/1	1/1	1/1		No	No	No									
Isodecyl Salicylate	85252-25-1	1	20/1	1/1	1/1		No	No	No									
Magnesium Salicylate	18917-89-0; 551-37-1	1	36/2	14/1	1/1		Yes	No	No									
Methyl Salicylate	119-36-8	1	427/34	19/3	60/1		No	60s and 70s data	No									
Myristyl Salicylate	19666-17-2	1	15/1	1/1	1/1		No	No	No									
Potassium Salicylate	578-36-9	1	205/0	3/1	1/1		Yes	No	No									
Salicylic Acid	69-72-2	1	331/21	105/3	176/7		Yes	70s and 90s data	No									
Sodium Salicylate	54-21-7	1	283/8	29/2	19/2		Yes	80s and 2000s data	No									
TEA-Salicylate	2174-16-5	1	310/4	1/1	2/1		Yes	No	No									
Tridecyl Salicylate	19666-16-1	1	30/0	2/1	1/1		No	No	No									
ADD ONS - Below																		
Amyl Salicylate	2050-08-0	1	487/13	4/1	4/1	Yes	No	Yes	No									
Hexyl Salicylate	6259-76-3	1	45/15	10/3	2/1	Yes	No	Yes	No									
Isotridecyl Salicylate	1863871-63-9	1	1/0	0/0	0/0	Yes	No	No	No									
Silver Salicylate	19025-97-9	1	149/5	3/0	1/1	Yes	No	No	No									

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*] years 1999-2018 for previously reviewed ingredients; all years for 4 new ingredients

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

LINKS

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

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

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

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

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

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

September 9-10, 1999 (72nd) Meeting of the CIR Expert Panel

Salicylic Acid, Calcium Salicylate, Magnesium Salicylate,
MEA-Salicylate, Potassium Salicylate, Sodium Salicylate,
TEA-Salicylate, Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate,
Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate,
Myristyl Salicylate, Octyl Salicylate,Tridecyl Salicylate, Butyloctyl Salicylate,
and Hexyldodecyl Salicylate

Dr. Schroeter said that his Team determined that additional data are needed to complete the safety assessment on this group of ingredients. He noted that the data requests on this group of ingredients, also known as beta hydroxy acids, would be similar to those that were issued on the alpha hydroxy acids. Dr. Schroeter's Team issued the following informal data request:

(1) Data to determine dermal irritation that is pH-dependent, as well as concentration-dependent
(2) Data that determines the possibility of promotion of carcinogenicity, such as a dermal sunburn cell study of pyrimidine dimers and/or MEDs to determine the photosensitivity that may occur
(3) Request that industry submit information on any additional uses or expected uses of these ingredients

Dr. Schroeter indicated that a discussion of his Team's concern regarding the use of these ingredients on children and any toxicity needs to be developed, but this can be done at a later date, after data requested informally have been reviewed.

Dr. Belsito said that no additional studies on irritancy are needed because of data indicating that these ingredients can be used at a level that is nonirritating. He also said that the Panel could take the same approach that was used during its review of alpha hydroxy acids, to indicate that products containing these ingredients should be formulated so as to be nonirritating. Dr. Belsito indicated that the following data are needed:

(1) Risk assessment for teratogenicity
(2) Some type of study (similar to alpha hydroxy acid sunburn cell study or thymidine dimers study)
(3) Update on the ways in which these ingredients are being used in cosmetics

The combined list of data requests (both Teams) is as follows:
(1) A risk assessment for developmental/reproductive toxicity of concentrations delivered by cosmetic products alone and in combination with salicylic acid from other common sources (e.g., acne medications, aspirin, etc.)
(2) Additional uses intended by industry, i.e., exfoliant use
(3) Dermal irritation data using pH vs. concentration (like in the AHA report)
(4) Studies similar to those requested for the AHA report examining the effect of use and sun exposure, i.e., sunburn cell or pyrimidine dimer studies

Dr. Andersen said that the list of data requests will be provided to Dr. McEwen as an informal request for industry data.

Dr. Belsito noted that his Team had also discussed the possible exclusion of MEA and TEA Salicylate from the present report, given the ongoing research activities on the ethanolamines.

The Panel agreed that MEA and TEA Salicylate should remain in the present report.

February 14-15, 2000 (74th) Meeting of the CIR Expert Panel

Salicylic Acid, Calcium Salicylate, Magnesium Salicylate,
MEA-Salicylate, Potassium Salicylate, Sodium Salicylate,
TEA-Salicylate, Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate,
Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate,
Myristyl Salicylate, Octyl Salicylate, Tridecyl Salicylate, Butyloctyl Salicylate,
and Hexyldodecyl Salicylate

Dr. Belsito noted that the following data on these ingredients were requested (informal data request) at the September 9-10, 1999 Panel meeting:

(1) A risk assessment for developmental/reproductive toxicity of concentrations delivered by cosmetic products alone and in combination with salicylic acid from other common sources (e.g., acne medications, aspirin, etc.)
(2) Additional uses intended by industry, i.e., exfoliant use
(3) Dermal irritation data using pH vs. concentration (like in the AHA report)
(4) Studies similar to those requested for the AHA report examining the effect of use and sun exposure, i.e., sunburn cell or pyrimidine dimer studies

Dr. Belsito also recalled that data on mutagenicity, phototoxicity, and skin irritation potential were received since the September Panel meeting. After considering these data along with the ingredient use concentration data, Dr. Belsito's Team concluded that Salicylic Acid and the other ingredients in this group are safe as used when formulated to avoid irritation and when formulated to avoid increased sun sensitivity. Furthermore, it was concluded that if these ingredients have an effect on sun sensitivity, it is expected that directions for use would include the daily use of sun protection.

Dr. Shank noted that the new data indicate that exposure to low ingredient concentrations in cosmetics leads to blood levels that would be considered insignificant.

Concerning the issue of exfoliant use, Dr. Bailey recalled that data on sunburn cell formation and MED's were included in the Panel's original request for data. He wanted to know whether the Panel plans to issue a safe as used conclusion in the absence of these data.

Dr. Belsito noted that Salicylic Acid and its salts are sunscreens to some extent. He also speculated that if the sunburn cell study were done, the results would indicate either no increase in sun sensitivity or protection against sun exposure; however, concern about the need for photoprotection would remain. Dr. Belsito also considered that the study results may be similar to those reported for AHA's, which would serve as the basis for restrictions/qualifications (proposed by Belsito Team) relating to the safe use of Salicylic Acid and its salts and esters.

Dr. Bailey expressed the view that the expectation is that industry will test products to determine whether or not there is any increase in sun sensitivity. This would entail the performance of both MED and sunburn cell studies.

The Panel voted unanimously in favor of issuing a Tentative Report with the conclusion that Salicylic Acid and its salts and esters are safe as used when formulated to avoid irritation, and when formulated to avoid increased sun sensitivity. It was also concluded that if enhanced sun sensitivity is expected, then directions for use including the daily use of sun protection should be provided.

September 11-12, 2000 (76th) Meeting of the CIR Expert Panel

Salicylic Acid, Calcium Salicylate, Magnesium Salicylate,
MEA-Salicylate, Potassium Salicylate, Sodium Salicylate,
TEA-Salicylate, Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate,
Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate,
Myristyl Salicylate, Octyl Salicylate, Tridecyl Salicylate, Butyloctyl Salicylate,
and Hexyldodecyl Salicylate

Dr. Belsito stated that a tentative conclusion on the safety of these ingredients was issued at the February 14-15, 2000 Panel meeting. He then indicated that it has been requested that the Panel consider adding the ingredient, Amyl Salicylate to this review, with the understanding that this is the only salicylate listed in the International Cosmetic Ingredient Dictionary and Handbook that is not included. However, Dr. Belsito's Team noted that Amyl Salicylate is listed only as being used as a fragrance ingredient in cosmetics, and that assessing the safety of ingredients that function only as fragrance materials is not within the Panel's purview. Dr. Belsito recalled that Benzyl Salicylate (used as fragrance ingredient and UV light absorber) also is not included in the present review.

Dr. Schroeter asked for Dr. McEwen's opinion on the proposed addition of other salicylates to the present review.

Dr. McEwen said that one might expect that Amyl Salicylate might be used in a fashion that is similar to that of the other salicylates in the group. He added that because Benzyl Salicylate is an aromatic compound, it probably is not in the same family of use.

Dr. McEwen stated that his reason for requesting the addition of Amyl Salicylate to the present review is based on the observation that the data already in the report are applicable to this ingredient. He added that if an additional function(other than that of a fragrance material) is assigned to this ingredient in the future, it would then be a candidate for the CIR review process. Thus, issuing a conclusion on Amyl Salicylate now would be more feasible.

Dr. Andersen said that the CIR Procedures are very specific in terms of exempting fragrance ingredients from the review process, and that this is the reason why Amyl Salicylate is not included in the report that is being reviewed.

Dr. Bergfeld asked if it would be appropriate to include information on the chemistry of Amyl Salicylate in the current report even though its safety in cosmetics is not being evaluated, and to also indicate why this decision was made in the report discussion.

Dr. Andersen said that the rationale for excluding Amyl Salicylate from this review could be stated in the introduction and report discussion, and that the chemical structure could also be included in the report.

The Panel agreed that the current report should be revised to reflect the preceding comments on Amyl Salicylate by Drs. Bergfeld and Andersen.

The Panel voted unanimously in favor of issuing a Final Report on this group of ingredients with the following conclusion: Based on the available information, the CIR Expert Panel concluded that Salicylic Acid, the salts Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate, the esters Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate, and Tridecyl Salicylate, and the compounds Butyloctyl Salicylate and Hexyldodecyl Salicylate are safe as used when formulated to avoid irritation and when formulated to avoid increasing sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

Amended Safety Assessment of Salicylates as Used in Cosmetics

Status: Re-Review for Panel Review
Release Date: May 23, 2018
Panel Date: June 4-5, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, 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 Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst

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INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) published a Safety Assessment of Salicylic Acid and 16 salicylates in 2003.[1] Based on the available data, the Panel issued the following conclusion: based on the available information, the CIR Expert Panel concluded that Salicylic Acid, the salts Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA- Salicylate; the esters Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate; and Tridecyl Salicylate, and the compounds Butyloctyl Salicylate and Hexyldodecyl Salicylate are safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection. The complete report is available on the CIR website. (http://www.cir-safety.org/ingredients) 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.

Also, according to the CIR Procedures, if the Panel concludes that a re-review is warranted, the Panel may consider adding ingredients during the re-review process. Furthermore, if the Panel concludes that the data in the original Final Report substantially address the safety of the expanded list of ingredients, a Tentative Amended Report shall be issued that includes a summary of the data in the original Final Report plus all available new published and unpublished data for the expanded list of ingredients. The following ingredients, in addition to those included in the original Final Report, are proposed herein for review: Amyl Salicylate, Hexyl Salicylate, Isotridecyl Salicylate, and Silver Salicylate. The addition of these ingredients is being proposed because Amyl Salicylate, Hexyl Salicylate, and Isotridecyl Salicylate are esters of Salicylic Acid, and Silver Salicylate is a salt of Salicylic Acid. All four add-ons are structurally similar to ingredients in the original report. The expanded list of ingredients (from the original Final Report + proposed additions) appears below:

Butyloctyl Salicylate
Calcium Salicylate
C12-15 Alkyl Salicylate
Capryloyl Salicylic Acid
Ethylhexyl Salicylate
Hexyldodecyl Salicylate
Isocetyl Salicylate
Isodecyl Salicylate
Magnesium Salicylate
MEA-Salicylate
Methyl Salicylate
Myristyl Salicylate
Potassium Salicylate
Salicylic Acid
Sodium Salicylate
TEA-Salicylate
Tridecyl Salicylate
Amyl Salicylate*
Hexyl Salicylate*
Isotridecyl Salicylate*
Silver Salicylate*

*Proposed add-ons

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI *Dictionary*), some of the functions that are associated with this group of salicylates include hair and skin conditioning agents, and, less frequently, preservatives and fragrance ingredients.[2] The complete list of functions is presented in Table 1.

The published data in this re-review document were identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that CIR evaluates, is available on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

Excerpts from the summaries of the 2003 report 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.) The original reports that was published in 2003 is available on the CIR website (http://www.cir-safety.org/ingredients).

A published toxicological and dermatological assessment of salicylates, when used as fragrance ingredients, by the Research Institute for Fragrance Materials (RIFM) is available, and the RIFM Expert Panel's lengthy conclusion on these fragrance ingredients (some of which are not included in this CIR safety assessment), are stated at the end of this report.[3] This conclusion is based, in part, on a review of safety test data on some of the salicylates included in this safety assessment that were available before and after issuance of the CIR published Final Report.

After reviewing the available new data on the original group of ingredients and the available data on the proposed 4 additional ingredients, the Panel will determine whether or not the published Final Report should be re-opened to revise the original conclusion. If there is no reason to amend the original conclusion, the Panel should then decide whether or not the Final Report should be reopened to add the 4 proposed salicylates to the safety assessment.

CHEMISTRY

Definition and General Characterization

Salicylic Acid, a lipophilic mono-β-hydroxybenzoic acid, is a colorless, crystalline organic acid that can be derived from salicin (a β-glucoside in willow bark; similar to aspirin (acetylsalicylic acid); Figure 1). The rest of the ingredients in this report (salicylates) are esters or salts comprising, in part, Salicylic Acid (Figure 2). However, there is one exception, Capryloyl Salicylic Acid (Figure 3), wherein the ester is actually the product of caprylic acid and *the hydroxyl group* of Salicylic Acid. The definitions of the salicylates that are being reviewed in this safety assessment are included in Table 1.

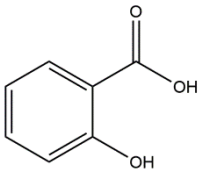


Figure 1. Salicylic Acid

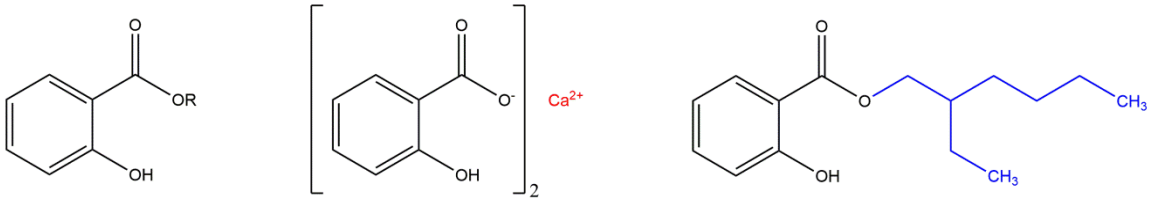


Figure 2. Salicylates generic structure (wherein R is a salt cation or an alcohol residue), and examples: Calcium Salicylate and Ethylhexyl Salicylate

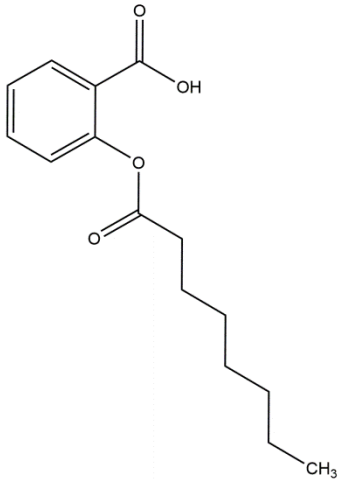


Figure 3. Capryloyl Salicylic Acid

Chemical and Physical Properties

Chemical and physical properties of Salicylic Acid and salicylates are presented in Table 2.[4,5,6,7]

Silver Salicylate

Silver Silicylate is a promoter in the classical Koenigs-Knorr reaction for the synthesis of glycosides.[8]

Method of Manufacture

Amyl Salicylate

According to one method in the literature, the production of primary normal Amyl Salicylate, a mixture of Salicylic Acid, primary normal amyl alcohol, and concentrated sulfuric acid is heated under a reflux condenser for approximately 4 h.[9] After the unreacted alcohol had been removed by distillation at atmospheric pressure, the residue is washed with 10% potassium carbonate and dissolved in ether, and the ether solution is dried over anhydrous sodium sulfate. The high-boiling material that remains after removal of the ether is fractionated under reduced pressure. The Amyl Salicylate fraction boils at 116 to 121ºC and 1.4 mmHg.

According to another source, Amyl Salicylate can be synthesized from Salicylic Acid and n-pentanol, using sodium hydrogen sulfate as a catalyst.[10]

USE

Cosmetic

The safety of the cosmetic ingredients included in this safety assessment is evaluated based on data received from the United States (U.S.) Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics.[11] 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 cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2018 VCRP data, the following salicylates in this safety assessment are being used in cosmetic products: Butyloctyl Salicylate, Capryloyl Salicylic Acid, Ethylhexyl Salicylate, Isodecyl Salicylate, Magnesium Salicylate, Methyl Salicylate, Salicylic Acid, Sodium Salicylate, TEA-Salicylate, Tridecyl Salicylate, Amyl Salicylate (addition to safety assessment proposed), and Hexyl Salicylate (addition to safety assessment proposed).[11] The greatest use frequency of 3,474 uses is reported for Ethylhexyl Salicylate, followed by 1,300 reported uses for Salicylic Acid. Reported use frequencies for the remaining ingredients are ≤ 165. When 2018 VCRP data are compared with 1998 VCRP data from the published CIR on salicylates, the uses of Ethylhexyl Salicylate in 1998 (83 uses) increased to 3,474 uses (~ 42 x greater) in 2018 and the uses of Salicylic Acid in 1998 (107 uses) increased to 1300 uses (~ 12 x greater) in 2018. Furthermore, in 1998, there were no reported uses of Magnesium Salicylate, but 10 uses are being reported in 2018.

The results of a concentration of use survey conducted in 2018 indicate that Capryloyl Salicylic Acid is being used at concentrations up to 62.9% in leave-on products (body and hand products [not spray]), which is the highest maximum use concentration that is being reported for the salicylates that are being reviewed in this safety assessment.[12] The following use concentration data are included in the published CIR final report on salicylates: Salicylic Acid (≤ 3%), Butyloctyl Salicylate (≤ 5%), Capryloyl Salicylic Acid (≤1%), Isocetyl Salicylate (≤ 5%), Methyl Salicylate (≤ 0.6%), Ethylhexyl Salicylate (≤ 8%), Sodium Salicylate (≤ 2%), TEA Salicylate (≤ 0.75%), and Tridecyl Salicylate (0.01%).[1] Further use frequency and concentration of use data are presented in Table 3.

The 2018 VCRP data indicate that the following salicylates are not being used in cosmetic products:

Calcium Salicylate
C12-15 Alkyl Salicylate
Hexyldodecyl Salicylate
Isocetyl Salicylate
Myristyl Salicylate
Potassium Salicylate
Isotridecyl Salicylate*
Silver Salicylate*

*Proposed add-ons

Cosmetic products containing salicylates may be applied to the skin or, incidentally, may come in contact with the eyes. These ingredients are also applied to mucous membranes, and could be incidentally ingested. Products containing salicylates may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Both Ethylhexyl Salicylate and Salicylic Acid are being used in aerosolized hair sprays and in fragrance preparations, which may result in incidental inhalation exposure. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[13,14,15,16] Therefore, most droplets/particles incidentally inhaled

from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[13,14]

Ethylhexyl Salicylate is used in face and dusting powders and Salicylic Acid is used in face powders. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[17,18,19]

Noncosmetic

Methyl Salicylate

Non-aspirin salicylates (i.e., not acetyl salicylic acid), such as methyl salicylate, are found in many over-the-counter brands of creams, ointments, lotions, liniments and medicated oils intended for topical application to relieve musculoskeletal aches and pains.[20]

Salicylic Acid

Salicylic Acid is a non-steroidal anti-inflammatory drug (NSAID) that is a precursor is a precursor of aspirin.[21] The FDA has issued a final rule for over-the-counter (OTC) drug products that permits the use of Salicylic Acid as an active ingredient at concentrations of 0.5 to 2%. [21 CFR 333.310]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Ethylhexyl Salicylate

A mathematical method was used to estimate the total body absorption of some salicylate esters including Ethylhexyl Salicylate.[22] Rate constants were calculated from the relevant physicochemical properties. The applied dose of the active ingredient used in the simulation was 40µg/cm^2 based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1µm/cm^2/h. The simulations were conducted on a 12-h time scale. The estimated total body absorption of Ethylhexyl Salicylate was 0.022 µg/1.4 m^2 at 2 h, 0.5 µg/1.4 m^2 at 6 h, and 3.3 µg/1.4 m^2 at 12 h.

The skin penetration of a sunscreen formulation containing Ethylhexyl Salicylate was evaluated using human full-thickness skin (from 3 women) that was mounted in a Franz diffusion cell with a receptor volume of 12.4 ml.[23] The sunscreen formulation tested was either in an oil-in-water emulsion gel or in petrolatum jelly. The receptor compartment was filled with an aqueous solution containing sodium chloride (0.9%) and bovine serum albumin (1.5%). The cell allowed skin (1.76 cm^2) to be exposed to the sunscreen formulation (in oil-in-water emulsion or in petrolatum jelly), and the formulation (3.0 ± 0.4 µg/cm^2) was applied to the skin for either 30 min or 6 h. Each value for skin penetration is reported as the mean value (n = 4). After either duration, Ethylhexyl Salicylate was not detected in the dermis. At 30 min, the value for skin penetration of Ethylhexyl Salicylate into the epidermis was 0.4 µg/cm^2, and 0.2% of the applied dose was detected in the epidermis. At 6 h, the value for penetration of Ethylhexyl Salicylate (in oil-in-water emulsion gel) into the epidermis was 0.4 µg/cm^2, and 0.2% of the applied dose was detected in the epidermis. The 6-h value for skin penetration of Ethylhexyl Salicylate (in petrolatum jelly) into the epidermis was 0.6 µg/cm^2, and 0.3% of the applied dose was detected in the epidermis.

Methyl Salicylate

The skin penetration of Methyl Salicylate was evaluated using rat full-thickness skin (cleared of excess subcutaneous tissue) from male Wistar rats.[24] The skin was cut into 15 x 15 mm pieces and mounted in Franz-type glass diffusion cells (surface area = 1.3 cm^2). The receptor fluid consisted of degassed, 20% ethanol:80% distilled water. A formulation containing 20% Methyl Salicylate (1 g) was placed on the skin and receptor fluid was removed and replaced over a 28-h period. Approximately 25% of the Methyl Salicylate that was absorbed through the skin was hydrolyzed to salicylate. At 24 h, the total amount of salicylate that penetrated through the skin was within 20%.

In vitro skin penetration tests on Methyl Salicylate were performed using fresh dermatomed (0.3 to 0.4 mm thick) female breast skin and leg skin in Bronaugh flow-through polytetrafluoroethylene diffusion cells.[25] Each dose of the test

substance was applied to a 0.38 cm^2 skin area in each cell. Exposure of skin samples to Methyl Salicylate was described as brief (30-min), and there was a 6.5-h reservoir collection period. The skin penetration of Methyl Salicylate was described as rapid. There was 32% absorption at the low dose (2 mM Methyl Salicylate), 17% absorption at the medium dose (20 mM Methyl Salicylate), and 11% absorption at the high dose (200 mM Methyl Salicylate).

Percutaneous absorption of Methyl Salicylate was evaluated in the isolated perfused porcine skin flap (IPPSF).[26,27] A dose of 400 µg/cm^2 of radio labeled [^{14}C]-Methyl Salicylate was applied non-occluded to a 7.5 cm^2 Stomadhesive® dosing template on the IPPSF. Skin flaps were allowed to equilibrate for 1 h prior to chemical application. A total of 16 flaps were dosed and terminated at 2, 4, or 8 h. Percutaneous absorption into IPPSF was 2.39% of the applied dose at 8 h. With the amount in skin and fat added, the penetration was 3.04% of the applied dose. The rate of absorption was also evaluated. Radiolabeled Methyl Salicylate showed a rapid absorptive flux profile that peaked at approximately 30 min at 0.016% dose/min.

The ester cleavage of Methyl Salicylate to Salicylic Acid in hairless mouse skin, in vitro, following topical application of 1% Methyl Salicylate in acetate buffer to the skin was evaluated.[28] Less than 5% of the applied dose was metabolized to Salicylic Acid.

Salicylates

In vitro data on pig, mouse, and rat skin indicate that salicylates are percutaneously absorbed.[1]

Animal

Methyl Salicylate

Twenty-seven 10-week-old Yorkshire-Landrace cross barrow pigs were used in a skin absorption study.[29] A circular plastic cup with two holes pierced through it to accept an 18-gauge needle was positioned over a piece of gauze cloth that was cut to a diameter slightly smaller than the cup and that was placed over the skin. Four sites were challenged including ear, epigastrium, perineum, and inguinal crease with total area of exposure of 49.3, 132.4, 49.3 and 88.2 cm^2, respectively. Neat Methyl Salicylate was introduced into the cup through one of the holes at volumes of 848µl for the ear, 2544 µl for the epigastrium, 848 µl for the perineum and 1696 µl for the inguinal crease. Arterial blood samples were taken every 10 min for the first 60 min and then every 15 min up to 360 min. The average dose absorbed through the skin at the ear region after 6 h was 11 µg/cm^2; at the perineum regions, the average dose absorbed was 8 µg/cm^2, and, through the epigastrium and inguinal crease regions, the average dose absorbed was 3 µg/cm^2. The initial flux (permeation rate) of Salicylic Acid through the skin after application of neat Methyl Salicylate was 0.063 µg/cm^2/ min at the ear region, 0.025 µg/cm^2/ min at the epigastrium region, 0.044µg/cm^2/ min at the perineum region and 0.012 µg/ cm^2/ min at the inguinal crease region.

Salicylates

In vivo percutaneous absorption data on rabbits, guinea pigs, rats, mice (including hairless mice), dogs, and monkeys are available.[1] *These data describe the following percutaneous absorption patterns: rate of penetration is proportional to concentration applied; absorption is dependent on the vehicle (e.g., ethanol > water); absorption varies as a function of pH; and absorption is greater through damaged skin when compared to normal skin. Approximately 10% of applied salicylates can remain in the skin.*

Human

Ethylhexyl Salicylate

The skin penetration of two Ethylhexyl Salicylate sunscreen formulations was evaluated in a study involving 6 subjects.[23] Penetration was determined by tape-stripping. Each sunscreen formulation was applied to 2 x 2 cm areas on the volar side of the forearm. At 30 minutes post-application, the remaining product formulation was removed from the skin using cotton swabs and the skin was tape-stripped 16 times. The mean value for Ethylhexyl Salicylate (in oil-in-water emulsion gel; n = 6) penetration into the stratum corneum was 28.4 ± 6.6 µg/cm^2, and 25.6% of the applied dose penetrated into the stratum corneum. The mean value for Ethylhexyl Salicylate (in petrolatum jelly; n =6) was 10.1 ± 3.5 µg/cm^2, and 11% of the applied dose penetrated into the stratum corneum. The authors noted that the concentration of Ethylhexyl Salicylate in the upper part of the stratum corneum was significantly higher (p value not stated) after application of the emulsion gel formulation than after application of the petrolatum jelly formulation. In the deeper parts of the stratum corneum, the concentration of Ethylhexyl Salicylate delivered form the emulsion gel formulation was significantly lower (p value not stated), but still greater than that achieved with the petrolatum jelly formulation.

Methyl Salicylate

The systemic exposure to Methyl Salicylate following the application of a number of adhesive patches (each containing 74.88 mg Methyl Salicylate) to the skin of 8 human subjects was evaluated.[30] The patches remained in place for 8 h. Blood samples were obtained for up to 12 h after placement of the patches. Exposure was quantified by determining the plasma concentration time profiles of the substance as a function of exposure to 2, 4, or 8 patches (or to very high doses). Data were presented as a plot of the average plasma concentration-time data as a function of dose. For the 2-patch application, the average maximum plasma concentration (C_{max}) value for Methyl Salicylate was 8.6 ± 3.8 ng/mL (range: 4.0-12.7 ng/mL). For the 4-patch application, the average C_{max} for Methyl Salicylate was 16.8 ± 6.8 ng/mL (range: 8.9-25.7 ng/mL). For the 8-patch application, the average C_{max} was 29.5 ± 10.5 ng/mL (range: 15.8-45.9 ng/mL). The authors noted that although it was not possible to determine the absolute dermal bioavailability of Methyl Salicylate, there appeared to be relatively low systemic exposure, even when an unrealistically large number of patches were applied for an unusually long time.

Salicylates

Data describing the penetration of salicylates through human skin are available.[1] These data describe the following percutaneous absorption patterns: rate of penetration is proportional to concentration applied; absorption is dependent on the vehicle (e.g., ethanol > water); absorption varies as a function of pH; and absorption is greater through damaged skin when compared to normal skin. Approximately 10% of applied salicylates can remain in the skin.

Penetration Enhancement

Salicylic Acid

Salicylic Acid is reported to enhance percutaneous penetration of vitamin A, ammoniated mercury, and triamcinolone acetonide, but not methyl nicotinate, (which itself rapidly penetrates the skin), hydrocortisone, diflucortolone-21-valerate, or cyclosporine.[1]

Absorption, Distribution, Metabolism, and Excretion

Animal

Dermal

Methyl Salicylate

The in vivo absorption of a formulation containing 20% Methyl Salicylate was studied using groups of 3 male Wistar rats.[24] The formulation (1 g) was applied to a 9.6 cm^2 area of abdominal skin, and a blood sample was removed from the tail vein at 0.5, 1, 2, 4, and 6 h thereafter. After 6 h, the animals were killed, the formulation was removed from the skin, and tissue samples (skin, subcutaneous tissue, superficial muscle, deep muscle, and fat) were excised. The levels of unhydrolyzed Methyl Salicylate in tissues below the treated site were low, i.e., only 2 to 3 µg/ml throughout the study period. The highest concentrations were observed in the dermal and subcutaneous sites in the first hour of application. At 0.5 to 1 h after application of the formulation, there was a significant increase in the concentration of total salicylate in contralateral dermal tissue, corresponding to 4 to 5 times above the circulating systemic plasma levels. At 2 h, the dermal levels were below the observed plasma salicylate concentration. The presence of unhydrolyzed Methyl Salicylate was only observed at the 0.5 h time point. The fraction of Methyl Salicylate observed in the tissues as a proportion of total salicylate varied from 0 to 0.26. The results of this study indicate that tissue and plasma concentrations of salicylate after the application of Methyl Salicylate increased rapidly within the first hour of application.

The site specificity of the percutaneous absorption of Methyl Salicylate (undiluted liquid) was examined in anaesthetized domestic swine (27 Yorkshire-Landrace cross barrows).[29] Four different anatomical sites (ear, perineum, inguinal crease, and epigastrium) were exposed to Methyl Salicylate, and serum levels were measured over a 6-h time period. Approximately 850 µl of Methyl Salicylate was used for each 45 to 50 cm^2 of surface area that was exposed to the test substance. The control group was anesthetized, but Methyl Salicylate was not administered. Arterial blood samples were taken every 10 min for the first 60 min, and then every 15 min up to 360 min. Of the four regions investigated, the ear and the epigastrium/inguinal crease were at the extremes in terms of the dose absorbed per unit area of exposure. The dose absorbed at the ear region was 11 µg/cm^2 - min, with an initial flux of 0.063 µg/cm^2 - min. In the epigastrium region, the dose absorbed was 3 µg/cm^2, with an initial flux of 0.025 µg/cm^2 - min.

Oral

Salicylates

Extensive data from oral delivery animals studies are available.[1] Metabolism by hepatic microsomal enzyme systems conjugates salicylates to glycine, forms glucuronides, or oxidizes them to hydroxybenzoic acids.

Human

Dermal

Ethylhexyl Salicylate

The systemic absorption of a sunscreen lotion, with the following composition, after dermal application was evaluated using 9 healthy volunteers: Ethylhexyl Salicylate (5% w/v), oxybenzone (6% w/v), octocrylene (7% w/v), and octyl methoxycinnamate (7.5% w/v).[31] All of these chemicals were identified as sun screening agents. The subjects were instructed to apply the product to the entire surface of their forearms generously in accordance with their normal sun protection behavior. In practice, "13.0 (1.0) g" of sunscreen product was applied to a surface area of "1051 (60.8) cm^2". The application density of the product was 12.4 mg/cm^2. The formulation remained unoccluded for 12 h prior to removal with soap and water. Urine samples were collected before product application and at 48 h post-application. Over the period of application, only 1 to 2% of the sunscreen in the applied product was absorbed. Data comparing the absorption of each ingredient were not provided.

Hexyl Salicylate

A mathematical method was used to estimate total body absorption of some salicylate esters including Hexyl Salicylate. Rate constants were calculated from the relevant physicochemical properties.[22] The applied dose of active ingredient used in the simulation was 40 $\mu g/cm^2$ based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 $\mu m/cm^2/h$. The simulations were conducted on a 12-h time scale. The estimated total body absorption of Hexyl Salicylate per µg over 1.4 m^2 was 0.18 at 2 h, 4.1 at 6 h and 27 at 12 h.

Oral

Methyl Salicylate

Reportedly, after oral ingestion, Methyl Salicylate is readily metabolized to salicylic acid.[20] No further details were provided.

Four (1 male/3 female) adult human volunteers participated in a study that was conducted as an open label, 4-way crossover design with randomized treatment order.[32] The subjects ingested 6.7 and 20 g of a Methyl Salicylate containing cream (commercial 15% cream containing 900 or 2700 mg salicylate). Plasma was collected at 0, 20, 40, 60, 120, 240, 480, 720, and 1440 min for the determination of salicylate concentrations by TDx immunoassay. The time to reach maximum salicylate concentration (T_{max}) and the peak plasma salicylate concentration (Cp_{max}) were determined. The T_{max} for the low-dose cream (900 mg salicylate) was 2.4 h (1.5 - 4 h), and the Cp_{max} was 42 mg/l (36–51 mg/l). The T_{max} for the high-dose cream was 7 h (4 - 12 h), and the Cp_{max} was 145 mg/l (120 - 201 mg/l). As a part of the same experiment, four fasting adults ingested 1 ml of wintergreen oil (which is primarily Methyl Salicylate; 14.2 mg/kg mean). Plasma was collected for salicylate determination at 0, 20, 40, 60, 120, 240, 480, 720 and 1440 min. Time to reach maximum concentration was 2.4 h with the maximum concentration of 70 mg/l.

Salicylates

Extensive data from oral delivery human studies are available.[1] Metabolism by hepatic microsomal enzyme systems conjugates salicylates to glycine, forms glucuronides, or oxidizes them to hydroxybenzoic acids.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Ethylhexyl Salicylate

Undiluted Ethylhexyl Salicylate was applied (under occlusion) to intact or abraded skin of 4 rabbits for 24 h.[4] The animals were observed for mortality and/or clinical signs for a 14-day period. No clinical signs were observed. The dermal LD_{50} in rabbits exceeded 5.0 g/kg.

Hexyl Salicylate

Ten rabbits received a single dermal application of neat Hexyl Salicylate at 5.0 g/kg.[5] The rabbits were observed for mortality and clinical symptoms. No clinical signs were observed. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. In another study involving rabbits (number and strain not stated), the acute dermal LD_{50} for Hexyl Salicylate (concentration not stated) was > 5 g/kg.[33]

Methyl Salicylate

A single dermal application of neat Methyl Salicylate at 5 g/kg was applied for 24 h under occlusion.[6] Animals were observed for a 14-day period. No clinical signs were observed. The dermal LD_{50} in rabbits exceeded 5 g/kg based on 1/10 deaths at that dose.

Butyloctyl Salicylate, Methyl Salicylate, Salicylic Acid, and Tridecyl Salicylate

Little acute toxicity (LD_{50} in rats; > 2 g/kg) via a dermal exposure route is seen for Butyloctyl Salicylate, Methyl Salicylate, Salicylic Acid, and Tridecyl Salicylate.[1] These compare with oral LD_{50} values for Salicylic Acid in rats ranging from a low of 0.891 g/kg to a high of 1.58 g/kg; for Sodium Salicylate, between 0.9 g/kg and 1.7 g/kg; for Isodecyl Salicylate, no toxicity at levels as high as 4.83 g/kg; for Methyl Salicylate, between 0.887 g/kg and 1.25 g/kg; for Ethylhexyl (Octyl) Salicylate, > 2 g/kg; for Tridecyl Salicylate, > 1.98 g/kg; and for Butyloctyl Salicylate, > 5 g/kg. Values for acute oral toxicity in other species are consistent with these values.

Oral

Ethylhexyl Salicylate

The acute oral LD_{50} of Ethylhexyl Salicylate exceeded 5.0 g/kg based on 1/10 deaths at that dose.[4] Animals (rats and rabbits) received a single oral administration of Ethylhexyl Salicylate at a dose level of 5.0 g/kg. Mortality and/or clinical signs were observed for 14 days. One animal died on the 6th day of study. No clinical reactions were observed.

Hexyl Salicylate

The acute oral LD_{50} of Hexyl Salicylate in rats exceeded 5.0 g/kg based on 1/10 deaths (on day 4) at that dose.[5] Each animal received a single oral administration of Hexyl Salicylate. The rats were observed for mortality and/or systemic effects for 14 days. Urinary incontinence was observed at 24 h.

Methyl Salicylate

The acute oral toxicity of Methyl Salicylate was determined in ddY male mice (10/dose).[34,6] Methyl Salicylate was administered at dose levels of 1.0, 1.2, 1.3, 1.5, or 1.7 g/kg. Mice were observed for a 7-day period. One animal died at 1.0 g/kg; 2/10 died at 1.2 g/kg; 4/10 died at 1.3 and 1.5 g/kg; and 9/10 died at 1.7 g/kg. Most animal deaths occurred on day 1. The LD_{50} was calculated to be 1.39 g/kg (95% CI 1.25 - 1.54 g/kg).

Methyl Salicylate was evaluated as a part of a study investigating the development of acute myocardopathy in dogs.[35] Healthy mongrel dogs were lightly anesthetized with pentobarbital sodium. Methyl Salicylate was intragastrically administered at a dose of 0.7 g/kg. After 4 - 5 h, animals either died or were sacrificed. Increases in arterial concentrations of

plasma salicylate, potassium and lactate were seen and a period of respiratory alkalosis was initially observed followed by metabolic acidosis after three hours. Microscopy studies revealed abnormalities in the mitochondria, swelling of cardiac muscles with separation of myofibrils and bulging of sarcolemma.

Inhalation

Animal

Methyl Salicylate

Methyl Salicylate administered by inhalation exposure is not lethal in rats and mice.[1]

Short-Term Toxicity Studies

Oral

Methyl Salicylate

Inconsistent results are seen regarding bone lesions with short-term oral exposures to salicylates, but reduced growth and feed consumption are consistently seen.[1]

The acute oral toxicity of Methyl Salicylate was determined in male and female CD-1 mice (8/sex/dose).[36] Methyl Salicylate was administered in corn oil by gavage once daily for 14 days at dose levels of 0.05, 0.1, 0.25, 0.50, and 1.00 g/kg. Two females died at 0.05 g/kg; 2 females and 3 males died at 0.10 g/kg; and 1 female and 1 male died at 1.00. Clinical signs observed prior to death were piloerection and dehydration. The LD_{50} was calculated to be 1.44 g/kg/day.

As a part of an associated reproductive toxicity study, a 2-week acute study was conducted using CD-1 mice (8/sex/dose).[37] Methyl Salicylate was administered by gavage at 0.05, 0.1, 0.25, 0.5, and 1 g/kg once a day for 14 days. The animals were observed for survival, body weights, and clinical signs. The maximum tolerated dose (MTD) was determined for the associated study. No effects were observed at 0.05, 0.1, 0.25, and 0.5 g/kg. Two (2/8) animals died at 0.05 g/kg but the deaths were diagnosed as possible gavage trauma. Three (3/8) animals died at 1 g/kg; one death was diagnosed as possible gavage trauma and the cause of death for the 2 remaining animals was diagnosed as pulmonary congestion or cardiac myodegeneration and tubular nephrosis. The dose of 0.5 g/kg was selected as the MTD.

Salicylic Acid

Salicylic Acid short-term oral delivery produces liver and plasma enzyme changes.[1]

Sodium Salicylate

Sodium Salicylate short-term oral exposures are linked with reduced growth and feed consumption, clear kidney damage, and some liver damage.[1]

Inhalation

Amyl Salicylate

The short-term inhalation toxicity of a fragrance mixture containing 5.8% Amyl Salicylate was evaluated using Groups of female CD rats or female Syrian hamsters.[38] The animals were exposed (whole body inhalation, in chamber) to the mixtures at 5 mg/m^3 (20 rats) or 9 mg/m^3 (12 rats and 12 hamsters) five days per week (4 h per day) for 6 weeks (26 exposures total). The doses used generally represented a 10- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. Particle sizes ranged from 0.5 to 7.5 µm. There were no exposure-related, toxicologically significant effects on the following: animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. Additionally, no test substance-related gross pathological or histopathological findings were observed.

Methyl Salicylate

No toxicity is seen with inhalation of Methyl Salicylate in a series of 20 exposures of 7 h each at 0.7 g/m^3.[1]

Groups of 6 male BALB/c mice were exposed (head/nose-only) to the following chemicals in a short-term exposure study (respiratory local lymph node assay (LLNA)): trimellitic anhydride (TMA, 30 mg/m^3), phthalic anhydride (PA, 15 mg/m^3), toluene diisocyanate (TDI, 7.5 mg/m^3), hexamethylene diisocyanate (HDI, 7.5mg/m^3), and isophorone diisocyanate (IPDI, 7.5mg/m^3) (all respiratory sensitizers); dinitrochlorobenzene (DNCB, 30 mg/m^3), oxazolone (OXA, 15 mg/m^3), and formaldehyde (FA, 3.6 mg/m^3) (all contact sensitizers); and the irritant Methyl Salicylate (30 mg/m^3).[39] The animals were exposed for 45, 90, 180, or 360 min/day on 3 consecutive days (days 0, 1, and 2). For inhalation exposure, the chemicals were evaporated in air without solvent (TDI, HDI, FA, and Methyl Salicylate) or first dissolved in acetone and nebulized in air, i.e. test atmospheres were generated as aerosols (TMA, PA, IPDI, DNCB, and OXA). Controls were exposed to air only (in case of evaporation of the test substance without solvent) or air containing at most 0.06% acetone (when the test chemical was dissolved in acetone) for 360 min/day. The vehicle and control groups consisted of 6 or 12 animals. Three days after the last inhalation exposure, the draining lymph nodes were excised and cytokine production was measured after ex vivo stimulation with Concanavalin A. It was noted that discrimination between contact and respiratory sensitizers can be achieved by the assessment of cytokine profiles.

Skin application was used as a positive control in this study. The dermal route (single ear application; *n* = 3 male BALB/c mice per chemical) was used as a positive control. Each chemical (25 µl), dissolved in acetone:olive oil (4:1) solution (AOO), was applied on the dorsum of both ears (50 µl per animal) for 3 consecutive days (days 0, 1, and 2). Controls (*n* = 6) were exposed to the vehicle, AOO, only. Concentrations for skin exposure (w/v) were: TMA (50%), PA (25%), TDI (1%), HDI (1%), IPDI (1%), DNCB (1%), OXA (0.1%), and Methyl Salicylate (25%). FA was used as 10% (v/v). On day 5, auricular lymph nodes were collected and used for ex vivo cell proliferation and cytokine measurements. After skin exposure, all respiratory sensitizers induced considerably higher interleukin-4 (IL-4) responses in the auricular lymph nodes when compared to the contact sensitizers DNCB, OXA, and FA. Methyl Salicylate did not induce a measurable IL-4 response.

After inhalation exposure, the respiratory sensitizers induced more IL-4 and interleukin-10 (IL-10) when compared to the contact sensitizers. Except for formaldehyde, the contact sensitizers induced relatively more interferon-γ (IFN-γ) production. When IL-4 and IFN-γ were plotted as a function of the proliferative response, it was shown that IL-4 could be used to identify respiratory sensitizers, except HDI, at concentration levels inducing intermediate stimulation indices. HDI could be distinguished from DNCB and OXA at high stimulation index (SI) values. In contrast, contact sensitizers could only be identified when IFN-γ was measured at high SIs. The skin positive control, tested at high concentrations, showed comparable results for IL-4 and IL-10, whereas IFN-γ levels could not be used to discriminate between respiratory and contact sensitizers. The contact sensitizer FA and the irritant Methyl Salicylate did not induce significant cytokine production after inhalation and skin exposure. The authors concluded that the respiratory LLNA is able to identify and distinguish strong contact and respiratory sensitizers when simultaneously proliferation and cytokine production are assessed in the upper respiratory tract draining lymph nodes.[39]

Subchronic Toxicity Studies

Animal

Dermal

Methyl Salicylate

Subchronic dermal exposures to Methyl Salicylate are associated with kidney damage.[1]

Oral

Isodecyl Salicylate

No toxicity is seen with subchronic oral exposure to Isodecyl Salicylate.[1]

Methyl Salicylate

Subchronic oral exposure to Methyl Salicylate results in reduced weight gain and bone lesions, which disappear when Methyl Salicylate is co-administered with calcium carbonate.[1]

Sodium Salicylate

Subchronic oral exposure to Sodium Salicylate is associated with reduced bone growth and feed consumption, and indication of some bone lesions and isolated muscle weakness.[1]

Tridecyl Salicylate

No toxicity is seen with subchronic oral exposure to Tridecyl Salicylate.[1]

Inhalation

Amyl Salicylate

The subchronic inhalation toxicity of a fragrance mixture containing 4% Amyl Salicylate was evaluated using groups of female CD rats or female Syrian hamsters.[38] The animals were exposed (whole body inhalation, in chamber) to the mixtures at 5 mg/m^3 (20 rats) or 9 mg/m^3 (12 rats and 12 hamsters) five days per week (4 h per day) for 13 weeks (62 to 67 exposures total). The doses used generally represented a 10- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. Particle sizes ranged from 0.5 to 7.5 µm. There were no exposure-related, toxicologically significant effects on the following: animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. Additionally, no test substance-related gross pathological or histopathological findings were observed.

Methyl Salicylate

Subchronic inhalation exposures to Methyl Salicylate are associated with kidney damage and pulmonary focal hemorrhages and hyperplasia.[1]

Chronic Toxicity Studies

Animal

Oral

Methyl Salicylate

In chronic oral exposure studies on Methyl Salicylate, adverse effects are seen as a function of the level of exposure in 2-year rat studies, with 2% producing bone lesions and 0.7% not doing so.[1] *Liver damage is seen in dogs exposed to 0.15 g/kg/day in one study, kidney and liver weight increases in another study, but no liver or kidney abnormalities in a study at 0.167 g/kg/day*.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Salicylic Acid

Post-implantation rat embryos of day 11 were cultured for 24 h with 10, 100, or 1000 µg/ml Salicylic Acid.[40] The growth and developmental of each embryo was evaluated and compared with control embryos for the presence of any malformations. Salicylic Acid decreased all growth and developmental parameters in a concentration-dependent manner, when compared with controls. However, exposure to Salicylic Acid at 10 µg/ml culture did not show any significant effect on embryonic growth and development. Parallel to this, flow cytometric analysis (cell cycle and annexin V binding) and DNA fragmentation assay were carried out followed by quantitation by 3′-OH labeling of cultured rat embryos to evaluate the role of apoptosis in bringing about Salicylic Acid-induced teratogenesis. All results were found to be dose-dependent and an increase in apoptosis in embryonic tissues may be related to the increased risk of congenital malformations. The data suggested that apoptosis might be involved in mediating teratogenesis of Salicylic Acid in vitro.

Salicylic Acid and Sodium Salicylate

The effects of Salicylic Acid and Sodium Salicylate on early organogenesis and the interaction of these chemicals with free radicals was investigated.[41] Post-implantation Wistar rat embryos were cultured in vitro) from day 9.5 of gestation for 48 h; each test substance was added to whole rat serum at concentrations between 0.1 and 0.6 mg/ml. Also, each test

substance (0.3 mg/ml) was added to the culture media in the presence of superoxide dismutase (30 U/ml) or glutathione (0.5 µmol/ml). The growth and development of embryos was compared, and each embryo was evaluated for the presence of malformations. When compared to the growth of control embryos, both chemicals decreased all growth and developmental parameters in a concentration-responsive manner. There was also a concentration-related increase in overall dysmorphology, including the following: hematoma in the yolk sac and neural system, open neural tube, abnormal tail torsion, and the absence of forelimb bud. When superoxide dismutase was added in the presence of Salicylic Acid, the incidence of malformations was decreased. However, the addition of superoxide dismutase did not affect the growth and developmental parameters of Salicylic Acid and Sodium Salicylate. The authors noted that the effects of salicylates might involve free oxygen radicals by the non-enzymatic production of the highly teratogenic metabolites 2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid. Furthermore, they noted that an enhanced production of these metabolites in embryonic tissues may be directly related to the increased risk of congenital malformations.

Animal

Dermal

Methyl Salicylate

Dermal exposure to Methyl Salicylate is associated with reproductive and developmental toxicity as a function of blood levels reached as a result of exposure.[1]

Oral

Methyl Salicylate, Salicylic Acid, and Sodium Salicylate

Salicylic Acid, produced when aspirin is rapidly hydrolyzed to Salicylic Acid after absorption from the gut, was reported to be the causative agent in aspirin teratogenesis in animals.[1] *Oral exposure to Methyl Salicylate, Salicylic Acid, and Sodium Salicylate are associated with reproductive and developmental toxicity as a function of blood levels reached as a result of exposure*.

Sodium Salicylate

Groups of 15 mated Crl:CD (SD)BR rats were given a single dose of 0 or 300 mg/kg (dose volume = 10 ml/kg) Sodium Salicylate (99.5% pure, in distilled water) on gestation day (GD) 9.[42] All fetal data, including all supernumerary ribs data, are presented as the percentage mean per litter. No statistical analysis was carried out on mean incidences of supernumerary ribs and the number of presacral vertebrae. In the treated group, adverse effects were noted on body weight changes and food consumption during the 2 days following dosing. At birth, a high majority of pups had extra ribs at the 300 mg/kg dose. Specifically, on postnatal day 1, 89% of pups from dams exposed to 300 mg/kg Sodium Salicylate had supernumerary ribs. For these pups, evidence of postnatal reversibility was observed in 10 out of 14 pups with rudimentary ribs and 26 presacral vertebrae. Radiographs done on postnatal days 1, 6, 14, 28 and 54 showed a reduction in the incidence of rudimentary ribs only, whereas extra ribs, often associated with 27 presacral vertebrae, had the same incidence from birth to adult stage. Furthermore, extra ribs seemed to exhibit similar growth evolution to the other thoracic ribs. The authors noted that dosing with Sodium Salicylate resulted in a significant increase in the incidence of supernumerary ribs. The length of gestation was not affected by treatment. At birth, the number of dead pups was slightly higher in the treated group (7 dead pups out of 15 litters) in comparison with the control group (3 out of 14 litters) but no external malformations were significantly increased in the treated group.

In a study involving mated female Sprague-Dawley rats, Sodium Salicylate was administered by gavage on GD 9 (day of vaginal plug or sperm in vaginal smears was designated gestation day (GD) 0) at a dose of 300 mg/kg (in distilled water).[43] Control animals received distilled water only. The females were killed on GD 13. The mean number of live embryos was slightly lower than the control group value (11.9 as compared to 14.7), mainly due to a slight, but non-significant, increased number of early resorptions in the treated group. Because Sodium Salicylate is known to cause an increased incidence of supernumerary ribs (see preceding study), the molecular basis of this defect was evaluated in this study by analyzing the possible involvement of *Hox* genes, known to specify vertebrae identity. On GD 13, the expression of several *Hox* genes, selected according to the position of their anterior limit of expression, namely upstream (*Hoxa9*), at the level (*Hoxa10*) and downstream (*Hoxd9*) to the morphological alteration, were analyzed. Posterior shifts in the anterior limit of expression of *Hoxa10* and *Hoxd9* were observed following exposure to Sodium Salicylate, which could explain an effect at the level of the axial skeleton. This finding suggests that the appearance of ectopic ribs can be attributed to an anterior transformation of lumbar vertebrae identity into thoracic vertebrae identity. The authors noted that whether this transformation occurs with all compounds inducing supernumerary ribs in rats remains to be determined.

Sodium Salicylate served as the positive control in an embryo-fetal developmental toxicity study.[44] The positive control (in distilled water) was administered intragastrically (dose = 250 mg/kg/day; once daily) to a group of 22 to 24 gravid female Sprague-Dawley rats on GDs 8 to 10. Sodium Salicylate was administered at a dose volume of 10 ml/kg/day. There were 4.8% malformations in fetuses from the positive control group, including exencephaly, cranial meningocele, spina bifida, gastroschisis, and subcutaneous ecchymosis. The rate of abnormality was significantly higher than that of the vehicle control group ($p < 0.01$). Additionally, there were significant difference in the body and tail length, and mean body weight of fetuses in positive control group compared with the vehicle control group ($p < 0.01$).

Human

Dermal

Salicylic Acid

In the third trimester, the use of Salicylic Acid can potentially cause early closure of ductus arteriosus and oligohydramnios. Therefore, it should not be applied over large surface areas for prolonged time periods, or under occlusive dressings that may enhance systemic absorption.[45,46] (The primary reference upon which these statements are based has been ordered for further details.)

Oral

Salicylic Acid

An exposure assessment of a representative cosmetic product (containing ≤ 2% Salicylic Acid) used on a daily basis is available which estimates that the exposure from the cosmetic product would be only 20% of the level seen with ingestion of a "baby" aspirin (81 mg) on a daily basis. This exposure assessment further contends that the reproductive and developmental toxicity from the daily use of a baby aspirin is not significant.[1]

GENOTOXICITY STUDIES

Butyloctyl Salicylate, Ethylhexyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Salicylic Acid, Sodium Salicylate, and Tridecyl Salicylate

Studies on the genotoxic potential of Butyloctyl Salicylate, Ethylhexyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Salicylic Acid, Sodium Salicylate, and Tridecyl Salicylate are negative, except that Salicylic Acid is positive in a B. subtilis rec assay (negative in seven other bacterial tests and one mammalian test); Methyl Salicylate is positive in S. typhimurium strains TA98, and TA100 with metabolic activation (negative in in 2 other Ames tests); and Sodium Salicylate is positive in an in vivo chromosome aberration study in mice (negative SCE in vivo in mice, and in 4 in vitro test systems).[1]

CARCINOGENICITY STUDIES

In Vitro

Salicylic Acid and Sodium Salicylate

In vitro predictors of carcinogenesis are also negative for Salicylic Acid and Sodium Salicylate.[1]

Human

Dermal

Methyl Salicylate

Methyl Salicylate, in a mouse skin painting study, does not induce neoplasms.[1]

Salicylic Acid

In a National Toxicology Program (NTP) carcinogenicity study, the effects of synthetic solar light on the skin of hairless mice that had been treated with creams containing Salicylic Acid were evaluated.[47] Creams containing Salicylic Acid (0%, 2%, or 4%), were applied to the skin of groups of 18 male and 18 female hairless mice in the mornings. Additional groups of 36 male and 36 female mice were not exposed to the cream. In the afternoons, groups of animals were exposed to one of three strengths of synthetic solar light for 4 h. Other groups were not exposed to light and were control groups. The treatment and exposures were performed five days per week for 40 weeks, during which time the animals were monitored for the development of skin cancers. Greater strengths of light increased the incidences of skin cancers in mice not given a cream or a cream with no acid included. Creams containing Salicylic Acid decreased the incidence of skin tumors in mice receiving the lower of the two light intensities. It was concluded that Salicylic Acid had some protective effect against photocarcinogenicity at lower intensities.

Parenteral

The tumor incidence in mice injected intraperitoneally with Methyl Salicylate was lower than that observed in vehicle and negative control groups.[1]

OTHER RELEVANT STUDIES

Estrogenic Activity

Butyloctyl Salicylate and Ethylhexyl Salicylate

The estrogenic activity of 2 UV filters, Butyloctyl Salicylate and Ethylhexyl Salicylate, was studied.[48] A consensus modeling method to predict their qualitative and quantitative binding activity towards the estrogen receptor (ER) was used. The consensus modeling comprised two Decision Forest (DF) models that were built using two different training data sets. The two DF models were validated using 5-fold cross validations and external chemicals. Similar predictions were made on unrelated compounds, to make reference comparisons as well to a few excipient ingredients that are frequently added to sunscreen formulations. Prediction confidence was defined as a number between 0 and 1, for indication of confidence for a prediction; the smaller the number, the less confident the prediction. The experimental ER binding affinities were given as logarithmic relative binding affinity (logRBA) values to the nature hormone estradiol whose logRBA was set to 2. Ethylhexyl Salicylate was classified as an estrogen receptor non-binder. Butyloctyl Salicylate was classified as having binding activity to the ER (prediction confidence value = 0.827; log RBA = -0.853).

A recombinant yeast estrogen assay was used to assess the activity of Ethylhexyl Salicylate.[49] The ERα gene, together with expression plasmids (containing estrogen responsive elements and the lac-Z reporter gene encoding the enzyme β-galactosidase), were incubated in medium containing Ethylhexyl Salicylate (10 µl, serially diluted in ethanol) and the chromogenic substrate, chlorophenol red-β-D-galactopyranoside (CPRG). Active ligands (which bind to the receptor) induce β-galactosidase (β-gal). The relative potency of the test substance was determined only when the dose–response curve was parallel to that of 17-β-estradiol. To do so, the concentration of the test substance required to produce a half-maximal response (absorbance at 540 nm (A540) between 1.7 and 2.0) was divided by the concentration of 17-β-estradiol required to produce the same response. Compounds displaying a submaximal response were compared at the 10% response level. Ethylhexyl Salicylate generated a dose-response curve that was shallower than the one for 17-β- estradiol, and had a submaximal response for estrogenic activity (estrogenic potency relative to 17β-estradiol = 1/2,000,000).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

Amyl Salicylate

The skin irritation potential of Amyl Salicylate (> 99.8%) was evaluated using 6 Albino angora rabbits.[50] The test substance (0.1 g) was applied (using a glass syringe) for 24 h to a 3 x 3 cm area on the dorsal surface of each animal. A 25 cm diameter plastic collar was wrapped around the neck of each animal. Application was repeated 30 min after the end of the initial 24-h application period. Reactions were scored 24 h after the first application and 48 h and 72 h after the second patch

application. Amyl Salicylate was severely irritating to the skin. When the irritation potential of the test substance was evaluated according to the same procedure (application to dorsal, mid-lumbar region) using 6 male Hartley guinea pigs, mild skin irritation was observed.

The skin irritation potential of Amyl Salicylate (> 99.8%) was evaluated using 6 miniature swine of the Pitman-Moore Improved strain.[50] The test substance (0.05 g) was applied, under a 15 mm diameter patch, to dorsal skin for 48 h. Skin irritation was not observed.

Ethylhexyl Salicylate

Irritation was evaluated during an acute dermal toxicity study on Ethylhexyl Salicylate (described earlier).[4] Mild erythema lasting 24 h was observed.

Hexyl Salicylate

As a part of a modified Draize sensitization study of Hexyl Salicylate, a preliminary irritation screen was conducted to determine the injection challenge concentration (ICC).[51] On the shaved flanks of four inbred Hartley strain albino guinea pigs, 0.1 ml aliquots of Hexyl Salicylate at a range of concentrations were given as intradermal injections. Reactions were read 24 h after injection. Hexyl Salicylate at 0.1% produced a positive irritation reaction, and 0.1% (vehicle not reported) was selected as the ICC.

As a part of a modified Draize sensitization study of Hexyl Salicylate, a preliminary irritation screen was conducted to determine the application challenge concentration (ACC).[51] On the shaved flanks of four inbred Hartley strain albino guinea pigs, 0.1 ml aliquots of Hexyl Salicylate at a range of concentrations were given as intradermal injections. Reactions were read 24 h after application. Hexyl Salicylate at 5% (vehicle not reported) produced no irritation and was selected as the ACC.

In a preliminary irritation screen conducted prior to a maximization test of Hexyl Salicylate, 4 male albino Dunkin/Hartley strain guinea pigs were treated topically with 8 mm diameter filter paper patches saturated with 10%, 25%, or 50% Hexyl Salicylate in acetone using 11 mm aluminum patch test cups.[5] The cups were applied to shaved flanks and held in place with adhesive plaster wound around the trunk of each animal. After 24 h, the patches were removed. Reactions were read 24 and 48 h after patch removal. No irritation was observed at 10%; very slight erythema was observed in three animals at concentrations of 25% and 50%.

In a preliminary irritation screen conducted prior to a maximization test of Hexyl Salicylate, 4 male albino Dunkin/Hartley strain guinea pigs were intradermally injected with 0.1 ml aliquots of 0.1%, 0.25%, 0.5%, 1.0%, and 2.0% Hexyl Salicylate in 0.01% dodecylbenzene sulphonate (Dobs)/saline.[5] After 24 h, the injection sites were examined for size (2 largest diameters), erythema, and edema. Very slight erythema was observed at 0.1%; slight erythema and edema were observed at 0.25%, 0.5%, 1%, and 2%.

Hexyl Salicylate was evaluated for primary irritation in male hrBR outbred hairless albino guinea pigs (5/group) as a part of a photoallergy screening study.[5] A single application of 0.3 ml Hexyl Salicylate at 1%, 5%, 10%, or 50% (in 3:1 diethyl phthalate (DEP):ethanol) or at 100% was applied to the dorsal skin of each animal using 25 mm Hilltop® Chambers. After 2 h (± 15 min), the chambers were removed and application sites were wiped with paper towels moistened with deionized water. Reactions were assessed 1 and 4 h later and at 1, 2, and 3 days after administration. No irritation was observed.

As part of a phototoxicity study, Hexyl Salicylate was evaluated for irritation in two miniature swine.[5] A 20 µl/5 cm^2 aliquot of neat Hexyl Salicylate was applied to the back of each animal. No irritation was observed.

Primary irritation was evaluated in a series of four tests which were conducted on either three or four healthy female New Zealand white rabbits.[5] A 0.5 ml aliquot of 10%, 25%, 50% Hexyl Salicylate in DEP, or 100% Hexyl Salicylate was applied to a 2.5 cm^2 piece of surgical lint. The lint square was then placed onto a 6 cm^2 area of clipped, intact dorsal skin for 4 h under semi-occlusion. After patch removal, the treated sites were cleansed by gentle swabbing with cotton wool soaked in warm water. Reactions were assessed at 1, 24, 48, 72, and 168 h after patch removal. No irritation was observed at 10% and 25%; irritation was observed with 50% and 100% Hexyl Salicylate.

Hexyl Salicylate was evaluated for irritation in the acute dermal LD_{50} study described earlier in this safety assessment.[5] Ten rabbits received a single dermal application of neat Hexyl Salicylate at a dose of 5.0 g/kg. Moderate (7/10) to slight (3/10) edema and moderate (8/10) to slight (2/10) erythema were observed.

As part of a phototoxicity study, Hexyl Salicylate was evaluated for irritation in 6 hairless mice.[5] A 20 µl/5 cm^2 aliquot of neat Hexyl Salicylate was applied to the back of each animal. No irritation was observed.

Methyl Salicylate

The irritation potential of wintergreen oil (containing 80–99% Methyl Salicylate) was evaluated at non-irradiated sites in an associated phototoxicity study.[6] An aliquot of 20 µl of neat Methyl Salicylate was applied to a 5 cm^2 area on the backs of 6 hairless mice and 2 miniature swine. Flaking, hyperkeratosis and dry desquamation were observed.

Methyl Salicylate was evaluated at various concentrations prior to an open epicutaneous test (OET).[52] A 0.1 ml aliquot of Methyl Salicylate was applied to an area measuring 8 cm^2 on the clipped flank of 6 - 8 male and female outbred Himalayan white-spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made. The minimal irritating concentration after 21 applications was 3% (vehicle not specified).

Prior to an OET test, Methyl Salicylate at a range of concentrations was evaluated for irritation in 6 - 8 male and female outbred Himalayan white-spotted guinea pigs.[52] A 0.025 ml aliquot was applied with a pipette to an area measuring 2 cm^2 on the clipped flank. The application site was left uncovered and reactions were read after 24 h. The concentration of 3% was the lowest concentration to produce mild erythema in at least 25% of the animals and this dose was selected as the minimal irritating concentration after 1 application.

As a part of an acute dermal LD_{50} study conducted in rabbits, a single application of 5 g/kg neat Methyl Salicylate produced slight (2/9 rabbits) to moderate (7/9 rabbits) erythema and edema.[6]

A primary irritation study was conducted to establish the concentration to be used for challenge in a mouse ear swelling test.[53] Methyl Salicylate was applied at a range of concentrations in 4:1 acetone to olive oil using a 4-day dosing protocol. The ear measurements were obtained before and after application of Methyl Salicylate. The minimal irritating concentration (MIC) was defined as the lowest concentration of Methyl Salicylate to produce a percent ear swelling significantly greater than the vehicle. The MIC was determined to be 20%.

Butyloctyl Salicylate, Ethylhexyl Salicylate,
Isodecyl Salicylate, Methyl Salicylate, and Tridecyl Salicylate

The application of 500 mg (in 0.5 ml) of Isodecyl Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate are not irritating.[1] Undiluted application of Ethylhexyl Salicylate produces minimal to mild irritation. Methyl Salicylate at concentrations greater > 50% is clearly irritating. One study of the effect of vehicle on Methyl Salicylate irritation shows irritation at concentrations as low as 1%, with a 70% ethanol vehicle producing the most irritation and polyethylene glycol producing little or no irritation at Methyl Salicylate concentrations up to 6%.

Human

Ethylhexyl Salicylate, Methyl Salicylate,
Salicylic Acid, TEA Salicylate, and Tridecyl Salicylate

Clinical tests for cumulative irritation are available for the following ingredients at the specified concentrations: Salicylic Acid (2% - minimal cumulative irritation; 1.5% - slight or no irritation); TEA Salicylate (8% - no irritation); Methyl Salicylate (> 12% - pain and erythema; 8% - no irritation; 1% aerosol - erythema); Ethylhexyl Salicylate (4% - no irritation); and Tridecyl Salicylate (no irritation).[1]

Amyl Salicylate

The skin irritation potential of Amyl Salicylate (> 99.8%) was evaluated using 50 adult male subjects.[50] Using a glass syringe, 0.05 ml of the test substance (32% Amyl Salicylate in acetone) was applied to an occlusive patch (15 mm diameter). The patch remained in place (on the back) for 48 h. Reactions were scored 30 minutes after patch removal. Skin irritation was not observed.

Ethylhexyl Salicylate

In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test was conducted with 4% Ethylhexyl Salicylate in petrolatum on the backs of 23 male volunteers.[4]

Hexyl Salicylate

Irritation was evaluated as a part of a human repeated insult patch test study (HRIPT) which was conducted in 103 volunteers (29 male/74 female).[5] A 0.3 ml aliquot of 30% Hexyl Salicylate in 3:1 DEP:ethanol was applied to 25 mm Hilltop Chambers® which were applied to the backs for 24 h under occlusion. A total of nine induction applications were made over a period of 3 weeks on a Monday, Wednesday, Friday schedule. No irritation was observed.

As a part of a maximization test, 3% Hexyl Salicylate was pre-tested on 22 male volunteers to determine whether sodium lauryl sulfate (SLS) pre-treatment was necessary.[5] Hexyl Salicylate was applied under occlusion to normal sites on the backs for 48 h. No irritation was observed.

Primary irritation was evaluated in 30 volunteers.[54] A 0.2 ml aliquot of neat Hexyl Salicylate was applied to 25 mm Hilltop Chambers®, which were then applied to the upper arm for 4 h. Reactions were read at 24, 48 and 72 h after patch removal. No irritation was observed.

Irritation was assessed as part of an associated phototoxicity study conducted in 56 volunteers (15 male/41 female).[5] Hexyl Salicylate, in 0.3 ml aliquots, at 0.3%, 3%, or 30% in 3:1 DEP:ethanol was applied to 25 mm Hilltop Chambers® that were applied to the back of each subject for a 24-h period. Each subject received duplicate patches that were placed on both sides of the spine on naive sites. The test sites were evaluated at approximately 1, 24, 48, and 72 h after patch removal. No irritation was observed.

Methyl Salicylate

Pre-tests were performed for human maximization studies using 48-h occlusive patches.[6] No irritation was observed with 8% Methyl Salicylate in petrolatum applied to the backs of 27 healthy male volunteers, or with 12% wintergreen oil (containing 80 – 99% Methyl Salicylate) in petrolatum applied to the backs of 25 volunteers.

The irritation potential of Methyl Salicylate was evaluated in nine volunteers (3 male/6 female).[55] A 25 ml aliquot of Methyl Salicylate in 80% ethanol and 20% deionized water was pipetted onto the skin of the forearm of each subject within a PTFE ring (1.2 cm ID, 1.6 cm OD) that was affixed to the skin with a bead of denture adhesive applied around the outside of its bottom edge. Immediately after Methyl Salicylate was applied, the ring was covered with a snug-fitting PTFE cap that prevented evaporation and left a headspace of only 0.21 cm^2. Each subject was tested six times. In each session, Methyl Salicylate was applied to one forearm and the vehicle to the other in random order. Subjects were tested no more frequently than every 48 hours. Irritation was observed with 30% and 60% Methyl Salicylate.

Salicylic Acid

Possible complications relating to the topical use of Salicylic Acid as a peeling agent include persistent erythema and pruritus (specific studies not included).[56]

Irritation/Comedogenicity

Animal

Salicylic Acid

Formulations containing Salicylic Acid were applied to groups of 6 Adult male albino New Zealand rabbits.[57] The formulations were applied daily on the concave side of the left ears, while distilled water (control) was applied to the right ears. A daily systematic macroscopic evaluation was performed to verify the possible occurrence of erythema, edema, desquamation, comedogenic and inflammatory reactions. The formulations containing 3.5%, 5.0%, and 7.5% Salicylic Acid exhibited significant macroscopic alterations compared to the untreated group in terms of desquamation, inflammatory reaction and comedogenic effect.

Sensitization

In Vitro

Hexyl Salicylate

Genomic Allergen Rapid Detection is a cell based alternative to animal testing for identification of skin sensitizers.[58] The assay is based on a biomarker signature comprising 200 genes measured in an in vitro model of dendritic cells following chemical stimulations, and consistently reports predictive performances of ~90% for the classification of external test sets. Hexyl Salicylate was predicted to be a skin sensitizer.

Salicylic Acid

A publication relating to an integrated testing strategy (ITS) for skin sensitization that focuses on three in vitro methods that cover the first three steps of the adverse outcome pathway (AOP) is available.[59] The three in vitro methods are: direct peptide reactivity assay (DPRA), keratinocyte activation assay (SENS-IS), and dendritic cell line activation assay (h-CLAT). The aim of this study was to compare these 3 methods due to the weight of evidence (WoE) approach, based on a 2-out-of-3-assessment. The results of 33 references (on 33 chemicals) were compared to in vivo data (especially human). The results for SLS and Salicylic Acid were equivocal, but ultimately, were considered positive results.

The modification of proteins by skin sensitizers is a pivotal step in T-cell mediated allergic contact dermatitis (ACD).[60] In this process small reactive chemicals interact covalently or non-covalently with cellular or extracellular skin self-proteins or self-peptides to become recognized by the human immune system. In an effort to develop a novel non-animal in vitro test system for predicting the sensitization potential of small reactive chemicals in human skin the allergen–peptide/protein interaction assay (APIA) has been developed. By applying modern proteomic technologies together with a target peptide containing all amino acids, this assay permits the profiling of all amino acid specific allergen–peptide interactions. Furthermore, potentially crucial allergen-specific Cys-modifications are qualitatively monitored by mass spectrometry and confirmed by a dual peptide approach. In this experiment, potential Salicylic Acid dependent peptide modifications were studied. Mass spectra of both target peptides revealed neither any modification of peptide-21 nor of peptide-20 by Salicylic Acid under skin-related in vitro conditions. It was noted that the data clearly reveal pH-dependent differential allergen DNCB and cinnamic aldehyde-specific peptide interaction and adduct formation, with concomitant partial peptide depletion, and selective modification of Cys-residues as demonstrated by peptide-21. Non-allergenic Salicylic Acid did not interfere with Cys containing peptide-21 or Cys-free peptide-20.

Animal

Butyloctyl Salicylate

Maximization test data on Butyloctyl Salicylate are negative.[1]

Ethylhexyl Salicylate

Maximization test data on Ethylhexyl Salicylate are negative.[1]

Hexyl Salicylate

A very low EC3 (0.18%) value was reported in a LLNA on Hexyl Salicylate.[59] The EC3 is defined as the effective concentration that induces a three-fold increase in local lymph node proliferative activity, and is said to correlate relatively well with known human thresholds for contact allergy. It was noted that this very low value may be due to its irritating properties (possibly sensitizing impurities).

Hexyl Salicylate was tested in a guinea pig sensitization study using a modified Draize procedure in 10 inbred Hartley albino guinea pigs.[51] Induction consisted of four intradermal injections with 0.1 ml of Hexyl Salicylate at 2.5 times the ICC (ICC = 0.1%) at four sites overlying the two auxiliary and the two inguinal lymph nodes. The animals were challenged 14 days later with an intradermal injection in one flank and a topical application in the other flank using 0.1 ml Hexyl Salicylate at 0.1% (ICC) and 5% (ACC), respectively. A second challenge was conducted 7 days later. Sensitization reactions were observed after the second challenge.

The sensitization potential of Hexyl Salicylate was evaluated during a photoallergy test using Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs (5/group).[5] During the induction phase, an intradermal injection with a 0.1 ml aliquot of a formulation of sterile water and Freund's complete adjuvant (FCA) (1:1 v/v) was made to a 2.5 cm^2 nuchal area of skin. The

skin area was then tape-stripped five times. For the topical induction, a 0.3 ml aliquot of 100% hexyl salicylate in 3:1 DEP:ethanol was applied using 25 mm Hilltop® chamber patches for 2 h. After patch removal, the application sites were gently wiped. This procedure was repeated on days 3, 5, 8, 10, and 12 of the induction phase. On day 22, the animals were topically challenged with 50% in 3:1 DEP:ethanol and 100% Hexyl Salicylate using the same procedure. The test sites were observed at 1 and 4 h, and at 1, 2, and 3 days after Hexyl Salicylate application. No sensitization reactions were observed.

A Magnusson-Kligman guinea pig maximization test was conducted on ten albino Dunkin/Hartley strain guinea pigs.[5] Induction consisted of intradermal injection followed one week later by a 48 h occluded patch. The six intradermal injections were made to a 2 x 4 cm clipped, shaved area in the dorsal shoulder region. There were two 0.1 ml injections of 1% hexyl salicylate in 0.01% DOBS/saline, two 0.1 ml injections of 1% Hexyl Salicylate in 50% FCA, and two 0.1 ml injections of 50% FCA. Seven days later, the site was clipped and shaved, and induction was supplemented topically with a 48 h occluded patch with 40% Hexyl Salicylate in acetone over the shoulder injection sites. Thirteen to 14 days after application of the shoulder patch, the guinea pigs were challenged on the clipped and shaved flank using an 8 mm diameter filter paper patch saturated with 10% hexyl salicylate in acetone which was applied for 24 h under occlusion. Reactions were assessed at 24 and 48 h after patch removal. Three additional challenge applications with 10% Hexyl Salicylate in acetone were made at weekly intervals on the contralateral flanks. No sensitization reactions were observed.

Methyl Salicylate

Results for Methyl Salicylate are negative at concentrations up to 25%, independent of vehicle, in the local lymph node assay (LLNA).[1] *Maximization tests on Methyl Salicylate are negative.*

The sensitization potential of Methyl Salicylate (0.7 µM) was evaluated using the LLNA.[61] The number of positive tests/the number of total tests was 1 in 4 (25% positive response). Overall, the results were classified as negative, and Methyl Salicylate was classified as a non-sensitizer. According to another source, 50% Methyl Salicylate was predicted to be a non-sensitizer using the LLNA.[62]

Salicylic Acid

Although results for Salicylic Acid are positive in the LLNA at a concentration of 20% in acetone, this is not true for Salicylic Acid at a concentration of 20% in acetone/olive oil.[1]

Salicylic Acid has been tested and found to be a non-sensitizer in the LLNA.[63]

Human

Ethylhexyl Salicylate, Methyl Salicylate, and Salicylic Acid

In normal skin, Salicylic Acid, Methyl Salicylate, and Ethylhexyl Salicylate are not sensitizers.[1]

Hexyl Salicylate

Hexyl Salicylate has been classified as a Category 4 substance (infrequent cause of contact allergy in relation to level of exposure) with regard to its human skin sensitization potential.[63] Substances in Category 4 are rarely important clinical allergens, because they require considerable/prolonged exposure to higher dose levels to produce sensitization, which even then is unlikely to exceed 0.01% of those exposed. Furthermore, a human skin sensitization no-observed-effect-level (NOEL) of 35,433 µg/cm^2 has been reported for Hexyl Salicylate. Actual data relating to these results are not included.

In a human maximization test on Hexyl Salicylate, no induction was observed at a dose of 20,654 µg/cm^2.[59] Again, actual data relating to these results are not included.

A maximization test was carried out with 3% Hexyl Salicylate in petrolatum on 22 adult volunteers.[5] Application was under occlusion to the same site on the volar forearms or backs for five alternate-day 48-h periods. Patch test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated for 30 min with 2% aqueous SLS under occlusion on the left side of the back whereas hexyl salicylate was applied without SLS on the right side. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were produced.

A HRIPT was conducted in 103 subjects (29 males and 74 females).[5] During the induction phase, a 0.3 ml aliquot of 30% Hexyl Salicylate in 3:1 DEP:ethanol was applied to Webril/adhesive patches (25 mm Hilltop® Chamber System) on the left side of the back of each subject. Patches remained in place and were kept dry for approximately 24 h and then removed. A series of nine induction applications were completed over a period of three weeks. A rest period of

approximately 2 weeks followed the last induction. At the challenge phase, patches were applied as in the induction phase and kept in place for 24 h, after which time they were removed and the challenge sites were scored. The test sites were also scored at 48, 72, and 96 h post-patching. No sensitization reactions were observed.

Methyl Salicylate

Methyl Salicylate has been classified as a Category 5 substance (a rare cause of contact allergy except perhaps in special circumstances, e.g., use in topical medicaments) with regard to its human skin sensitization potential. [63] It was also noted that there are insufficient data (availability of specific data not mentioned) to define a human skin sensitization NOEL. Category 5 consists of substances that have a very low intrinsic ability to cause skin sensitization. Here, typically only exceptionally prolonged exposure in combination with high use levels will lead to skin sensitization, for example, routine use in medicaments for treatment of chronic skin conditions. For these materials, sensitization in the general population is likely to be (extremely) rare.

A human maximization test was conducted on 25 healthy volunteers. Wintergreen oil (containing 80–99% Methyl Salicylate) in petrolatum was applied under occlusion, to the same site on the volar forearms of 25 subjects for 5 alternate-day 48-h periods.[6] The patch sites were pretreated for 24 h with 5% aqueous SLS under occlusion for the initial patch only. Following a 10 - 14-day rest period, a challenge patch of Methyl Salicylate (concentration not stated) was applied to a fresh site for 48 h under occlusion. Prior to the challenge, 5% SLS was applied to the test sites for 30 min under occlusion on the left side of the back, while Methyl Salicylate was applied without SLS treatment on the right side. Additional SLS controls were placed on the left and petrolatum on the right, and labeled as the fifth site. No reactions were observed with 12% wintergreen oil.

A maximization test was carried out on 27 healthy volunteers using 8% Methyl Salicylate in petrolatum.[6] An occluded patch with Methyl Salicylate was applied to the same sites of the forearms of each subject for five alternate 48-h periods. Patch sites were pre-treated with 5% aqueous SLS under occlusion. Following a 10 - 14-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. An application of 10% aqueous solution of SLS under occlusion was applied 1 h prior to challenge. Reactions were read at patch removal and 24 h later. No sensitization reactions were observed.

An HRIPT was conducted in 39 male and female volunteers (13 male/26 female) with 1.25% Methyl Salicylate.[6] A 0.5 ml aliquot of Methyl Salicylate (vehicle not specified) was applied to a 1-inch square Webril patch fixed to the center of 1 x 3 in. strip of adhesive elastic bandage and placed on the upper arm of each subject. Patches were removed 24 h later. A total of nine applications were made over a three week period. The patches were applied to the same sites unless a reaction was observed. A challenge patch was applied to a fresh site on the Monday of the sixth week and removed 24 h later. Reactions were scored at 24 and 72 h after patch removal. No sensitization was observed.

Salicylic Acid

Salicylic Acid has been classified as a Category 6 substance with regard to its human skin sensitization potential.[63] Substances in Category 6 are essentially free from skin sensitizing activity (i.e., nonsensitizers).

Photosensitization/Phototoxicity

In Vitro

Ethylhexyl Salicylate

The phototoxicity of Ethylhexyl Salicylate was evaluated in the 3T3 neutral red uptake phototoxicity test, involving 100 µl of a cell suspension of 3T3 fibroblasts in Dulbecco's Modification of Eagle's Medium (DMEM) containing new born calf serum and antibiotics (1 x 10^5 cells/mL, 1 x 10^4 cells/well).[64] Different concentrations of the test substance (0.1 to 316 µg/mL) were applied, in sextuplicate in the 96-well plates. After 1 h incubation, the +UVA plate was irradiated for 50 minutes with 1.7 mW/cm^2 of UVA (equal to 5 J/cm^2 of UVA**).** Following a second incubation of the cell suspension, neutral red medium was added. Neutral red extracted from viable cells formed a homogeneous solution and the +UVA and $^-$UVA plates were analyzed in a microliter plate reader at 540 nm. The photoirritation factor (PIF) was calculated and a value for the mean photoeffect (MPE) was determined. A test substance is predicted as having a potential phototoxic hazard if the PIF, calculated as the ratio of toxicity with and without UV light, is > 1. The MPE is a statistical comparison of the dose-response curves obtained with and without UV. A test substance is predicted to be phototoxic if the MPE is > 0.1. Phototoxicity test results for Ethylhexyl Salicylate were classified as negative (PIF = 1.756 (1st run) and 1.043 (2nd run); MPE = 0.109 (1st run) and 0.061 (2nd run)).

Animal

Hexyl Salicylate

Neat Hexyl Salicylate was not phototoxic when tested in Skh:hairless-1 mutant mice.[5,65] Twelve animals received a single application of 20 µl of Hexyl Salicylate on a 2 cm^2 area of the back. Six mice treated with Hexyl Salicylate were exposed to a 6-kW long arc xenon lamp (distance = 1 m; intensity = 0.1667 W/m^2) for 40 min and four fluorescent blacklight lamps, type F40BL, with exposure for 1 h with an intensity of 3 W/m^2. The remaining six mice treated with Hexyl Salicylate, served as a control for primary irritation reactions. The irradiation area was defined by a 1-cm diameter hole punched in an aluminum foil adhesive tape, and the tape masked the skin surrounding the exposure area. One group of controls was treated with 8-methoxypsoralen in methanol (0.01% w/v). The sites were assessed at 4, 24, 48, 72, and 96 h. No reactions were observed.

Hexyl Salicylate was evaluated for phototoxic potential. Two miniature swine were given a single application of neat Hexyl Salicylate (20µl) on the back, to an area measuring approximately 5 cm^2.[5,65] Irradiation was conducted using a 6-kW long-arc xenon lamp with exposure time of 40 min (distance = 1 m; intensity = 0.1667 W/m^2) and four fluorescent blacklight lamps, type F40BL, with exposure for 1 h (intensity = 3 W/m^2). Each irradiation area was defined by 1-cm diameter hole punched in an aluminum foil adhesive tape, and the tape masked the skin surrounding the exposure area. The reactions were graded at 4, 24, 48, 72, and 96 h after the irradiation exposure. The positive control was a 0.01% solution of 8-methoxypsoralen (8-MOP) in methanol, and the negative control was the vehicle alone. No phototoxicity was observed.

The phototoxic potential of Hexyl Salicylate was evaluated in two groups (five/group) of Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs.[5] A 0.3 ml aliquot of Hexyl Salicylate at 0%, 5%, 10%, 50%, or 100% in 3:1 DEP: ethanol was applied to 25 mm Hilltop Chambers® which were then applied to the dorsal skin along the midline of each guinea pig and occluded with dental dam. Two hours later the patches were removed and the application sites were gently wiped. The animals were exposed to ultraviolet radiation (UVR) using a 6.5 kW long-arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). A dose of approximately 2.25 minimal erythema doses (MED) was delivered for each exposure session (approximately 2.25 h). Observations were made immediately, 1 and 4 h later, and 1, 2, and 3 days after administration and UVR exposure. No phototoxic effects were observed.

Photoallergy was evaluated in two groups of 5 Crl:IAF (HA)-hrBR outbred albino hairless guinea pigs.[5] A nuchal area of skin approximately 2.5 cm^2 was defined by intradermal injections (0.1 ml/corner) with a formulation of sterile water and Freund's complete adjuvant (1:1 v/v) in each animal. This skin area was then tape-stripped five times. A 0.3 ml aliquot of Hexyl Salicylate in 3:1 DEP:EtOH was applied to Hilltop® Chamber patches (25 mm diameter), then applied to the nuchal area, and occluded with a dental dam. After 2 h the patches were removed, and the application sites were gently wiped with disposable paper towels moistened with reverse osmosis, membrane-processed deionized water. The nuchal area of animals was exposed to UVR for approximately 2.25 h. The UVR source was a 6.5 kW long-arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). Exposures were monitored by a customized detector that records both intensity and UVR dose. A dose of about 2.25 instrumental Minimal Erythema Doses (MED) was delivered for each exposure session. Procedures were repeated once daily on days 3, 5, 8, 10 and 12 of the induction phase of the study. On day 22, using the induction procedure, Hexyl Salicylate at 50% and 100% was topically applied to each animal. Animals were exposed to UVR for 2.25 h after 2 h of patch application. The sites were scored 1 and 4 h after dosage administration and/or UVR exposure. Photoallergy was not observed.

Methyl Salicylate

The phototoxicity of wintergreen oil (which contained 80–99% Methyl Salicylate) was evaluated in 2 miniature swine.[6] A 20µl aliquot of neat wintergreen oil was applied to a 5 cm^2 area on the back of each animal. Animals were exposed to UV from a fluorescent black light lamps F4OT12BL (filtered to limit exposure to long wave ultraviolet light only) at a dose of UVA 10 watts/m^2 for 1 h or from a Xenon XBF 6000 W (filtered to stimulate sea level sun light), ½ solar constant for 40 min. The negative control was methanol and the positive was 8-MOP in methanol. No phototoxicity was observed. As a part of the same experiment, undiluted wintergreen oil was applied to the skin of 6 hairless mice. No phototoxic reactions were observed.

Salicylic Acid

Salicylic Acid is not a photosensitizer.[1]

Tridecyl Salicylate

Tridecyl Salicylate is not a photosensitizer.[1]

Human

Hexyl Salicylate

Phototoxicity of Hexyl Salicylate was evaluated in 56 subjects (41 females and 15 males), when used at concentrations of 0.3%, 3% and 30% in 3:1 DEP:ethanol. Hexyl Salicylate was applied to a 25 mm Hilltop® Chamber, which was applied to the back of each subject.[5] Each subject received duplicate patches that were placed on both sides of the spine: three patches with Hexyl Salicylate and three control patches (vehicle control 3:1 DEP:ethanol and saline control). Patches remained in place for 24 h. Following 24 h, the patches on the left paraspinal region were removed and the skin sites were irradiated with 16 J/cm^2 of UVA irradiation for 10 min. Then the sites were irradiated with 0.75 MED UVB. A 150-W Berger Solar Ultraviolet Simulator was used as the ultraviolet radiation source in the study. Patches were removed from the non-irradiated test sites on the right paraspinal region after the UVA/ UVB dosing was complete. The non-irradiated sites were used as controls to assess irritation potential of Hexyl Salicylate. Reactions were assessed at 1, 24, 48, and 72 h following UVA and UVB irradiation. No reactions were observed.

Ethylhexyl Salicylate and Salicylic Acid

Salicylic Acid is not a photosensitizer, nor is it phototoxic. Salicylic Acid and Ethylhexyl Salicylate are low-level photoprotective agents.[1]

Computational Analyses/Predictions

Amyl Salicylate, Hexyl Salicylate, and Methyl Salicylate

A database of 259 heterogeneous organic compounds (including Amyl Salicylate, Hexyl Salicylate, and Methyl Salicylate) evaluated in the guinea pig maximization test was subjected to multivariate quantitative structure-activity relationship (QSAR) analysis, utilizing principal component analysis and linear discriminant analysis.[66] These 2 techniques are commonly associated with the analysis of qualitative biological data, and this study is an effort to correlate the sensitization potential of a chemical with its physicochemical parameters. Forty-four chemical descriptors (molecular weight included) were calculated. To utilize the guinea pig sensitization test data for QSAR analysis, chemicals were classified as either non-sensitizers (non- or weak) or sensitizers (moderate or strong). Amyl Salicylate, Hexyl Salicylate, and Methyl Salicylate were classified as non-sensitizers. The authors noted that principal component analysis was only moderately useful as a predictive tool when physicochemical descriptors were chosen rationally. Linear discriminant analysis predicted the sensitization potential of only 82.6% of compounds in the database correctly, and, results indicated that there is a trend for this model to predict that compounds are non-sensitizers. This is due to the structural alerts and physicochemical properties not being able to distinguish between sensitizers and non-sensitizers. In other words, the model puts so much weight on the structural alerts that, if one is not present, the compound will be classified as a non-sensitizer.

A QSAR system for estimating skin sensitization potency that incorporates skin metabolism and considers the potential of parent chemicals and/or their activated metabolites to react with skin proteins has been developed.[67] A training set of diverse chemicals was compiled and their skin sensitization potency assigned to one of three classes. The sensitization potential of chemicals is determined in two stages. The first model discriminates whether the query structure is a sensitizer or non-sensitizer. If the chemical is identified as a sensitizer, a second model is applied to discriminate between strong and moderate-weak sensitizers. Amyl Salicylate was one of the chemicals that was identified to fall within the model domain accounting for the first neighbors of centered atoms, and was predicted to be a non-sensitizer. A set of 96 chemicals tested for skin sensitization using the LLNA (47 chemicals) and guinea pig maximization test (49 chemicals) and not used in the training set were used for external validation of the model. It was noted that the model predicts the external data fairly well if the model domain was determined based on the second neighbors of centered atoms. In this case, the correctness of predictions is 87%, compared with 71% if the model domain was determined accounting for first neighbors only. It should be mentioned that ignoring the model domain reduced the predictability of the model to 52%.

A study was performed to validate a QSAR rank model for grading allergenic potency using a database of 74 known allergens and non-allergens that were chosen among fragrance chemicals in common use.[68] The model's scoring system for class levels was: Class 1 (non-allergic; scores = 0.63 to 1.97), Class 2 (weak to mild; scores = 1.24 to 3.10), Class 3 (moderate; scores = 1.81 to 4.14), and Class 4 (strong to extreme; scores = 2.66 to 4.88). Hexyl Salicyalate and Methyl Salicylate were classified as non-allergic.

Hexyl Salicylate

An exposure-based QRA methodology has been used to determine acceptable exposure limits (in finished product) for Hexyl Salicylate and a new International Fragrance Association (IFRA) standard has been issued.[69] Limitations for various finished product categories have been established, ranging from 1.3% to 25.7%. The following relevant sensitization data were used for implementation of the QRA: LLNA weighted mean EC3 value (45 µg/cm^2), human data: NOEL – HRIPT (induction) (35,433 µg/cm^2), experimental NOEL – MAX (induction) (2069 µg/cm^2), and WoE NESIL (35,400 µg/cm^2).[5]

OCULAR IRRITATION STUDIES

In Vitro

Sodium Salicylate

Sodium Salicylate was evaluated using the EpiOcular™ reconstructed human cornea-like tissue model.[70] The tissues are cultured from primary non-transformed human epidermal keratinocytes (NHEK) obtained from individual donors. The tissues were incubated with Sodium Salicylate (50 µl) for 30 minutes, and tissue viability was assessed using the MTT assay. If the test chemical-treated tissue viability was ≤ 60% relative to negative control-treated tissue viability, the test chemical was predicted as "in vitro irritant." Values for % viability were 5% (run #1) and 5.1% (run #2) for Sodium Salicylate, classifying the chemical as an ocular irritant.

Animal

Methyl Salicylate

A rabbit eye irritation test was conducted in 5 healthy albino rabbits. A 0.005 ml aliquot of neat Methyl Salicylate was applied to the center of the cornea while the lids were retracted.[71] One minute later the lids were released. The eyes were examined 18 - 24 h later in strong diffuse daylight and then stained with fluorescein. Methyl Salicylate caused necrosis on 13 to 37% of the cornea (visible after staining).

A rabbit eye test was conducted in 3 healthy albino rabbits.[6] A One-tenth ml of 1.25% Methyl Salicylate in specially denatured alcohol (SDA) 39C was instilled into the right eye of each rabbit with no further treatment. The untreated left eye served as control. Observations were made every 24 h for 4 days and then again on day 7 according to the Draize method. Intense conjunctival irritation accompanied by chemosis and considerable discharge was observed in all 3 rabbits. The treated eyes were normal on day 7 of observation.

Butyloctyl Salicylate, Ethylhexyl Salicylate,
Isodecyl Salicylate, Methyl Salicylate, and Tridecyl Salicylate

The ocular irritation potential is negative for the following ingredients: Butyloctyl Salicylate, Ethylhexyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, and Tridecyl Salicylate.[1]

CLINICAL STUDIES

Retrospective and Multicenter Studies

Amyl Salicylate

A total of 1323 patients (from 11 centers combined) were patch tested with fragrances.[72] Patch testing was performed with Finn chambers on Scanpor tape, applied to the back for 2 days. Readings were made according to International Contact Dermatitis Research Group (ICDRG) guidelines on days 2 and 3, or on days 2 and 4. Twenty-eight irritant or doubtful reactions (on day 3 or 4) to a total of 19 fragrance materials were reported. Two reactions (irritant or doubtful) were reported for 1% Amyl Salicylate.

A population of 1855 patients (6 European dermatology departments combined), was patch tested with fragrances. [73] Finn Chambers on Scanpor tape were used in all centers except 1(at which van der Bend chambers were used). Readings were taken at most centers on days 2 and 4. The reading at D3 or D4 was used for overall evaluation of positive test results. Three patients had a positive reaction (+) to 5% Amyl Salicylate, and 5 had doubtful reactions.

Hexyl Salicylate

In a multicenter study, 218 fragrance sensitive patients with proven contact dermatitis were patch tested with various fragrance materials according to internationally accepted criteria.[74] No reactions were observed with 5% Hexyl Salicylate in petrolatum.

Case Reports

Capryloyl Salicylic Acid

A female patient woman who used day and night creams containing Capryloyl Salicylic Acid presented with dermatitis of the face, first observed 3 months earlier.[75] Positive patch test reactions (+) to both products and to Capryloyl Salicylic Acid (1% in alcohol) were reported. Another female patient who used the same night cream containing Capryloyl Salicylic Acid also presented with facial dermatitis and had a positive path test reaction to this ingredient (1% in alcohol).

A female patient presented with a pruritic erythematous rash that arose on her face 10 days after application of a cream containing Capryloyl Salicylic Acid.[76] Patch testing was performed, and reactions were scored at 48 h and 96 h. At 96 hours, a positive reaction (++) to the cream was reported. A positive allergic reaction (++) to 1% Capryloyl Salicylic Acid in alcohol was observed in the patient (at 48 h and 96 h), but not in 15 healthy control subjects.

In a comment on the preceding 2 case reports, it is stated that Capryloyl Salicylic Acid is unlikely to be significantly allergenic, and is therefore unlikely to be the cause of the contact allergy reported.[77] However, the structural isomer, 3-capryloyl salicylic acid, is a highly plausible contaminant of Capryloyl Salicylic Acid, and is likely to be sufficiently allergenic to account for the observed contact allergy.

Amyl Salicylate, Ethylhexyl Salicylate, Methyl Salicylate, Salicylic Acid, and Sodium Salicylate

A 48-year-old woman with a 12-year history of rosacea was advised to use a sunscreen that contained Ethylhexyl Salicylate during several months prior to intense pulsed-light treatment for facial telangiectasia.[78] One-half year later, the patient developed facial dermatitis. She had a positive (++) patch test reaction to 2% Ethylhexyl Salicylate in petrolatum, a positive (+) patch test reaction to 5% Ethylhexyl Salicylate in petrolatum, and a positive (++) patch reaction to the sunscreen product. Results of repeated open application tests (ROATs) with Ethylhexyl Salicylate, 2% and 5%, were positive from day 4 on. A total of 29 consecutive eczema patients acting as controls were negative to Ethylhexyl Salicylate (at 5% and 2% in petrolatum). The patient was retested after 1 year, and the (+) reaction to Ethylhexyl Salicylate was reproduced. Patch test results for the following other salicylates were negative: Amyl Salicylate (5% in petrolatum), Methyl Salicylate (2% in petrolatum), Salicylic Acid (2% in petrolatum), and Sodium Salicylate (2% in petrolatum).

Ethylhexyl Salicylate, Methyl Salicylate, Salicylic Acid, and Sodium Salicylate

A woman who used a sunscreen containing Ethylhexyl Salicylate and had a history of rhinitis and intrinsic bronchial asthma developed erythematous micropapules (that progressed to microvesicles and vesicles) on the back, chest, and abdomen.[79] A skin biopsy of the lesions revealed a dermal hypersensitivity reaction that was consistent with contact dermatitis. Epicutaneous tests of the components of the sunscreen spray product were performed. Results were positive for

Ethylhexyl Salicylate (test concentration not stated), but not for any of the other ingredients tested. Patch test results for the following other salicylates were negative: Methyl Salicylate, Sodium Salicylate, and Salicylic Acid. Photopatch test results were positive for Ethylhexyl Salicylate (test concentration not stated), but not for Methyl Salicylate, Sodium Salicylate, or Salicylic Acid.

Methyl Salicylate, Salicylic Acid, and Salicylates

Numerous case studies report toxic reactions to oral ingestion of salicylates.[1] *Dermal toxicity is described in the case literature as follows: dermal application of Salicylic Acid with concomitant oral administration of a nonsteroidal anti-inflammatory drug; following dermal application of a Salicylic Acid ointment in an elderly subject recovering from acute renal failure; topical application of Methyl Salicylate (and methanol) followed by the application of heat (skin and muscle necrosis and interstitial nephritis); and severe urticarial and angioedema with Methyl Salicylate exposure. In 20 patients with eczema or contact dermatitis, Methyl Salicylate at 67% is reported to cause irritation in 8 subjects; at 40%, 2 subjects; and at 38%, 15%, and 3.75% - no irritation in any subject. In 2 case studies of reactions to a wart paint containing Salicylic Acid, Salicylic Acid (tested at 3% in petrolatum) was not the causative agent. Methyl Salicylate (2%) in arachis oil and 2% aqueous Sodium Salicylate produced positive patch results in a patient with acute dermatitis who had been using an ointment containing menthol and camphor. Methyl Salicylate (12%) and Salicylic Acid (5%) in yellow soft paraffin produced positive patch tests in 4 patients with dermatitis and one with psoriasis, all with some history of exposure to salicylates.*

Adverse Event Reports

Salicylic Acid

Although rare, salicylic acid toxicity (salicylism) can occur from topical application.[80] Salicylism, the syndrome of salicylic acid toxicity, can be acute or chronic and develops when blood concentrations of salicylate are greater than 35 mg/dL. Symptoms of salicylism include nausea, confusion, dizziness, delirium, psychosis, stupor, and coma.

Methyl Salicylate

A man became acutely ill (within less than an hour) after using an herbal skin cream containing Methyl Salicylate (high concentration, value not stated) for the treatment of psoriasis.[81] The area of application was covered with an occlusive wrap. Signs of metabolic acidosis superimposed on respiratory alkalosis and a serum salicylate level of 48.5 mg/dL were reported. These signs declined with treatment. The author noted that the transcutaneous absorption (described as rapid) of Methyl Salicylate was enhanced due to the abnormal areas of skin and use of an occlusive dressing. It was concluded that acute salicylate toxicity may result from the topical administration of Methyl Salicylate.

Other Clinical Reports

Capryloyl Salicylic Acid

In a split-face study, 44 female volunteers with mild to moderate facial hyperpigmentation and fine lines/wrinkles were randomized and Capryloyl Salicylic Acid peel was applied to one side of the face.[82] Increasing peel concentrations were applied (5 - 10% Capryloyl Salicylic Acid) based on the tolerance level of the subjects and clinical observations of an expert dermatologist for 12 weeks at biweekly intervals. Results indicated that there were no significant changes in erythema for Capryloyl Salicylic Acid from baseline values when compared with pre-peel to pre-peel and post-peel to post-peel at different weeks.

Salicylic Acid

In patients with venous leg eczema, Salicylic Acid augments histidine release in 3/320 challenged with ragweed pollen.[1] *Salicylic Acid exacerbates urticarial reactions to aspirin; 13 of 18 patients in one studyand 6 of 20 in another. At 5% in petrolatum, however, Salicylic Acid does not cause any urticarial reactions in atopic, urticarial, nonatopic, and nonallergic patients. Salicylic Acid is well-documented to have keratolytic action on normal human skin. It had a small therapeutic effect in patients with various forms of ichthyosiform dermatoses, but decreased clearing in 8 of 11 psoriasis patients when compared to UV therapy alone. Therapeutic toxicities include nausea, vomiting, tinnitus, dizziness, headache, dullness, confusion, sweating, rapid pulse and breathing, skin eruptions, and fever. One estimate is that a blood concentration > 300 µg/ml of a salicylate should be considered toxic. Toxic reactions occur more frequently in children. Care must be taken in prescribing salicylate-containing medications because systemic clearance of salicylates may be*

reduced with age. Severe poisoning can result in delirium, hallucinations, convulsions, coma, and respiratory or cardiovascular collapse. Reversible hearing loss and tinnitus are reported side effects of salicylates at therapeutic levels.

Methyl Salicylate

Methyl Salicylate taken in quantities greater than or equal to 1 teaspoon are reported to be quite toxic (equivalent of the salicylate that could be derived from 20+ adult aspirin tablets.[1] *Accidental poisoning is not uncommon, especially in children; symptoms of poisoning include kidney irritation, vomiting, and convulsions. The average lethal dose of Methyl Salicylate is 10 ml for children and 30 ml for adults.*

Sodium Salicylate

Sodium Salicylate injected in the skin of aspirin intolerant individuals affected several parameters as follows: 1/23 had a positive skin test to Sodium Salicylate; 2/31 were positive in the passive cutaneous anaphylaxis test; and 2/26 were positive in the lymphocyte transformation test.[1]

Salicylates

A review of radiographs taken in 155 cases of juvenile arthritis in which various forms of salicylates had been administered at concentrations ranging from 0.1 to 3.24 g for several months did not find any evidence of bone lesions.[1]

RIFM SAFETY ASSESSMENT CONCLUSION ON SALICYLATES

A published toxicologic and dermatologic assessment of salicylates, when used as fragrance ingredients, by the RIFM is available, and the RIFM Expert Panel's lengthy conclusion on these fragrance ingredients is stated in the paragraphs below.[3] This conclusion is based on a review of safety test data on salicylates that were available before and after publication of the CIR published final report. Many of the studies are found in the original CIR Final Report on salicylates and in this re-review document. Studies on salicylates with aromatic sidechains (i.e., benzyl salicylate) are also mentioned in the RIFM safety assessment conclusion. Such studies (on salicylates with aromatic sidechains) are not included in this re-review document or the original CIR Final Report, and, thus, are not relevant to this safety assessment. It should be noted that the conclusion stated in the paragraphs below should not be considered alone, but along with the more recent data summaries that are included in this re-review document.

The salicylates are well absorbed by the oral route, and oral bioavailability is assumed to be 100%. Absorption by the dermal route in humans is more limited with bioavailability in the range of 11.8 - 30.7%.

The salicylates are expected to undergo extensive hydrolysis, primarily in the liver, to salicylic acid. In the liver, salicylic acid is conjugated with either glycine or glucuronide and is excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. The hydrolyzed side chains are metabolized by common and well-characterized metabolic pathways leading to the formation of innocuous end products. The expected metabolism of the salicylates does not present toxicological concerns.

The acute dermal toxicity of the salicylates is very low, with LD_{50} values in rabbits reported to be greater than 5000 mg/kg body weight. The acute oral toxicity of the salicylates is moderate, with toxicity generally decreasing with increasing size of the ester side-hain and with LD_{50s} between 1000 and > 5000 g/kg.

In dermal subchronic toxicity studies, extreme doses of Methyl Salicylate (~5 g/kg body weight/day) possibly were nephrotoxic but the data were minimal. The subchronic oral no-observable-adverse-effect level (NOAEL) is concluded to be 50 mg/kg body weight/day. At higher doses, in excess of 300 – 450 mg/kg body weight/day, Methyl Salicylate is associated with increased density of the metaphyses of the long bones in rats. The oral NOAEL of 50 mg/kg body weight/day can be used in the risk assessment of the use of the salicylates as fragrance ingredients.

Oral chronic toxicity data for Methyl Salicylate are consistent with the oral subchronic toxicity data in that the lowest NOAEL value identified was 50 mg/kg body weight/day in both rats and dogs.

Genetic toxicity data, for Methyl Salicylate, a few other salicylates and for structurally related alkyl- and alkoxy-benzyl derivatives are negative for genotoxicity. Since the metabolites of the salicylates are simple alcohols and acids, the salicylates as a group are considered to be non-genotoxic.

Limited long-term oral studies in rats and an i.p. injection study in mice using Methyl Salicylate provided no evidence of carcinogenicity. Given the metabolism of salicylate and the evidence that they are non-genotoxic, it can be concluded that the salicylates are without carcinogenic potential.

The reproductive and developmental toxicity data on Methyl Salicylate demonstrate that high, maternally toxic doses result in a pattern of embryotoxicity and teratogenesis similar to that characterized for Salicylic Acid. The NOAELs for reproductive toxicity (e.g., fertility, neonatal growth and survival, etc.) are lower than doses reported to be teratogenic and are consistent with the NOAELs available from subchronic and chronic toxicity studies. The Cosmetic Ingredient Review Board has concluded that use of salicylates and Salicylic Acid in cosmetic products would not pose a risk for reproductive or developmental effects in humans.

At concentrations likely to be encountered by humans through the use of the salicylates as fragrance ingredients, these chemicals are considered to be non-irritating to the skin.

The salicylates are non-phototoxic and have no photoirritant or photoallergenic activity.

The use of the salicylates in fragrances produces low levels of exposure relative to doses that elicit adverse systemic effects in laboratory animals exposed by the dermal or oral route. The estimates for maximum systemic exposure to salicylates of humans using cosmetic products range from 0.0002 to 0.4023 mg/kg/day based on the assumption of 100% bioavailability. Considering that bioavailability of the salicylates is actually likely in the range of 11.8–30.7%, systemic exposures are likely lower, in the range of 0.00002–0.124 mg/kg body weight/day.

Based on the above considerations, and using the NOAEL values of 50 mg/kg body weight/day identified in the subchronic (Webb and Hansen, 1963; Abbott and Harrisson, 1978; Drake et al., 1975) and the chronic toxicity studies (Packman et al., 1961; Webb and Hansen, 1962, 1963), a margin of safety for systemic exposure of humans to the individual salicylates in cosmetic products may be calculated to range from 125 to 2,500,000 (depending upon the assumption of either 12–30% or 100% bioavailability following dermal application) times the maximum daily exposure.

SUMMARY

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) published a Final Report on the Safety Assessment of Salicylic Acid and 16 salicylates in 2003. This ingredient group includes Salicylic Acid and its salts and esters. The re-review document relating to this final report that is being considered contains data on the 17 ingredients in the published final report as well as data on 4 additional salicylates that may be added to the safety assessment. It is possible that the final report will be reopened to revise the original conclusion or add the 4 ingredients, or both actions could result.

Of the 21 ingredients that are included in the re-review document, the greatest use frequency of 3,474 uses is being reported for Ethylhexyl Salicylate. In the absence of current use concentration data, it should be noted that this ingredient has been used at concentrations up to 8% in lipstick (leave-on product). This is the highest ingredient use concentration that is reported in the published final report.

In vitro skin penetration data (human or rat skin) indicated that Ethylhexyl Salicylate and Methyl Salicylate were percutaneously absorbed. Additionally, the conversion of Methyl Salicylate to Salicylic Acid in hairless mouse skin *in vitro* following topical application of 1% Methyl to the skin was evaluated. Less than 5% of applied dose was metabolized to Salicylic Acid.

In *in vivo* studies, the percutaneous absorption of Methyl Salicylate has been demonstrated in pigs and humans, and the percutaneous absorption of Ethylhexyl Salicylate has been demonstrated in humans. The *in vivo* absorption of a formulation containing 20% Methyl Salicylate was studied using male Wistar rats. The levels of unhydrolyzed Methyl Salicylate in tissues below the treated site were low, i.e., only 2 to 3 µg/ml throughout the study period. A mathematical method was used to estimate total body absorption of some salicylate esters including Hexyl Salicylate. The estimated total body absorption of Hexyl Salicylate per µg over 1.4 m^2 was 0.18 at 2 h, 4.1 at 6 h and 27 at 12 h. Reportedly, after oral ingestion, Methyl Salicylate is readily metabolized to Salicylic Acid.

In acute dermal toxicity studies of Ethylhexyl Salicylate, Hexyl Salicylate, and Methyl Salicylate involving rabbits, the LD_{50} was > 5 g/kg for each salicylate. The same was true in acute oral toxicity studies on Ethylhexyl Salicylate and Hexyl Salicylate involving rats and rabbits. In acute oral toxicity studies on Methyl Salicylate involving mice, the LD_{50} was calculated to be 1.39 g/kg (95% CI of 1.25 to 1.54 g/kg) and a dose of 0.5 g/kg was selected as the maximum tolerated dose.

In a short-term inhalation toxicity study involving mice, there were no test substance-related gross pathological or histopathological findings after inhalation of a fragrance mixture containing 5.8% Amyl Salicylate. Also, in a short-term inhalation toxicity study evaluating respiratory sensitization potential, Methyl Salicylate did not induce a measurable IL-4 response. There were no test substance-related gross pathological or histopathological findings in rats in a subchronic inhalation toxicity study of a fragrance mixture containing 4% Amyl Salicylate.

In a toxicological and dermatological assessment of salicylates, when used as fragrance ingredients, a margin of safety for systemic exposure is mentioned. Based on NOAEL values of 50 mg/kg body weight/day in subchronic and chronic toxicity studies, a margin of safety for systemic exposure of humans to the individual salicylates in cosmetic products may be calculated to range from 125 to 2,500,000 (depending upon the assumption of either 12 - 30% or 100% bioavailability following dermal application) times the maximum daily exposure.

In an in vitro developmental toxicity study involving Salicylic Acid, post-implantation rat embryos were cultured with Salicylic Acid concentrations of 10 to 1000 µg/ml. Salicylic Acid decreased all growth and developmental parameters in a concentration-dependent manner. The same results were reported for rat embryos cultured with Salicylic Acid or Sodium Salicylate at concentrations in the 0.1 to 0.6 mg/ml range. On postnatal day 1, 89% of the pups from dams (rats) that had received a single oral dose of 300 mg/kg Sodium Salicylate had supernumerary ribs. No external malformations of pups was observed. In another study, a 4.8% malformations (including exencephaly and spina bifida) incidence was reported for fetuses from rats dosed with Sodium Salicylate (250 mg/kg/day) on gestation days 8 to 10. It has been reported that the use of Salicylic Acid in the third trimester can potentially cause closure of the ductus arteriosus and oligohydramnios.

Hairless mice were evaluated for skin cancer in a study in which the effects of synthetic solar light on the skin after application of a cream containing 2% or 4% Salicylic Acid were evaluated. It was concluded that Salicylic Acid had a protective effect against the photocarcinogenicity of light at lower intensities.

In an estrogen receptor binding study using a consensus modeling method, Ethylhexyl Salicylate was classified as an estrogen receptor non-binder, whereas Butyloctyl Salicylate was classified as having binding activity to the estrogen receptor. When the estrogenic activity of Ethylhexyl Salicylate was compared to 17-ß-estradiol in a recombinant yeast estrogen assay, the dose response curve for Ethylhexyl Salicylate was shallower than the one for 17-ß-estradiol and Ethylhexyl Salicylate had a submaximal response for estrogenic activity.

Undiluted Amyl Salicylate (0.1 g) was severely irritating to the skin of rabbits, but mildly irritating to the skin of guinea pigs. Undiluted Amyl Salicylate (0.05 g) did not cause skin irritation in miniature swine. Mild erythema was observed in the acute dermal toxicity study on Ethylhexyl Salicylate that is summarized in this safety assessment.

Following intradermal injection, 0.1% Hexyl Salicylate(vehicle not reported) produced an irritation reaction in guinea pigs, but 5% Hexyl Salicylate (vehicle not reported) did not. An explanation for these results was not provided. In an irritation test in which patches containing up to 2% Hexyl Salicylate (0.1 ml) were applied to Dunkin/Hartley albino guinea pigs, slight erythema and edema were observed at concentrations of 0.25%, 0.5%, 1%, and 2%; very slight erythema was observed at a concentration of 0.1%. Patches saturated with concentrations up to 50% Hexyl Salicylate were applied to Dunkin/Hartley albino guinea pigs in another test, and slight skin irritation was observed at concentrations of 25% and 50%, but not 10%. The patch testing of hairless guinea pigs with Hexyl Salicylate (0.3 ml per patch) at concentrations up to 100% yielded negative results. Skin irritation also was not observed in miniature swine tested with undiluted Hexyl Salicuylate (20 µl/5 cm^2). When the irritation potential of Hexyl Salicylate at concentrations up to 100% was evaluated using rabbits, patch test (0.5 ml per patch) results for 10%, 25%, 50%, and 100% Hexyl Salicylate were negative. Slight to moderate edema and erythema was observed rabbits in an acute dermal toxicity study on Hexyl Salicylate that is summarized in this safety assessment. Skin irritation was not observed in hairless mice tested with Hexyl Salicylate (20 µl/5 cm^2).

Flaking, hyperkeratosis, and dry desquamation were observed after an aliquot of 20 µl of undiluted wintergreen oil (contained 80 to 99% Methyl Salicylate) was applied to miniature swine. When Methyl Salicylate was applied repeatedly (twenty-one 0.1 ml applications) to guinea pigs in an open epicutaneous test, the minimal irritating concentration was determined to be 3% Methyl Salicylate. A minimally irritating concentration of 20% was determined in a skin irritation test on Methyl Salicylate. Slight to moderate edema and erythema was observed rabbits in an acute dermal toxicity study on 5 g/kg Methyl Salicylate that is summarized in this safety assessment.

Skin irritation was not observed in a 48-h occlusive patch test on 32% Amyl Salicylate (in acetone) involving 50 subjects. Skin irritation also was not observed in a 48-h closed patch test on 4% Ethylhexyl Salicylate (in petrolatum) involving 23 subjects.

Using Hilltop® chambers on 30% Hexyl Salicylate involving 103 subjects, skin irritation was not observed. Skin irritation also was not observed in a 48-h patch test on 3% Hexyl Salicylate involving 22 subjects, in a 4-h patch (Hilltop® chamber) test on undiluted Hexyl Salicylate involving 30 subjects, or in a 24-h patch (Hilltop® chamber) test on Hexyl Salicylate at concentrations up to 30% in a study involving 56 subjects.

Skin irritation was not observed in a 48-h occlusive patch test involving 27 subjects or in a 48-h occlusive patch test on 12% wintergreen oil (containing 80 to 99% Methyl Salicylate) involving 25 subjects. In a study evaluating the skin irritation potential of Methyl Salicylate (in 80% ethanol and 20% deionized water) pipetted (25 ml) onto the skin of 9 subjects, 30% and 60% Methyl Salicylate caused skin irritation. It has been noted that possible complications relating to the topical use of Salicylic Acid as a peeling agent include persistent erythema and pruritus.

Formulations for vitiligo treatment containing up to 7.5% Salicylic Acid were applied to groups of 6 rabbits. The 3.5%, 5%, and 7.5% formulations cause desquamation, an inflammatory reaction, and a comedogenic effect.

Hexyl Salicylate was predicted to be a skin sensitizer in the Genomic Allergen Rapid Detection assay. Using an integrated testing strategy for skin sensitization that focuses on 3 in vitro methods that cover the first 3 steps of the adverse outcome pathway, results for the sensitization potential of Salicylic Acid were considered equivocal, but ultimately were considered positive results.

In the LLNA, a very low EC3 value (0.18%) was reported for Hexyl Salicylate, which may have been due to possibly sensitizing impurities. When Hexyl Salicylate was tested for sensitization potential in guinea pigs using a modified Draize procedure, sensitization was observed after intradermal challenge with 0.1% Hexyl Salicylate and topical challenge with 5% Hexyl Salicylate. In a photoallergy test involving hairless albino guinea pigs, sensitization reactions were not observed after challenge with 50% and 100% Hexyl Salicylate. In a Magnusson-Kligman guinea pig maximization test, skin sensitization was not observed in guinea pigs challenged with 10% Hexyl Salicylate in acetone.

Methyl Salicylate (50%) was predicted to be a non-sensitizer in the LLNA. The same was true for Salicylic Acid and 0.7 µM Methyl Salicylate.

A human skin sensitization no-observed-effect-level of 35,433 µg/cm^2 (study details not provided) has been reported for Hexyl Salicylate. Also in a human maximization test on Hexyl Salicylate, no induction was observed at a dose of 20,654 µg/cm^2 (study details not included). In an HRIPT (Hilltop® chamber system) involving 103 subjects, sensitization reactions to 30% Hexyl Salicylate were not observed. Maximization test results for 3% Hexyl Salicylate in petrolatum were negative in 22 subjects.

In a human maximization test on wintergreen oil (contains 80 to 99% Methyl Salicylate) involving 25 volunteers, sensitization was not observed at a concentration of 12%. Maximization test results for 8% Methyl Salicylate were also negative in 27 subjects. In an HRIPT involving 39 subjects, 1.25% Methyl Salicylate did not induce skin sensitization.

Results for Ethylhexyl Salicylate were classified as negative in the 3T3 neutral red uptake phototoxicity test at concentrations ranging from 0.1 to 316 µg/ml. Undiluted Hexyl Salicylate was not phototoxic in studies involving mice or miniature swine. At concentrations ranging from 5% to 100%, Hexyl Salicylate was not phototoxic to albino hairless guinea pigs. Hexyl Salicylate did not induce photoallergenicity in groups of albino hairless guinea pigs tested with concentrations of 50% and 100%.

The phototoxicity of undiluted wintergreen oil (contained 80% to 99% Methyl Salicylate) was evaluated using miniature swine, and results were negative. There also was no evidence of phototoxicity in 56 subjects tested with Hexyl Salicylate at concentrations of 0.3%, 3%, and 30%.

Amyl Acetate was classified as a non-sensitizer in a QSAR system for estimating sensitization potency that incorporates skin metabolism and considers the potential of parent chemicals and their activated metabolites to react with skin proteins. Hexyl Salicylate and Methyl Salicylate were classified as non-allergenic in a study that was performed to validate a QSAR rank model for grading allergenic potency. An exposure-based QRA methodology has been used to determine acceptable exposure limits (in finished product) for Hexyl Salicylate. Limitations for various finished product categories have been established, ranging from 1.3% to 25.7%.

Sodium Salicylate was classified as an ocular irritant using the EpiOcular™ reconstructed human cornea-like tissue model, whereby the tissues were incubated with 50 µl of Sodium Salicylate. In an ocular irritation test involving rabbits, the instillation of Methyl Salicylate (0.0005 ml) caused a grade 3 reaction (necrosis on 13 to 37% of the cornea). Intense

conjunctival irritation, accompanied by chemosis and considerable discharge, was observed in rabbits in which 1.25% Methyl Salicylate (0.1 ml) was instilled into the eyes.

In multicenter studies, an irritant or doubtful reaction was observed in 2 of 1323 patients patch (Finn chamber) tested with 1% Amyl Salicylate and 3 positive reactions and 5 doubtful reactions were observed in a population of 1855 patients patch tested with 5% Amyl Salicylate. No reactions were observed in a multicenter study in which 218 fragrance-sensitive patients with contact dermatitis were patch tested with 5% Hexyl Salicylate.

Positive patch test reactions to 1% Capryloyl Salicylic Acid have been reported in case reports, one of which reported no reactions in a control group of 15 subjects. It has been suggested that it is unlikely that 5-Capryloyl Salicylate is significantly allergenic, but that the structural isomer, 3-capryloyl salicylic acid, is a highly plausible contaminant and is likely to be sufficiently allergenic. Positive patch test reactions to 2% and 5% Ethylhexyl Salicylate were reported in another case report (patient with facial telangiectasia and history of rosacea), but reactions to these test concentrations were negative in the 29 consecutive eczema patients that served as controls. Also, patch test reactions to the following salicylates were negative in this case report: 5% Amyl Salicylate, 2% Methyl Salicylate, 2% Salicylic Acid, and 2% Sodium Salicylate. A contact dermatitis patient had a positive patch test reaction to Ethylhexyl Salicylate (concentration not stated), but not to Salicylic Acid, Methyl Salicylate, or Sodium Salicylate.

DISCUSSION FROM PUBLISHED CIR FINAL REPORT ON SALICYLATES

The CIR Expert Panel considered that the available information is sufficient to evaluate the safety of these ingredients in cosmetic formulations. In reaching its conclusion, the Panel considered three primary issues: 1) increased sun sensitivity (e.g., UV radiation induced skin damage); 2) skin irritation; and 3) reproductive and developmental toxicity.

The Panel expects that these ingredients will have application as exfoliating agents in cosmetic formulations at concentrations of use at the high end of the currently reported use levels, in addition to the other uses that have been specified. In that regard, the Panel expressed concern that repeated use of Salicylic Acid and the various salicylates may effectively increase exposure of the dermis and epidermis to UV radiation. On the other hand, information is available suggesting that these ingredients absorb UV radiation, which would decrease the exposure. Data are not available that suggest what the balance of these two influences would be vis a vis UV radiation-induced skin damage. Drawing on its previous experience in reviewing the safety of alpha hydroxyl acids (AHAs), the Panel compared the relatively mild exfoliating action of Salicylic Acid and the various salicylates with that of AHAs, factored in the ultraviolet radiation absorption by salicylates, and estimated that the small increase in sun sensitivity associated with use of AHAs would likely be smaller still with salicylates.

The Panel considered requesting additional safety testing of these ingredients to resolve this question of the existence and/or magnitude of an increase in sun sensitivity, but was convinced that the exfoliant action alone would always raise the possibility that some increase in UV radiation-induced skin damage would be detected, e.g., if more animals had been used, if a more sensitive assay for damage were available, etc. Were there to be evidence of a small increase in sun sensitivity associated with the use of Salicylic Acid and the several salicylates at exfoliant concentrations, or were the available data to be equivocal, the Panel reasoned that the appropriate conclusion would be that these ingredients could be used safely as exfoliants, if expressly formulated to avoid increasing a user's sun sensitivity. Accordingly, the Panel concluded that the prudent course of action would be to advise the cosmetics industry that there can be a risk of increased UV radiation damage with the use of any exfoliant, including Salicylic Acid and the listed salicylates, and that steps need to be taken to formulate cosmetic products with these ingredients as exfoliating agents so as not to increase sun sensitivity, or when increased sun sensitivity would be expected, to include directions for the daily use of sun protection.

The Panel was concerned that the available data were not sufficient to establish a limit on concentration of these ingredients, or to identify the minimum pH of formulations containing these ingredients, such that no skin irritation would occur. Such limits were established with AHAs. Because the available animal and clinical safety test data demonstrate that these ingredients are generally milder than AHAs, the Panel was convinced that it is possible to formulate cosmetic products in a way such that significant irritation would not be likely. Therefore, the Panel concluded that the cosmetics industry should formulate products containing these ingredients so as to be non-irritating.

Reproductive and developmental toxicity associated with exposures to large, therapeutic serum concentrations of Salicylic Acid (as a metabolite of aspirin) have been extensively demonstrated. The Panel considered the possibility that use of Salicylic Acid or the various salicylates could produce serum levels of Salicylic Acid or, with other sources (e.g., aspirin), contribute to serum levels and thereby present a reproductive and developmental toxicity risk. Beginning with the premise

that ingestion of a low-dose regimen (81 mg) aspirin by a 58-kg female would result in exposure of ~1.4 mg/kg/day and that this exposure level is not considered to present any reproductive or developmental toxicity risk, the Panel considered that a representative exposure to a cosmetic product containing Salicylic Acid could result in exposure to ~0.4 to 0.5 mg/kg/day and would not present a risk. Although simultaneous use of several products containing Salicylic Acid could produce exposures greater than would be seen with a baby aspirin, the Panel did not consider it likely that consumers would simultaneously use multiple cosmetic products containing Salicylic Acid. Thus, the serum levels of Salicylic Acid that would result from dermal application would likely be less than serum levels from ingestion.

TABLES

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.[2; *CIR Staff*]

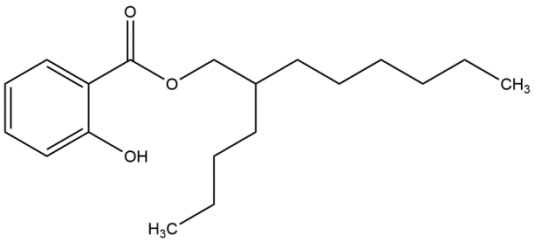
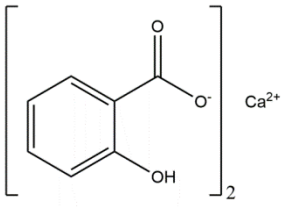
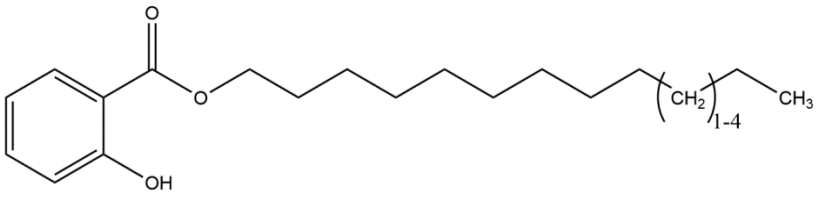
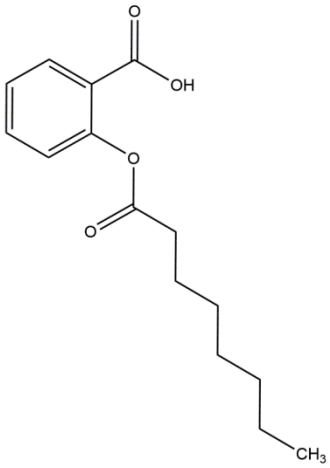
Ingredient CAS No.	Definition & Structures	Function(s)
Butyloctyl Salicylate 190085-41-7	Butyloctyl Salicylate is the organic compound that conforms to the formula: 	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Solvents
Calcium Salicylate 824-35-1	Calcium Salicylate is the calcium salt of Salicylic Acid that conforms to the formula: 	Preservatives
C12-15 Alkyl Salicylate	C12-15 Alkyl Salicylate is the ester of C12-15 Alcohols and salicylic acid. It conforms generally to the formula: where R represents the C12-15 alkyl group. 	Skin-Conditioning Agents - Miscellaneous
Capryloyl Salicylic Acid 78418-01-6	Capryloyl Salicylic Acid is the ester of Salicylic Acid and Caprylic Acid. 	Skin-Conditioning Agents - Miscellaneous

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.[2; *CIR Staff*]

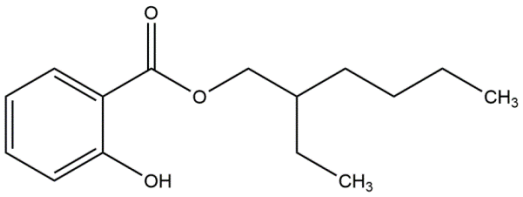
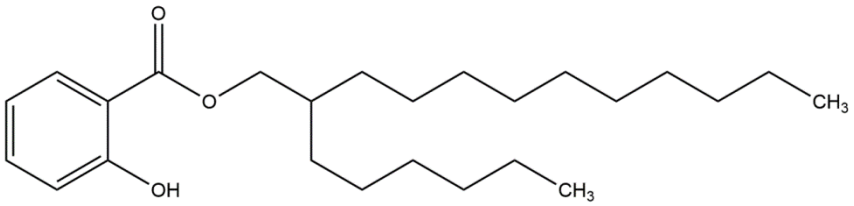
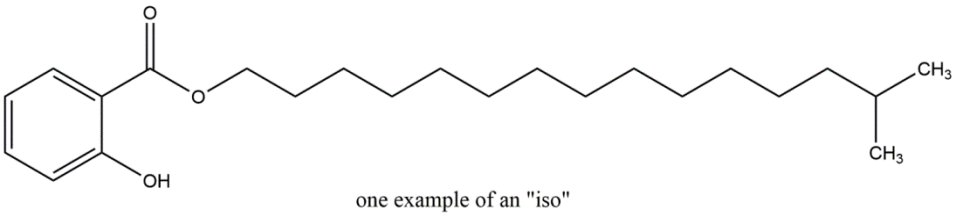
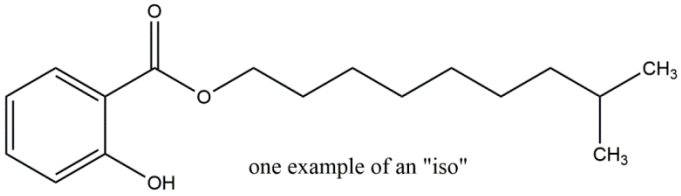
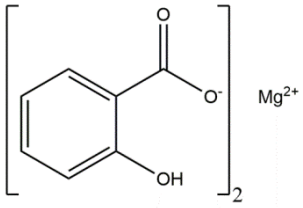
Ingredient CAS No.	Definition & Structures	Function(s)
Ethylhexyl Salicylate 118-60-5	Ethylhexyl Salicylate is the ester of 2-ethylhexyl alcohol and salicylic acid. It conforms to the formula: 	Fragrance Ingredients; Light Stabilizers; Sunscreen Agents
Hexyldodecyl Salicylate 220778-06-3	Hexyldodecyl Salicylate is the organic compound that conforms to the formula: 	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Solvents
Isocetyl Salicylate 138208-68-1	Isocetyl Salicylate is the ester of Isocetyl Alcohol and Salicylic Acid. It conforms to the formula:  one example of an "iso"	Skin-Conditioning Agents - Miscellaneous
Isodecyl Salicylate 85252-25-1	Isodecyl Salicylate is the ester of branched chain decyl alcohols and salicylic acid that conforms to the formula:  one example of an "iso"	Skin-Conditioning Agents - Miscellaneous
Magnesium Salicylate 18917-89-0 551-37-1	Magnesium Salicylate is the magnesium salt of Salicylic Acid that conforms to the formula: 	Preservatives

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.[2; *CIR Staff*]

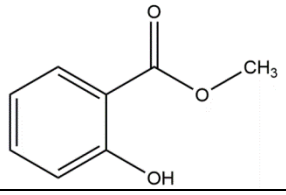
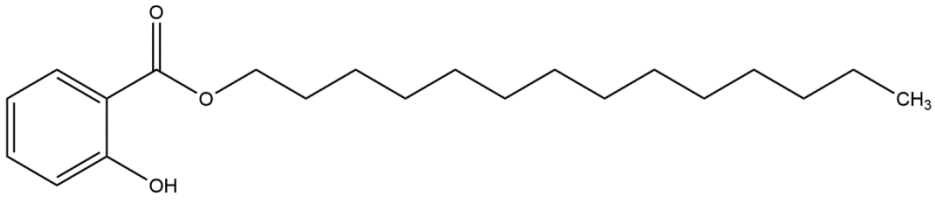
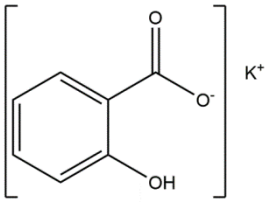
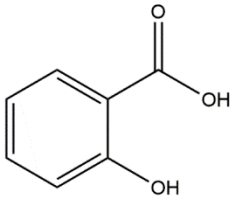
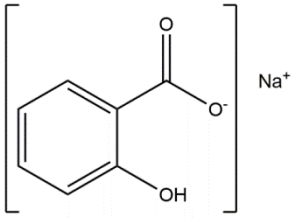
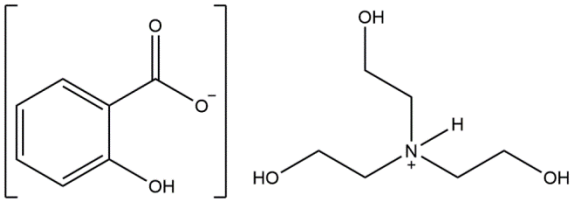
Ingredient CAS No.	Definition & Structures	Function(s)
Methyl Salicylate 119-36-8	Methyl Salicylate is the ester of methyl alcohol and salicylic acid. It conforms to the formula: 	Denaturants; External Analgesics; Flavoring Agents; Fragrance Ingredients; Oral Health Care Drugs
Myristyl Salicylate 19666-17-2	Myristyl Salicylate is the ester of myristyl alcohol and salicylic acid. It conforms to the formula: 	Not Reported
Potassium Salicylate 578-36-9	Potassium Salicylate is the potassium salt of Salicylic Acid that conforms to the formula: 	Cosmetic Biocides; Preservatives
Salicylic Acid 69-72-7	Salicylic Acid is the aromatic acid that conforms to the formula: 	Antiacne Agents; Antidandruff Agents; Corn/Callus/Wart Removers; Denaturants; Exfoliants; Fragrance Ingredients; Hair Conditioning Agents; Hair-Waving/Straightening Agents; Skin-Conditioning Agents - Miscellaneous
Sodium Salicylate 54-21-7	Sodium Salicylate is the sodium salt of salicylic acid that conforms to the formula: 	Denaturants; Preservatives
TEA-Salicylate 2174-16-5	TEA-Salicylate is the triethanolamine salt of salicylic acid that conforms generally to the formula: 	Light Stabilizers; Sunscreen Agents

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.[2; *CIR Staff*]

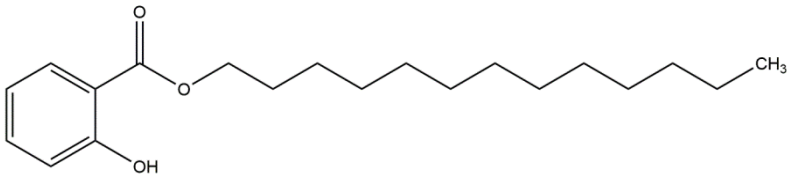
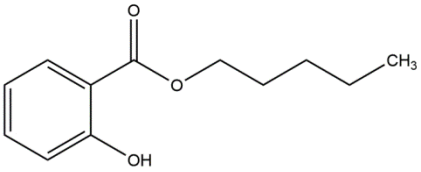
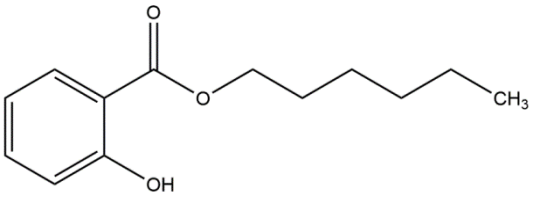
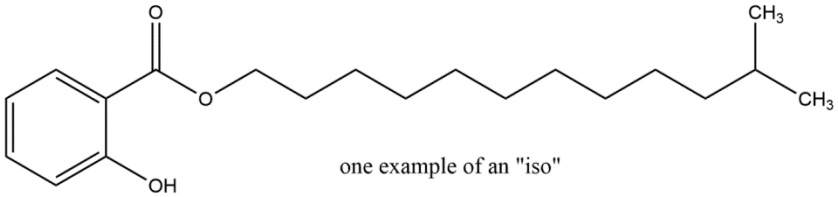
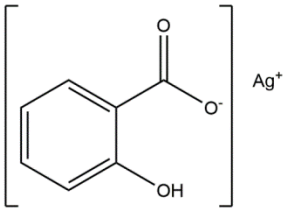
Ingredient CAS No.	Definition & Structures	Function(s)
Tridecyl Salicylate 19666-16-1	Tridecyl Salicylate is the ester of Tridecyl Alcohol and Salicylic Acid. It conforms to the formula: 	Skin-Conditioning Agents - Miscellaneous
POTENTIAL ADD-ONS		
Amyl Salicylate 2050-08-0	Amyl Salicylate is the ester of amyl alcohol and salicylic acid that conforms to the formula: 	Fragrance Ingredients
Hexyl Salicylate 6259-76-3	Hexyl Salicylate is the organic compound that conforms to the formula: 	Fragrance Ingredients; Skin-Conditioning Agents - Occlusive
Isotridecyl Salicylate 1863871-63-9	Isotridecyl Salicylate is the organic compound that conforms to the formula:  one example of an "iso"	Antistatic Agents; Skin-Conditioning Agents - Miscellaneous
Silver Salicylate 19025-97-9	Silver Salicylate is the silver salt of Salicylic Acid. 	Skin Protectants

Table 2. Chemical and Physical Properties of Salicylic Acid and Salicylates

Property	Value/Results	Reference
Butyloctyl Salicylate		
Molecular weight (Da)	306.45	7
log P	6.03 (estimated)	7
pK_a	10.3 (estimated)	7
Calcium Salicylate		
Formula weight (Da)	314.31	7
C12-15 Alkyl Salicylate		
Molecular weight (Da)	306.45 – 348.53	7
Capryloyl Salicylic Acid		
Molecular weight (Da)	264.32	7
log P	3.92 (estimated)	7
pK_a	3.29 (estimated)	7
Ethylhexyl Salicylate		
Form	Colorless liquid	4
Molecular weight (Da)	250.34	7
Water solubility (mg/l at 25ºC)	0.7171 (estimated)	4
Vapor pressure (mm Hg at 25ºC)	0.00000436	4
Flash point (ºC)	> 200	4
Log K_{ow}	6.02 (estimated)	4
Hexyldodecyl Salicylate		
Molecular weight (Da)	390.61	7
log P	8.53 (estimated)	7
pK_a	10.3 (estimated)	7
Isocetyl Salicylate		
Molecular weight (Da)	326,55	7
log P	7.63 (estimated)	7
pK_a	10.4 (estimated)	7
Isotridecyl Salicylate		
Molecular weight (Da)	320.47	7
log P	6.37 (estimated)	7
pK_a	10.4 (estimated)	7
Magnesium Salicylate		
Formula weight (Da)	298.53	7
Methyl Salicylate		
Form	Clear, colorless liquid	6
Molecular weight (Da)	152.15	7
Specific gravity	1.18	6
Water solubility (mg/l at 25ºC)	1875 (estimated)	6
Vapor pressure (mm Hg at 25ºC)	0.09 (estimated)	6
Boiling point (ºC)	222	6
Flash point (ºF)	>212	6
Log K_{ow}	2.6 (estimated)	6
Myristyl Salicylate		
Molecular weight (Da)	334.50	7
log P	6.88 (estimated)	7
pK_a	10.4 (estimated)	7

Property	Value/Results	Reference
Potassium Salicylate		
Formula weight (Da)	176.21	7
Salicylic Acid		
Molecular weight (Da)	138.12	7
log P	1.2 (estimated)	7
pK_a	3.01 (1^{st}; estimated)	7
Sodium Salicylate		
Formula weight (Da)	160.10	7
TEA Salicylate		
Formula weight (Da)	287.31	7
Tridecyl Salicylate		
Molecular weight (Da)	320.47	7
log P	6.46 (estimated)	7
pK_a	10.4 (estimated)	7
ADD-ONS		
Amyl Salicylate		
Molecular weight (Da)	208.26	7
log P	3.12 (estimated)	7
pK_a	10.4 (estimated)	7
Hexyl Salicylate		
Form	Colorless, oily liquid	5
Molecular weight (Da)	222.28	7
Water solubility (mg/l at 25ºC)	6.084 (estimated)	5
Vapor pressure (mm Hg at 20ºC)	< 0.001	5
Boiling Point (ºC)	> 200	5
Log K_{ow}	5.06 (estimated)	5
Isodecyl Salicylate		
Molecular weight (Da)	278.39	7
log P	5.12 (estimated)	7
pK_a	10.4 (estimated)	7
Silver Salicylate		
Formula weight (Da)	244.98	7

Table 3. Frequency and Concentration of Use of Salicylates According to Duration and Exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Amyl Salicylate				**Butyloctyl Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**10**	NR	***0.0023-0.26***	NR	**19**	NR	***1-35.9***	**0.5-5**
Duration of Use								
Leave-On	***1***	***NR***	***0.0023-0.23***	***NR***	***18***	***NR***	***1-35.9***	***0.5-5***
Rinse-Off	***9***	***NR***	***0.02-0.26***	***NR***	***1***	***NR***	***NR***	***NR***
Diluted for (Bath) Use	**NR**	***NR***	***NR***	***NR***	**NR**	***NR***	***NR***	***NR***
Eye Area	NR	NR	NR	NR	1	NR	3.6	NR
Incidental Ingestion	NR	NR	NR	NR	6	NR	35.9	NR
Incidental Inhalation-Spray	NR	NR	0.0023-0.12	NR	3[b]	NR	1-3	4-5[a]
Incidental Inhalation-Powder	NR	NR	NR	NR	3[b]	NR	3.6	0.5
Dermal Contact	1	NR	0.02-0.26	NR	13	NR	1-10	0.5-5 NR
Deodorant (underarm)	NR	NR	0.23	NR	NR	NR	NR	NR
Hair - Non-Coloring	9	NR	0.0023-0.12	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	0.26	NR	6	NR	35.9	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Capryloyl Salicylic Acid				**Ethylhexyl Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**100**	**5**	**0.1 -62.9**	**0.1-1**	**3474**	**83**	**0.0003-5.1**	**0.001-8**
Duration of Use								
Leave-On	***89***	***5***	***0.1-62.9***	***1***	***2762***	***80***	***0.0003-5.1***	***0.001-8***
Rinse-Off	***11***	***NR***	***0.1-0.29***	***0.1***	***701***	***3***	***0.001-0.21***	***0.001-0.005***
Diluted for (Bath) Use	***NR***	***NR***	***NR***	***NR***	***11***	***NR***	***0.2***	***NR***
Eye Area	9	NR	NR	NR	3	NR	0.1	NR
Incidental Ingestion	NR	NR	0.1	NR	54	2	4-4.5	8
Incidental Inhalation-Spray	26[b]	1[b]	0.1	0.1-1[b]	2307;98[b]	18;2[b]	0.00099-5;0.012-0.05[a]	0.001-0.01;0.001-5[b]
Incidental Inhalation-Powder	26[b]	1[b]	0.3	0.1-1[b]	3;98[b]	2[b]	NR	5; 0.001-5[b]
Dermal Contact	100	5	0.1-62.9	0.1-1	3280	45	0.0003-5.1	0.5-5
Deodorant (underarm)	NR	NR	0.3	NR	6	NR	0.0016	NR
Hair - Non-Coloring	NR	NR	0.1	NR	129	35	0.00099-0.2	0.001-0.01
Hair-Coloring	NR	NR	NR	NR	5	NR	0.012	NR
Nail	NR	NR	NR	NR	6	1	0.15	0.1
Mucous Membrane	NR	NR	0.3	NR	676	2	0.0012-4.5	8
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Hexyl Salicylate				**Isodecyl Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**2**	NR	**0.013-0.52**	NR	**19**	**3**	**2.5**	NR
Duration of Use								
Leave-On	***2***	***NR***	***0.013-0.12***	***NR***	***19***	***2***	***2.5***	***NR***
Rinse-Off	***NR***	***NR***	***0.032-0.52***	***NR***	***NR***	***1***	***NR***	***NR***
Diluted for (Bath) Use	***NR***	***NR***	NR	***NR***	***NR***	***NR***	***NR***	***NR***
Exposure Type								
Eye Area	NR	NR	0.00074	NR	1	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1[b]	NR	0.013-0.023; 0.11[a]	NR	7[b]	2[a]	NR	NR
Incidental Inhalation-Powder	1[b]	NR	NR	NR	7[b]	NR	NR	NR
Dermal Contact	2	NR	0.02-0.52	NR	19	3	2.5	NR
Deodorant (underarm)	NR	NR	0.097	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	0.013-0.21	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	0.5	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	0.52	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

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

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

[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

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

NR - no reported use

Table 3. Frequency and Concentration of Use of Salicylates According to Duration and Exposure.

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Magnesium Salicylate				**Methyl Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**10**	NR	**0.2**	NR	**36**	**25**	**0.00000006-1**	**0.0001-0.6**
Duration of Use								
Leave-On	***10***	***NR***	***0.2***	***NR***	***18***	***4***	***0.0000013-1***	***0.02***
Rinse-Off	***NR***	***NR***	***NR***	***NR***	***17***	***20***	***0.00000006-0.038***	***0.0001-0.6***
Diluted for (Bath) Use	***NR***	***NR***	***NR***	***NR***	***1***	***1***	***0.0016***	***NR***
Exposure Type								
Eye Area	10	NR	0.2	NR	NR	NR	0.0000013-0.000026	NR
Incidental Ingestion	NR	NR	NR	NR	12	14	0.038	0.03-0.2
Incidental Inhalation-Spray	NR	NR	NR	NR	8[b]	1[b]	0.0000051-0.5;0.000065-0.0004[b]	0.1;0.02-0.2[b]
Incidental Inhalation-Powder	NR	NR	NR	NR	8[b]	1[b]	0.000065-0.0004[b]	0.02-0.2[b]
Dermal Contact	2	NR	0.2	NR	23	6	0.00000006-1	0.0001-0.6
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	1	3	0.0000051-0.0011	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	0.00000002	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	17	17	0.000018-0.038	0.0001-0.2
Baby Products	NR	NR	NR	NR	1	NR	NR	NR
	Salicylic Acid				**Sodium Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**1300**	**107**	**0.00001-30**	**0.0008-3**	**165**	**7**	**0.0008-0.5**	**0.09-2**
Duration of Use								
Leave-On	***608***	***62***	***0.00001-30***	***0.02-3***	***70***	***5***	***0.0015-0.1***	***2***
Rinse-Off	***689***	***45***	***0.01-4***	***0.0008-3***	***95***	***2***	***0.0008-0.5***	***0.09-0.3***
Diluted for (Bath) Use	***3***	***NR***	***NR***	***NR***	***NR***	***NR***	***NR***	***NR***
Exposure Type								
Eye Area	26	2	0.00001-0.2	0.2-2	5	NR	NR	NR
Incidental Ingestion	1	NR	NR	1	NR	2	NR	0.09-0.2
Incidental Inhalation-Spray	5;248[b]	3;10[b]	0.1-0.2;0.004-0.5[a]	0.02-3[b]	41[b]	1[b]	NR	0.09-2[b]
Incidental Inhalation-Powder	7;248[b]	1;10[b]	NR	0.2-0.6; 0.02-3[b]	41[b]	1[b]	NR	0.09-2[b]
Dermal Contact	999	77	0.00001-30	0.0008-3	155	3	0.0015-0.5	2
Deodorant (underarm)	6	1	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	254	28	0.004-4	0.002-0.2	9	2	0.0008-0.5	0.2
Hair-Coloring	42	2	0.015-0.1	0.1	1	NR	NR	NR
Nail	3	NR	NR	0.2	NR	NR	NR	NR
Mucous Membrane	190	2	0.064-0.2	0.0008-2	82	2	0.25-0.37	0.09-0.2
Baby Products	2	NR	NR	NR	NR	NR	0.31	NR
	TEA Salicylate				**Tridecyl Salicylate**			
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**	**2018[11]**	**1998[1]**	**2018[12]**	**2000[1]**
Totals*	**5**	**5**	**NR**	**0.0001-0.75**	**14**	**2**	**NR**	**0.01**
Duration of Use								
Leave-On	***4***	***5***	***NR***	***0.0001-0.75***	***12***	***2***	***NR***	***0.01***
Rinse-Off	***1***	***NR***	***NR***	***0.0002***	***2***	***NR***	***NR***	***NR***
Diluted for (Bath) Use	***NR***	***NR***	***NR***	***NR***	***NR***	***NR***	***NR***	***NR***
Exposure Type								
Eye Area	NR	NR	NR	NR	2	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	1[b]	NR	0.001[b]	4[b]	2[b]	NR	0.01[b]
Incidental Inhalation-Powder	NR	1[b]	NR	0.001[b]	4[b]	2[b]	NR	0.01[b]
Dermal Contact	NR	5	NR	0.0001-0.75	14	2	NR	0.01
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	5	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	0.0002	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

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

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

[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

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

NR - no reported use

Table 3. Frequency and Concentration of Use of Salicylates According to Duration and Exposure.

	# of Uses		*Max Conc of Use (%)*					
	Isocetyl Salicylate							
	2018[11]	**1998[1]**	**2018[12]**	**2000[1]**				
Totals*	NR	NR	**NR**	**3-5**				
Duration of Use								
Leave-On	***NR***	***NR***	***NR***	***3-5***				
Rinse-Off	***NR***	***NR***	***NR***	***NR***				
Diluted for (Bath) Use	***NR***	***NR***	***NR***	***NR***				
Exposure Type								
Eye Area	NR	NR	NR	NR				
Incidental Ingestion	NR	NR	NR	NR				
Incidental Inhalation-Spray	NR	NR	NR	5[a]				
Incidental Inhalation-Powder	NR	NR	NR	NR				
Dermal Contact	NR	NR	NR	3-5				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	NR	NR	NR	NR				
Hair-Coloring	NR	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.

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

[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

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

NR - no reported use

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Safety Assessment of Salicylic Acid, Butyloctyl Salicylate, Calcium Salicylate, C12–15 Alkyl Salicylate, Capryloyl Salicylic Acid, Hexyldodecyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Magnesium Salicylate, MEA-Salicylate, Ethylhexyl Salicylate, Potassium Salicylate, Methyl Salicylate, Myristyl Salicylate, Sodium Salicylate, TEA-Salicylate, and Tridecyl Salicylate[1]

Salicylic Acid is an aromatic acid used in cosmetic formulations as a denaturant, hair-conditioning agent, and skin-conditioning agent—miscellaneous in a wide range of cosmetic products at concentrations ranging from 0.0008% to 3%. The Calcium, Magnesium, and MEA salts are preservatives, and Potassium Salicylate is a cosmetic biocide and preservative, not currently in use. Sodium Salicylate is used as a denaturant and preservative (0.09% to 2%). The TEA salt of Salicylic Acid is used as an ultraviolet (UV) light absorber (0.0001% to 0.75%). Several Salicylic Acid esters are used as skin conditioning agents—miscellaneous (Capryloyl, 0.1% to 1%; C12–15 Alkyl, no current use; Isocetyl, 3% to 5%; Isodecyl, no current use; and Tridecyl, no current use). Butyloctyl Salicylate (0.5% to 5%) and Hexyldodecyl Salicylate (no current use) are hair-conditioning agents and skin-conditioning agents—miscellaneous. Ethylhexyl Salicylate (formerly known as Octyl Salicylate) is used as a fragrance ingredient, sunscreen agent, and UV light absorber (0.001% to 8%), and Methyl Salicylate is used as a denaturant and flavoring agent (0.0001% to 0.6%). Myristyl Salicylate has no reported function. Isodecyl Salicylate is used in three formulations, but no concentration of use information was reported. Salicylates are absorbed percutaneously. Around 10% of applied salicylates can remain in the skin. Salicylic Acid is reported to enhance percutaneous penetration of some agents (e.g., vitamin A), but not others (e.g., hydrocortisone). Little acute toxicity (LD_{50} in rats; >2 g/kg) via a dermal exposure route is seen for Salicylic Acid, Methyl Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate. Short-term oral, inhalation, and parenteral exposures to salicylates sufficient to produce high blood concentrations are associated primarily with liver and kidney damage. Subchronic dermal exposures to undiluted Methyl Salicylate were associated with kidney damage. Chronic oral exposure to Methyl Salicylate produced bone lesions as a function of the level of exposure in 2-year rat studies; liver damage was seen in dogs exposed to 0.15 g/kg/day in one study; kidney and liver weight increases in another study at the same exposure; but no liver or kidney abnormalities in a study at 0.167 g/kg/day. Applications of Isodecyl, Tridecyl, and Butyloctyl Salicylate were not irritating to rabbit skin, whereas undiluted Ethylhexyl Salicylate produced minimal to mild irritation. Methyl Salicylate at a 1% concentration with a 70% ethanol vehicle were irritating, whereas a 6% concentration in polyethylene glycol produced little or no irritation. Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate were not ocular irritants. Although Salicylic Acid at a concentration of 20% in acetone was positive in the local lymph node assay, a concentration of 20% in acetone/olive oil was not. Methyl Salicylate was negative at concentrations up to 25% in this assay, independent of vehicle. Maximization tests of Methyl Salicylate, Ethylhexyl Salicylate, and Butyloctyl Salicylate produced no sensitization in guinea pigs. Neither Salicylic Acid nor Tridecyl Salicylate were photosensitizers. Salicylic Acid, produced when aspirin is rapidly hydrolyzed after absorption from the gut, was reported to be the causative agent in aspirin teratogenesis in animals. Dermal exposures to Methyl Salicylate, oral exposures to Salicylic Acid, Sodium Salicylate, and Methyl Salicylate, and parenteral exposures to Salicylic Acid, Sodium Salicylate, and Methyl Salicylate are all associated with reproductive and developmental toxicity as a function of blood levels reached as a result of exposure. An exposure assessment of a representative cosmetic product used on a daily basis estimated that the exposure from the cosmetic product would be only 20% of the level seen with ingestion of a "baby" aspirin (81 mg) on a daily basis. Studies of the genotoxic potential of Salicylic Acid, Sodium Salicylate, Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate were generally negative. Methyl Salicylate, in a mouse skin-painting study, did not induce neoplasms. Likewise, Methyl Salicylate was negative in a mouse pulmonary tumor system. In clinical tests, Salicylic Acid (2%) produced minimal cumulative irritation and slight or no irritation(1.5%); TEA-Salicylate (8%) produced no irritation; Methyl Salicylate (>12%) produced pain and erythema, a 1% aerosol produced erythema, but an 8%

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solution was not irritating; Ethylhexyl Salicylate (4%) and undiluted Tridecyl Salicylate produced no irritation. In atopic patients, Methyl Salicylate caused irritation as a function of concentration (no irritation at concentrations of 15% or less). In normal skin, Salicylic Acid, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate are not sensitizers. Salicylic Acid is not a photosensitizer, nor is it phototoxic. Salicylic Acid and Ethylhexyl Salicylate are low-level photoprotective agents. Salicylic Acid is well-documented to have keratolytic action on normal human skin. Because of the possible use of these ingredients as exfoliating agents, a concern exists that repeated use may effectively increase exposure of the dermis and epidermis to UV radiation. It was concluded that the prudent course of action would be to advise the cosmetics industry that there is a risk of increased UV radiation damage with the use of any exfoliant, including Salicylic Acid and the listed salicylates, and that steps need to be taken to formulate cosmetic products with these ingredients as exfoliating agents so as not to increase sun sensitivity, or when increased sun sensitivity would be expected, to include directions for the daily use of sun protection. The available data were not sufficient to establish a limit on concentration of these ingredients, or to identify the minimum pH of formulations containing these ingredients, such that no skin irritation would occur, but it was recognized that it is possible to formulate cosmetic products in a way such that significant irritation would not be likely, and it was concluded that the cosmetics industry should formulate products containing these ingredients so as to be nonirritating. Although simultaneous use of several products containing Salicylic Acid could produce exposures greater than would be seen with use of baby aspirin (an exposure generally considered to not present a reproductive or developmental toxicity risk), it was not considered likely that consumers would simultaneously use multiple cosmetic products containing Salicylic Acid. Based on the available information, the Cosmetic Ingredient Review Expert Panel reached the conclusion that these ingredients are safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

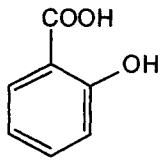
INTRODUCTION

This report reviews the safety of Salicylic Acid; its Calcium, Magnesium, MEA, Potassium, Sodium, and TEA salts; its acid ester, Capryloyl Salicylic Acid; and its Butyloctyl, C12–15 Alkyl, Ethylhexyl, Isocetyl, Hexyldodecyl, Isodecyl, Methyl, Myristyl, and Tridecyl alcohol esters. This family of ingredients was determined based on similarity of structure and/or function in cosmetics. Ethylhexyl Salicylate was formerly known as Octyl Salicylate. Amyl Salicylate, although structurally similar to the other salicylate esters (see next section), was not included because its only listed function (Pepe, Wenninger, and McEwen 2002) is as a fragrance ingredient, which excludes it from review according to Cosmetic Ingredient Review (CIR) procedures.

CHEMISTRY

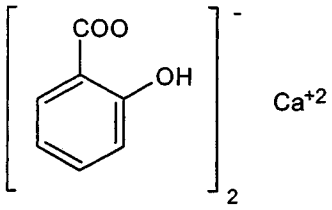
Definition and Structure

Salicylic Acid (CAS no. 69-72-7) is the aromatic acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

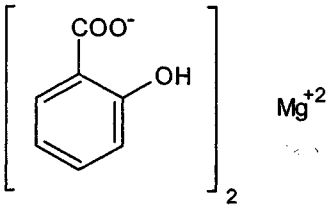


It is also known as 2-Hydroxybenzoic Acid (Pepe, Wenninger, and McEwen 2002; Lide 1993; Lewis 1993a; Budavari 1989); Benzoic Acid, 2-Hydroxy (Pepe, Wenninger, and McEwen 2002; Gennaro 1990); *o*-Hydroxybenzoic Acid (Pepe, Wenninger, and McEwen 2002; Lewis 1993a, 1993b; Gennaro 1990); *o*-Hydroxy Benzoic Acid (Sax 1979); and Orthohydroxybenzoic Acid (Lewis 1993a).

Calcium Salicylate (CAS no. 824-35-1) is the calcium salt of Salicylic Acid (q.v.) that is also known as Salicylic Acid, Calcium Salt; Calcium 2-Hydroxybenzoate; and Benzoic Acid, 2-Hydroxy-, Calcium Salt (Pepe, Wenninger, and McEwen 2002). It conforms to the formula (Pepe, Wenninger, and McEwen 2002):

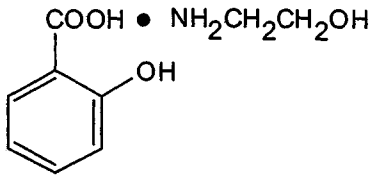


Magnesium Salicylate (CAS no. 18917-89-0) is the magnesium salt of Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



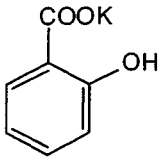
It is also known as Salicylic Acid, Magnesium Salt; Magnesium 2-Hydroxybenzoate (Pepe, Wenninger, and McEwen 2002); 2-Hydroxybenzoic Acid Magnesium Salt (Budavari 1989); Benzoic Acid, 2-Hydroxy-, Magnesium Salt (Pepe, Wenninger, and McEwen 2002); and Magnesium, Bis(2-Hydroxybenzoato-O^1,O^2)- (Gennaro 1990).

MEA-Salicylate (CAS no. 59866-70-5) is the monoethanolamine salt of Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

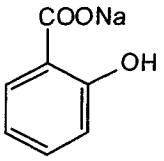


It is also known as Ethanolamine Salicylate; Salicylic Acid, Monoethanolamine Salt; Monoethanolamine 2-Hydroxybenzoate; and Benzoic Acid, 2-Hydroxy-, Monoethanolamine Salt (Pepe, Wenninger, and McEwen 2002).

Potassium Salicylate (CAS no. 578-36-9) is the potassium salt of Salicylic Acid (q.v.) that is also known as Salicylic Acid, Potassium Salt; Potassium 2-Hydroxybenzoate; and Benzoic Acid, 2-Hydroxy-, Potassium Salt (Pepe, Wenninger, and McEwen 2002). It conforms to the formula (Pepe, Wenninger, and McEwen 2002):

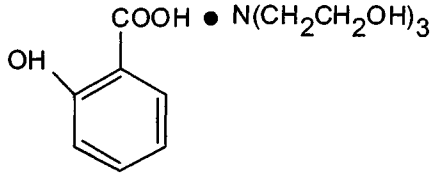


Sodium Salicylate (CAS no. 54-21-7) is the sodium salt of Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



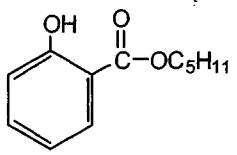
It is also known as Sodium Salicylic Acid; Salicylic Acid, Sodium Salt; Sodium *o*-Hydroxybenzoate; *o*-Hydroxybenzoic Sodium Salt (Lewis 1993a); 2-Hydroxybenzoic Acid, Monosodium Salt (Pepe, Wenninger, and McEwen 2002; Lewis 1993a; Budavari 1989); and Benzoic Acid, 2-Hydroxy-, Monosodium Salt (Pepe, Wenninger, and McEwen 2002).

TEA-Salicylate (CAS no. 2174-16-5) is the triethanolamine salt of Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

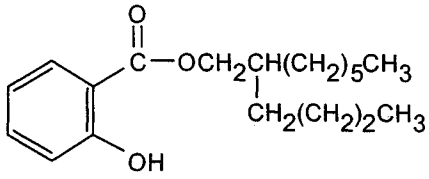


It is also known as Triethanolamine Salicylate; Trolamine Salicylate; 2-Hydroxybenzoic Acid, Compound with 2,2′,2″-Nitrilotris[Ethanol] (1:1); and Benzoic Acid, 2-Hydroxy-, Compound with 2,2′,2″-Nitrilotris[Ethanol] (1:1) (Pepe, Wenninger, and McEwen 2002).

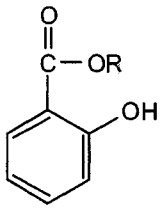
Amyl Salicylate is not addressed in this report because its only current use is as a fragrance ingredient; it is the ester of amyl alcohol and salicylic acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



Butyloctyl Salicylate (CAS number not available), also known as Salicylic Acid, 2-Butyloctyl Ester, is the compound that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



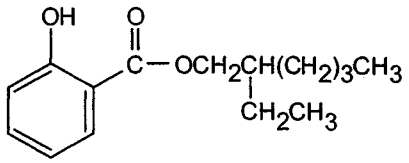
C12–15 Alkyl Salicylate (CAS number not available) is the ester of C12–15 alcohols (q.v.) and Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



where R represents the C12–15 alkyl group.

Capryloyl Salicylic Acid is the ester of Salicylic Acid (q.v.) and caprylic acid (q.v.) (Pepe, Wenninger, and McEwen 2002). (CAS number and structure not available.)

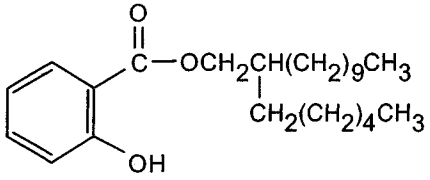
Ethylhexyl Salicylate (CAS no. 118-60-5) is the ester of 2-ethylhexyl alcohol and Salicylic Acid that is also known as Benzoic Acid, 2-Hydroxy-, 2-Ethylhexyl Ester; 2-Ethylhexyl 2-Hydroxybenzoate; Ethyl hexyl salicylate; 2-Ethylhexyl Salicylate; Octyl Salicylate; and Salicylic Acid, 2-Ethylhexyl Ester (Pepe, Wenninger, and McEwen 2002). It conforms to the formula (Pepe, Wenninger, and McEwen 2002):



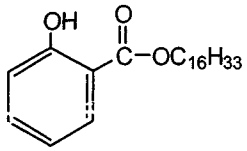
Because this cosmetic ingredient was previously known as Octyl Salicylate (Pepe, Wenninger, and McEwen 2002) and many of the references refer to Octyl Salicylate, this ingredient will be identified as Ethylhexyl (Octyl) Salicylate in the text. Headings will refer to Ethylhexyl Salicylate, the current accepted cosmetic ingredient name.

Hexyldodecyl Salicylate (CAS number not available), also known as Salicylic Acid, 2-Hexyldodecyl Ester, is the compound

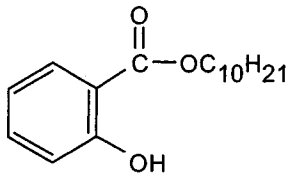
that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



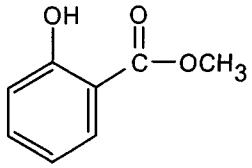
Isocetyl Salicylate (CAS number not available), also known as Salicylic Acid, Isocetyl Ester, is the ester of isocetyl alcohol (q.v.) and Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



Isodecyl Salicylate (CAS number not available) is also known as Salicylic Acid, Isodecyl Ester (Pepe, Wenninger, and McEwen 2002). It is the ester of branched chain decyl alcohols and Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

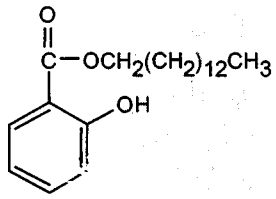


Methyl Salicylate (CAS no. 119-36-8) is the ester of methyl alcohol and Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

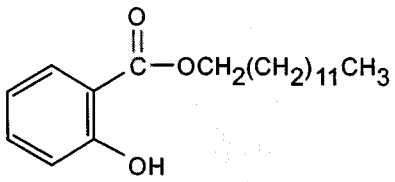


It is also known as Salicylic Acid, Methyl Ester (Lewis 1993a); Methyl 2-Hydroxybenzoate; Benzoic Acid, 2-Hydroxy-, Methyl Ester; (Pepe, Wenninger, and McEwen 2002); 2-Hydroxybenzoic Acid, Methyl Ester (Pepe, Wenninger, and McEwen 2002; Lewis 1993a; Budavari 1989); *o*-Hydroxybenzoic Acid, Methyl Ester; 2-Methoxybenzoic Acid; *o*-Methoxybenzoic Acid; Methyl-o-Hydroxybenzoate; Natural Wintergreen Oil; Synthetic Wintergreen Oil (Lewis 1993a); Oil of Wintergreen (Pepe, Wenninger, and McEwen 2002; Lewis 1993a); Birch Oil, Sweet (Pepe, Wenninger, and McEwen 2002); Sweet Birch Oil (Pepe, Wenninger, and McEwen 2002; Lewis 1993a, 1993b; Budavari 1989); Wintergreen Oil; Betula Oil; Teaberry Oil (Lewis 1993a; Budavari 1989); Gaultheria Oil (Lewis 1993b; Grant 1972); Gaultheria Oil, Artificial; and *o*-Anisic Acid (Lewis 1993a).

Myristyl Salicylate (CAS no. 19666-17-2) is the ester of myristyl alcohol and Salicylic Acid that is also known as Tetradecyl Salicylate; Salicylic Acid, Tetradecyl Ester; Tetradecyl 2-Hydroxybenzoate; 2-Hydroxybenzoic Acid, Tetradecyl Ester; and Benzoic Acid, 2-Hydroxy-, Tetradecyl Ester (Pepe, Wenninger, and McEwen 2002). Myristyl Salicylate conforms to the formula (Pepe, Wenninger, and McEwen 2002):



Tridecyl Salicylate (CAS no. 19666-16-1), also known 2-Hydroxybenzoic Acid, Tridecyl Ester and Benzoic Acid, 2-Hydroxy-, Tridecyl Ester, is the ester of tridecyl alcohol (q.v.) and Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



Physical and Chemical Properties

The physical and chemical properties of Salicylic Acid and Calcium, Magnesium, Potassium, Sodium, Methyl, and Ethylhexyl (Octyl) Salicylate are described in Table 1. Salicylic Acid and its salts are powders, but its esters appear to be liquids.

Manufacture and Production

Salicylic Acid is found in the bark of the willow tree, *Salix alba* (Lin and Nakatsui 1998). It can be prepared using the Kolbe-Schmidt process in which carbon dioxide is reacted with sodium phenolate under pressure at approximately 130°C to form Sodium Salicylate, which is then treated with mineral acid (Gennaro 1990).

Ethylhexyl Salicylate [Ethylhexyl (Octyl) Salicylate] is prepared from 2-ethylhexanol and Salicylic Acid by azeotropic esterification (Anonymous 1976).

Magnesium Salicylate is prepared by reacting magnesium oxide in a hot mixture of isopropanol and water, and the hydrated salt crystallizes on cooling (Gennaro 1990).

Methyl Salicylate is present in wintergreen leaves, *Gaultheria procumbens* L., *Ericacese*, and in the sweet birch bark, *Betula lenta* L., *Betulaceae,* (United States Pharmacopeial Convention

TABLE 1
Physical and chemical properties of Salicylic Acid and Salicylate salts and esters

Property	Description	Reference
	Salicylic Acid	
Physical characteristics	Needles with water and monoclinic prisms with alcohol	Lide 1993
	White powder with an acrid taste	Lewis 1993b
	White, fine, needle-like crystals or a fluffy, white, crystalline powder; the synthetic form is white and odorless with a sweetish, afterward acrid, taste	Gennaro 1990
	Acicular crystal or crystalline powder that is virtually odorless	Nikitakis and McEwen 1990a
Molecular formula	$C_7H_6O_3$	Gennaro 1990; Budavari 1989
Molecular weight	138.12	Lide 1993; Gennaro 1990
Boiling point	211°C (20 mm Hg); sublimes at 76°C	Lewis 1993b; Budavari 1989
Melting point	158–161°C	USP* 1995a; Nikitakis and McEwen 1990a
	157–159°C	Budavari 1989; Kabara 1984
Solubility	Soluble in acetone, oil of turpentine, alcohol, ether, benzene; slightly soluble in water	Lewis 1993b
	Soluble in ethanol, acetone, chloroform, ether, and boiling water; only slightly soluble in cold water	Nikitakis and McEwen 1990a
	Solubility in water is increased by the addition of sodium phosphate, borax, alkali acetates or citrates	Kabara 1984
Octanol/Water partition coefficient (log P)	1.96	Sheu et al. 1975
	2.25	Higo et al. 1995
Refractive index	1.565	Lide 1993
Density	1.443 (20°/4°C)	Lide 1993; Lewis 1993a
pH of saturated aqueous solution	2.4	Budavari 1989
Flash point	315°F	Sax 1979
Stability	Discolors in sunlight	Nikitakis and McEwen 1990a
	Emits acrid smoke and irritating fumes when heated to decomposition	Lewis 1993a
	Decomposes into phenol and CO_2 when rapidly heated at atmospheric pressure	Kabara 1984
Reactivity	Incompatible with iron salts, spirit nitrous ether, lead acetate, and iodine; colored reddish by ferric salts	Budavari 1989
Autoignition temperature	1013°F	Sax 1979
	Calcium Salicylate	
Physical characteristics	Colorless crystals	Grant 1972
Molecular formula	$Ca(OCC{\cdot}C_6H_4{\cdot}OH)_2{\cdot}2H_2O$	Grant 1972
Molecular weight	350.20	Grant 1972
Solubility	Soluble in carbonated water	Grant 1972
	Magnesium Salicylate	
Physical characteristics	White to slightly pink, free-flowing crystalline powder with no or a faint characteristic odor	Gennaro 1990
	Tetrahydrate, white, odorless, efflorescent, crystalline powder	Budavari 1989
Molecular formula	$C_{14}H_{10}MgO_6$	Budavari 1989
Molecular weight	298.54 (anhydrous)	USP 1995a
	298.53 (anhydrous)	Gennaro 1990

(Continued on next page)

TABLE 1
Physical and chemical properties of Salicylic Acid and Salicylate salts and esters *(Continued)*

Property	Description	Reference
	Potassium Salicylate	
Physical characteristics	White odorless powder	Budavari 1989
	White crystals	Grant 1972
Molecular formula	$C_7H_5KO_3$	Budavari 1989
Molecular weight	176.21	Budavari 1989
Solubility	Very soluble in water and alcohol	Budavari 1989
Reactivity	Becomes pink on exposure to light	Budavari 1989
	Sodium Salicylate	
Physical characteristics	Colorless or faintly pink amorphous or microcrystalline powder or scales that has no or a faint characteristic odor and has a sweet, saline taste	Gennaro 1990
	White odorless crystals, scales, or powder	Budavari 1989
Molecular formula	$C_7H_5NaO_3$	Budavari 1989
Molecular weight	160.11	USP 1995a
Solubility	Soluble in water	Grant 1972
pH of aqueous solution	5–6	Budavari 1989
Reactivity	Incompatible with alkalies or iron; darkens	Gennaro 1990
	Becomes pinkish on long exposure to light; incompatible with ferric salts, lime water, spirit nitrous ether, mineral acids, iodine, lead acetate, silver nitrate, sodium phosphate in powder	Budavari 1989
	Methyl Salicylate	
Physical characteristics	Colorless, yellowish, or reddish liquid with the odor and taste of wintergreen	National Academy of Sciences (NAS) 1996
	Volatile oil having the characteristic odor and taste of wintergreen	Nikitakis and McEwen 1990a
	Colorless, yellowish, or reddish oily liquid with the odor and taste of gaultheria	Budavari 1989
Molecular formula	$C_8H_8O_3$	Budavari 1989
Molecular weight	152.15	USP 1995b; Lide 1993
	152.14	Sax 1979
Boiling point	223.3°C	Lide 1993; Sax 1979
	220–224°C	Budavari 1989
Melting point	−8°C	Lide 1993
	−8.6°C	Budavari 1989
Solubility	Soluble in alcohol and glacial acetic acid; slightly soluble in water	NAS 1996
	Soluble in alcohol and ether	Lide 1993
	Soluble in chloroform and ether; slightly soluble in water; miscible with alcohol and glacial acetic acid	Budavari 1989
	Insoluble in water	Grant 1972
log P	1.45	Sheu et al. 1975
	2.46	Higo et al. 1995
Index of refraction	1.5350–1.5380 (20°C)	USP 1995b; Nikitakis and McEwen 1990a
Acid value	0.5% maximum	Nikitakis and McEwen 1990a
Specific gravity	Synthetic: 1.180–1.185 (25°/25°C); natural: 1.176–1.182 (25°/25°C)	USP, 1995b; Nikitakis and McEwen 1990a
	Natural: 1.180	Budavari 1989

(Continued on next page)

TABLE 1
Physical and chemical properties of Salicylic Acid and Salicylate salts and esters *(Continued)*

Property	Description	Reference
Angular rotation	Synthetic and from *Betula*: inactive; natural: −1.5° maximum	USP 1995b
Flash point	210°F (closed cup)	Budavari 1989
	214°F (closed cup)	Sax 1979
Reactivity	Slight fire hazard when exposed to heat or flame; can react with oxidizing materials	Sax 1979
Autoignition temperature	850°F	Sax 1979
	Ethylhexyl (Octyl) Salicylate	
Physical characteristics	Clear, pale, straw-colored liquid having a faint characteristic odor	Nikitakis and McEwen 1990b
Molecular weight	250	Treffel and Gabard 1996
log P	6.02	Treffel and Gabard 1996
Solubility	Insoluble in water	Haarmann and Reimer 1992
Saponification value	200 minimum	Nikitakis and McEwen 1990b
Specific gravity	1.103–1.022 (25°/25°C)	Nikitakis and McEwen 1990b
	Butyloctyl Salicylate	
Boiling point	Decomposes at 251–334°C	Huntingdon Life Sciences 1998a
Freezing point	<−25°C	Huntingdon Life Sciences 1998a
Solubility	$<2.84 \times 10^{-5}$ g/L at 20°C	Huntingdon Life Sciences 1998a
log P	>6.2 (20°C)	Huntingdon Life Sciences 1998a
Density	0.971 (D_4^{20})	Huntingdon Life Sciences 1998a
Vapor pressure	14 Pa at 25°C	Huntingdon Life Sciences 1998a
Flash point	166°C	Huntingdon Life Sciences 1998a
Reactivity	Not explosive	Huntingdon Life Sciences 1998a
Autoignition temperature	263°C	Huntingdon Life Sciences 1998a

[USP] 1995b). Methyl Salicylate can be produced synthetically or obtained by maceration and subsequent distillation with steam from the leaves of *Gaultheria procumbens* L. or from the bark of *Betula lenta* L. (USP 1995b). Methyl Salicylate is synthesized by esterification of Salicylic Acid with methyl alcohol (Speer 1979).

Sodium Salicylate is mixed with sufficient distilled water to form a paste, then sufficient pure sodium carbonate is added in small portions to neutralize all but a fraction of the Salicylic Acid (Gennaro 1990). The resulting solution is filtered, and the filtered solution is evaporated.

Analytical Methods

A salicylate test system, a device intended to measure salicylates in humans, has been used in the diagnosis and treatment of salicylate overdose and in monitoring salicylate concentrations to ensure appropriate therapy (21 CFR 862.3830).

Salicylic Acid has been determined in human urine using colorimetry (Farid et al. 1975); in human serum using a liquid-liquid chromatographic system with a limit of detection of 1 ng (corresponding to 40 ppb Salicylic Acid in serum) (Terweij-Groen, Vahlkamp, and Kraak 1978) and with spectrofluorometric methods (Birmingham, Green, and Rhodes 1979); simultaneously with aspirin in plasma using gas-liquid chromatography (Walter, Biggs, and Coutts 1974); in human plasma and urine using gradient reverse-phase high-performance liquid chromatography (HPLC), with limits of detection of 0.2 and 5 μg/ml in plasma and urine, respectively (Vree et al. 1994a); and in human serum and urine using micellular electrokinetic chromatography, capillary zone electrophoresis, and capillary isotachophoresis (Caslavska, Lienhard, and Thormann 1993). Salicylic Acid was measured in rat cerebrospinal fluid and striatal tissue using HPLC with ultraviolet (UV) absorbance and electrochemical detection (Sloot and Gramsbergen 1995). HPLC with spectrophotometry was used to identify and quantify Salicylic Acid in biological fluids without organic extraction, with a limit of detection of 3.89 μmol/L (Coudray et al. 1996), and HPLC with UV detection of Salicylic Acid in biological fluids was also used by Krivosíková, Spustová, and Dzúrik (1996).

HPLC was used to determine the presence of Salicylic Acid in aspirin, with a limit of detection of 5 ng (Salako, Fadiran, and Thomas 1989), and Salicylic Acid was quantified in aspirin powders and its dosage forms using reverse-phase HPLC, with Salicylic Acid quantities as low as 0.1 μg being assayed

(das Gupta 1980). Second-derivative spectroscopy and HPLC have also been used to determine Salicylic Acid in aspirin, with limits of detection of 1.27 and 1.93 μg/ml, respectively (Torrado, Torrado, and Cadórniga 1994), as has a spectrofluorometric method, with sensitivity of the order of 10^{-8} g (Villari et al. 1994).

Simultaneous analysis of Salicylic Acid and aspirin in aspirin products was determined using reverse-phase HPLC with UV and fluorescence detection (Kirchhoefer 1980), and in pharmaceutical tablet preparations with two multicomponent UV-spectrophotometric methods using principal component regression and classical least-square algorithm and by an assay based on second-derivative spectroscopy (Glombitza and Schmidt 1994). Salicylates in buffer solutions have been determined using a voltametric method (Moore et al. 1995). Salicylic Acid was determined in an aerosol foot powder with gas chromatography (Palermo and Lundberg 1979).

Methyl Salicylate has been determined using HPLC (Boehnlein et al. 1994).

TEA-Salicylate has been assayed by thin-layer chromatography and nuclear magnetic resonance (Rabinowitz and Baker 1984).

Composition/Impurities

Salicylic Acid

USP-grade Salicylic Acid is to contain not less than 99.5% and not more than 101.0% $C_7H_6O_3$, calculated on the dry basis (USP 1995a).

Magnesium Salicylate

USP-grade Magnesium Salicylate is to contain not less than 98.0% and not more than 103.0% $C_{14}H_{10}MgO_6 \cdot 4H_2O$, and it should contain less than 0.004% heavy metals (USP 1995a).

Methyl Salicylate

USP-grade Methyl Salicylate is to contain not less than 98.0% and not more than 100.5% $C_8H_8O_3$, and it should contain less than 0.004% heavy metals (USP 1995b). Methyl Salicylate is to contain no more than 3 ppm arsenic (as As) or 10 ppm lead (as Pb) (Nikitakis and McEwen 1990a).

Sodium Salicylate

USP-grade Sodium Salicylate is to contain not less than 99.5% and not more than 100.5% $C_7H_5NaO_3$, calculated on the anhydrous basis, and it should contain less than 0.003% heavy metals (USP 1995a).

Ultraviolet Radiation Absorbance

Salicylic Acid

In the UVB range, Salicylic Acid has a peak absorbance at approximately 305-310 nm (Glombitza and Schmidt 1994; Kornreich et al. 1996). Coudray et al. (1996) reported maximal absorption at 295 nm.

Ethylhexyl Salicylate

Ethylhexyl (Octyl) Salicylate has an absorption band at 280 to 320 nm, with moderate absorptivity (Gennaro 1990). An aqueous solution of Ethylhexyl Salicylate was illuminated with light from a solar simulator and evaluated for singlet molecular oxygen formation (Allen, Gossett, and Allen 1996). Furfuryl alcohol, a chemical trap for singlet oxygen, was added to the solution. No loss of furfuryl alcohol was observed, indicating that no singlet oxygen was formed, and Ethylhexyl Salicylate did not produce any other toxic oxidant species capable of consuming furfuryl alcohol.

USE

Cosmetic

The ingredients reviewed in this report function in cosmetic formulations as reported in Table 2 (Pepe, Wenninger, and McEwen 2002).

Information on use of these ingredients in cosmetic formulations is available both from the Food and Drug Administration (FDA) and the Cosmetic, Toiletry, and Fragrance Association (CTFA). Information reported to FDA by manufacturers in 1998 listed the following uses: Salicylic Acid in a total of 107 cosmetic formulations, Sodium Salicylate in 7 formulations, TEA-Salicylate in 5 formulations, Capryloyl Salicylic Acid in 5 formulations, Isodecyl Salicylate in 3 formulations, Methyl Salicylate in 25 formulations, Ethylhexyl (Octyl) Salicylate in 83 formulations, and Tridecyl Salicylate in 2 formulations (FDA 1998). The product categories in which these ingredients were reportedly used are shown in Table 3. CTFA additionally reported use of Isocetyl Salicylate and Butyloctyl Salicylate (CTFA 2000). Neither FDA nor CTFA reported uses of Butyloctyl, Calcium, C12–15 Alkyl, Hexyldodecyl, Isocetyl, Magnesium, MEA-, Myristyl, and Potassium Salicylate (FDA 1998; CTFA 2000).

Concentration of use data submitted by industry (CTFA 2000) stated that Salicylic Acid was used at concentrations of $\leq$3%, Butyloctyl Salicylate was used at concentrations of $\leq$5%, Capryloyl Salicylic Acid was used at concentrations of $\leq$1%, Isocetyl Salicylate was used at concentrations of $\leq$5%, Methyl Salicylate was used at concentrations of $\leq$0.6%, Ethylhexyl (Octyl) Salicylate was used at concentrations of $\leq$8%, Sodium Salicylate was used at concentrations of $\leq$2%, TEA-Salicylate was used at concentrations of $\leq$0.75%, and Tridecyl Salicylate was used at a concentration of 0.01%. The product categories in which these ingredients reportedly were used and the concentrations of use for each are shown in Table 3.

Salicylic Acid and its salts appear in Annex VI, Part 1, of the Cosmetics Directive of the European Union, which names the preservatives which cosmetic products may contain (European Economic Community 1998). Salicylic Acid, Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate are allowed for use in cosmetics as preservatives at a maximum concentration of 5%

TABLE 2
Cosmetic ingredient functions of Salicylic Acid and its salts and esters

Ingredient	Function
Salicylic Acid	Antiacne agent
	Antidandruff agent
	Corn/callus/wart remover
	Denaturant
	Hair-conditioning agent
	Skin-conditioning agent—miscellaneous
Butyloctyl Salicylate	Hair-conditioning agent
	Skin-conditioning agent—miscellaneous
	Solvent
Calcium Salicylate	Preservative
C12–15 Alkyl Salicylate	Skin-conditioning agent—miscellaneous
Caprylcyl Salicylic Acid	Skin-conditioning agent—miscellaneous
Hexyldodecyl Salicylate	Hair-conditioning agent
	Skin-conditioning agent—miscellaneous
	Solvent
Isocetyl Salicylate	Skin-conditioning agent—miscellaneous
Isodecyl Salicylate	Skin-conditioning agent—miscellaneous
Magnesium Salicylate	Preservative
MEA-Salicylate	Preservative
Methyl Salicylate	Denaturant
	External analgesic
	Flavoring agent
	Fragrance ingredient
Myristyl Salicylate	Not reported
Ethylhexyl Salicylate	Fragrance ingredient
	Sunscreen agent
	UV light absorber
Potassium Salicylate	Cosmetic biocide
	Preservative
Sodium Salicylate	Denaturant
	Preservative
TEA-Salicylate	Sunscreen agent
	Ultraviolet light absorber
Tridecyl Salicylate	Skin-conditioning agent—miscellaneous

(acid). These ingredients are not to be used in preparations for children under 3 years of age, except for shampoo formulations, and this warning must be printed on the label.

According to the Ministry of Health, Labor and Welfare (MHLW) of Japan, these ingredients are not on the negative list of ingredients prohibited from use in cosmetics; Salicylic Acid and its salts as preservatives are restricted to a maximum content of 0.2 g/100 g in all cosmetics. Salicylic Acid and its salts are also listed as ingredients of quasi-drugs; and Ethylhexyl (Octyl) Salicylate as a UV filter is restricted to a maximum content of 10 g/100 g in Japan (MHLW 2001)

Noncosmetic

Salicylic Acid, Magnesium Salicylate, Sodium Salicylate, and Methyl Salicylate have use as indirect food additives (21 Code of Federal Regulations [CFR] 175.105; 177.1010; 178.2010). Salicylic Acid has been used in the treatment of ichthyosiform dermatoses (Van Scott and Yu 1974). Salicylic Acid is an approved active ingredient for use in topical over-the-counter (OTC) acne drug products at concentrations of 0.5% to 2% (21 CFR 333.310), in OTC wart remover drug products at concentrations of 12% to 40% in a plaster vehicle, 5% to 17% in a collodion-like vehicle, and 15% in a karaya gum, glycol plaster vehicle (21 CFR 358.110); in corn and callus remover OTC drug products at concentrations of 12% to 40% in a plaster vehicle and 12% to 17.6% in a collodion-like vehicle (21 CFR 358.510); and in OTC drugs for the control of dandruff, seborrheic dermatitis, and psoriasis at a concentration of 1.8% to 3% (21 CFR 358.710). Labeling requirements, including directions and warnings, for wart remover drug products are found in 21 CFR 358.150 and for corn and callus remover drug products in 21 CFR 358.550.

Salicylic Acid has been present in OTC topical acne preparations (at concentrations of 2% to 5%), external analgesics and skin protectants used for poison ivy, oak, and sumac, and topical antifungal drug products. Calcium Salicylate has been present in OTC internal analgesic drug products. Sodium Salicylate has been present in OTC dandruff/seborrheic dermatitis/psoriasis and digestive aid drug products. TEA-Salicylate has been present in OTC external analgesic—fever blister and cold sore; insect bite and sting; and poison ivy, oak, and sumac drug products (21 CFR 310.545). Methyl Salicylate has been present in OTC smoking-deterrent drugs (21 CFR 310.544), boil treatment (21 CFR 310.531) dandruff/seborrheic dermatitis/psoriasis, fever blister and cold sore treatment, oral health care, and skin protectant–astringent drug products (21 CFR 545). However, currently "there is a lack of adequate data to establish general recognition of the safety and effectiveness" of these ingredients for the specified OTC uses. Any drug product intended to be taken orally that contains any salicylate ingredient, except effervescent preparations, must bear a statement warning to keep the product out of the reach of children (21 CFR 201.314).

Because of the toxicity of Methyl Salicylate, the Department of Health and Human Services regards any drug containing >5% Methyl Salicylate as misbranded under the Federal Food, Drug, and Cosmetic Act if that product does not have labeling that warns that misdirected use may be dangerous and that the product should be kept out of the reach of children (21 CFR 201.314). A traditional use of Methyl Salicylate is as a counterirritant (Green and Flammer 1989).

Salicylic Acid is allowed for use in the removal of scar tissue from the teat canal of milk-producing cows (21 CFR 529.2090);

TABLE 3
Ingredient usage as a function of product type

Product type (Total number reported to FDA 1998)	Number of formulations with the ingredient (FDA 1998)	Concentration of use (CTFA 2000) (%)
Salicylic Acid		
Eye lotion (18)	—	2
Other eye makeup preparations (120)	2	0.2
Hair conditioners (636)	4	0.1–0.2
Hair straighteners (63)	—	0.002
Permanent waves (192)	1	—
Shampoos (noncoloring) (860)	11	0.2
Tonics, dressings, and other hair-grooming aids (549)	10	0.2
Other hair preparations (276)	2	0.2
Hair dyes and colors (all types requiring caution statement and patch test) (1572)	—	0.1
Hair tints (54)	—	0.1
Other hair coloring preparations (59)	2	—
Blushers (all types) (238)	1	0.5
Face powders (250)	1	0.2–0.6
Foundations (287)	2	0.5–3.0
Lipstick (790)	—	1
Makeup bases (132)	—	0.6
Makeup fixatives (11)	—	1
Other makeup preparations (135)	2	0.6
Nail creams and lotions (17)	—	0.2
Bath soaps and detergents (385)	1	0.0008–2.0
Deodorants (underarm) (250)	1	—
Other personal cleanliness products (291)	1	0.1
Skin cleansing (653)	18	0.04–3.0
Depilatories (28)	1	—
Face and neck preparations (excluding shaving) (263)	1	0.1–3.0
Body and hand preparations (excluding shaving) (796)	9	0.02–2.0
Foot powders and sprays (38)	3	—
Moisturizing creams, lotions, powders, and sprays (769)	10	0.2–0.5
Night preparations (188)	1	—
Paste masks (mud packs) (255)	6	0.2
Skin fresheners (184)	7	0.5–3.0
Other skin care preparations (692)	8	0.1–3.0
Indoor tanning preparations (62)	—	0.1
Other suntanning preparations (38)	2	—
Total Salicylic Acid uses and concentration ranges	**107**	**0.0008–3**
Capryloyl Salicylic Acid		
Skin cleansing (653)	—	0.1
Face and neck preparations (excluding shaving) (263)	1	1.0
Body and hand preparations (excluding shaving) (796)	—	0.5
Moisturizing creams, lotions, powders, and sprays (789)	2	—
Indoor tanning preparations (62)	2	0.1
Total Capryloyl Salicylic Acid uses and concentration ranges	**5**	**0.1–1**

(Continued on next page)

TABLE 3
Ingredient usage as a function of product type *(Continued)*

Product type (Total number reported to FDA 1998)	Number of formulations with the ingredient (FDA 1998)	Concentration of use (CTFA 2000) (%)
Butyloctyl Salicylate		
Face powders (250)	—	0.5
Foundations (287)	—	4.0
Moisturizing creams, lotions, powders, and sprays (769)	—	4.0
Suntan gels, creams, and liquids (136)	—	5.0
Total Butyloctyl Salicylate uses and concentration ranges	**0**	**0.5–5.0**
Isocetyl Salicylate		
Face and neck preparations (excluding shaving) (263)	—	3.0
Suntan gels, creams, and liquids (136)	—	5.0
Total Isocetyl Salicylate uses and concentration ranges	**0**	**3.0–5.0**
Isodecyl Salicylate		
Moisturizing creams, lotions, powders, and sprays (769)	2	—
Paste masks (mud packs) (255)	1	—
Total Isodecyl Salicylate uses and concentration ranges	**3**	—
Methyl Salicylate		
Dentifrices (38)	4	0.03
Mouthwashes and breath fresheners (49)	10	0.08–0.2
Other oral hygiene products (6)	—	0.2
Bath soaps and detergents (385)	—	0.0001
Bath oils, tablets, and salts (124)	1	—
Body and hand preparations (excluding shaving) (796)	1	0.05
Skin cleansing (653)	1	—
Douches (5)	2	—
Foot powders and sprays (35)	—	0.02
Hair conditioners (636)	1	—
Shampoos (noncoloring) (860)	1	—
Tonics, dressings, and other hair-grooming aids (549)	1	—
Paste masks (mud packs) (255)	1	0.6
Skin fresheners (184)	1	0.1
Other skin care preparations (692)	1	0.02
Suntan gels, creams, and lotions (136)	—	0.2
Total Methyl Salicylate uses and concentration ranges	**25**	**0.0001–0.6**
Ethylhexyl Salicylate		
Hair conditioners (636)	2	0.001–0.005
Hair sprays (aerosol fixatives) (261)	16	0.001–0.01
Other fragrance preparations (148)	2	—
Shampoos (noncoloring) (860)	1	0.001
Tonics, dressings and other hair-grooming aids (549)	12	0.001–0.01
Other hair preparations (276)	4	—
Foundations (287)	1	5.0
Lipstick (790)	2	8.0
Makeup bases (132)	2	—
Other makeup preparations (135)	1	5.0
Basecoats and undercoats (manicuring preparations) (48)	1	0.1
Men's talcum (8)	—	5.0
Face and neck preparations (excluding shaving) (263)	—	5.0
Body and hand preparations (excluding shaving) (796)	2	0.5–5.0

(Continued on next page)

TABLE 3
Ingredient usage as a function of product type *(Continued)*

Product type (Total number reported to FDA 1998)	Number of formulations with the ingredient (FDA 1998)	Concentration of use (CTFA 2000) (%)
Moisturizing creams, lotions, powders, and sprays (769)	10	2.0–5.0
Other skin preparations (692)	1	—
Suntan gels, creams, and lotions (136)	21	4.0–5.0
Indoor tanning preparations (62)	5	4.0–5.0
Total Ethylhexyl Salicylate uses and concentration ranges	**83**	**0.001–5.0**
Sodium Salicylate		
Tonics, dressings, and other hair-grooming aids (549)	1	0.2
Other hair preparations (276)	1	—
Dentifrices (38)	2	—
Mouthwashes and breath fresheners (liquids and sprays) (49)	—	0.09–0.2
Other oral hygiene products (6)	—	0.2
Skin cleansing (653)	—	0.3
Face and neck preparations (excluding shaving) (263)	—	2.0
Body and hand preparations (excluding shaving) (796)	1	—
Moisturizing creams, lotions, powders, and sprays (769)	2	—
Other skin care preparations (692)	—	2.0
Total Sodium Salicylate uses and concentration ranges	**7**	**0.09–2.0**
TEA-Salicylate		
Foundations (287)	3	0.0001
Makeup bases (132)	—	0.75
Other personal cleanliness products (291)	—	0.0002
Skin cleansing (653)	—	0.0001
Other skin care preparations (692)	1	—
Face and neck preparations (excluding shaving) (263)	—	0.0002
Body and hand preparations (excluding shaving) (796)	—	0.001
Suntan gels, creams and liquids (136)	1	—
Total TEA-Salicylate uses and concentration ranges	**5**	**0.0001–0.75**
Tridecyl Salicylate		
Face and neck preparations (excluding shaving) (263)	1	—
Body and hand preparations (excluding shaving) (796)	1	0.01
Total Tridecyl Salicylate uses and concentration ranges	**2**	**0.01**

however, a residue tolerance of 0 has been established for milk from dairy animals (21 CFR 556.590).

Salicylic Acid is used in the manufacture of aspirin (Lewis 1993b). The amount of free Salicylic Acid allowed in aspirin is 0.1%; in uncoated aspirin tablets is 0.3%; in aspirin capsules is 0.75%; in aspirin delayed-release tablets is 2.0%; in coated aspirin tablets, buffered aspirin tablets, aspirin extended-release tablets, aspirin delayed-release capsules, and aspirin suppositories is 3.0%; and in aspirin effervescent tablets for oral solution is 8.0% (USP 1995a).

Salicylic Acid is also used in the manufacture of salicylates and resins and as a dyestuff intermediate, prevulcanization inhibitor, analytical reagent, and fungicide (Lewis 1993b). Sodium Salicylate is used as a preservative for paste, mucilage, glues, and hides, and Methyl Salicylate is used in perfumery (Budavari 1989).

According to Markland (1976), TEA- and Ethylhexyl (Octyl) Salicylate, although not potent sunscreens, are effective sunscreens and have extraordinary stability. TEA-Salicylate is approved for use as an active ingredient in sunscreens at concentrations of <12%, whereas Ethylhexyl (Octyl) Salicylate is allowed at concentrations of <5% (FDA 1999).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Absorption of salicylates from the stomach is normally quite rapid (Andrews 1973). Salicylate is metabolized by the hepatic

microsomal enzyme system, which conjugates Salicylic Acid to glycine, forming salicyluric acid (SU), and to glucuronic acid, forming salicylic acid phenolic glucuronide (SAPG) and/or salicylic acid acyl glucuronide (SAAG) (Goldsmith 1979). Vree et al. (1994b) describe the conjugation reaction to salicylic acid acyl glucuronide as reversable if the urine is alkaline. Salicylic Acid may also be oxidized to gentisic acid (GA), which may, in turn, be conjugated with glucuronic acid to form gentisic acid phenolic glucuronide (GAPG) and/or gentisic acid acyl glucuronide (GAAG).

Figure 1 depicts these possible metabolites of Salicylic Acid, along with the several double conjugates that are possible (Vree et al. 1994b). Goldsmith (1979) states that urinary metabolites of salicylic acid obtained after percutaneous absorption of salicylate differ from those obtained after oral administration in that there is reported more salicylate glucuronides and less salicyluric acid (SUA) and Salicylic Acid.

To assist the reader with the large amount of information available on absorption, distribution, metabolism, and excretion, a series of tables have been constructed and will appear at the end of this section. Table 8a presents the information for the dermal route of administration, Table 8b for the oral route, Table 8c for administration via the oral mucosa, and Table 8d for parenteral administration.

Dermal Route of Administration

Salicylic Acid—In Vitro Animal Data

Loveday (1961) examined the in vitro percutaneous absorption of Salicylic Acid using whole skin from the external ears of Landrace pigs. At pH 2.2, the rate of penetration was proportional to the concentration of Salicylic Acid; the rate ranged from approximately 0.1 to 1.4 mg/cm^2/24 h with concentrations of 0.25 to 2.0 mg/ml. With a 1 mg/ml solution of Salicylic Acid, variation of the pH of the buffer solution did not affect penetration at a pH >4.4; however, at pH <4.4, a "rapid rise in rate occurred." The approximate penetration rates were 1.5, 0.75, 0.5, and 0.4 mg/cm^2/24 h at pH 2.6, 3.5, 4.2, and 4.4, respectively, and 0.375 mg/cm^2/24 h at pH 5.5 and 7.75. Addition of surfactant to the solution decreased the rate of penetration of Salicylic Acid. Treatment of excised skin with chloroform or petroleum ether for 30 min increased the rate of penetration from 0.67 mg/cm^2/24 h to 0.91 and 0.79 mg/cm^2/24 h, respectively.

The permeability coefficients for the steady-state diffusion of Salicylic Acid through hairless mouse skin was determined using six different vehicles (Sloan et al. 1986). The permeability coefficients (cm/h) (and flux [mg/cm^2/h]) for Salicylic Acid with the various vehicles were 21.2 (0.64) with oleic acid, 21.0 (0.87) with isopropyl myristate, 11.2 (1.6) with 1-octanol, 4.8 (1.09) with 1-propanol, 2.1 (0.43) with propylene glycol, and 7.9 (1.15) with formamide.

The in vitro percutaneous absorption and metabolism of Salicylic Acid was determined using back skin from female fuzzy rats (Bronaugh, Stewart, and Storm 1989; Bronaugh et al. 1989–1990). Approximately 5 μg/cm^2 skin of ^{14}C-Salicylic Acid, 53.8 mCi/mmol, was applied to a 0.64-cm^2 area of dermatomed skin (200 μm) in an acetone vehicle. Receptor fluid was collected at 6-h intervals for 24 h at a flow rate of 1.5 ml/h. The skin surface was then washed to remove unabsorbed test material. The metabolism of Salicylic Acid was also determined. Controls were included. To examine microsomal transformation, 100 μM Salicylic Acid, 16.6 μCi/μmol, was added to an incubation medium containing microsomal protein for 60 min. Most of the absorbed radioactivity was found in the receptor fluid; 12.2% and 7.7% of the penetrating dose was found in the fluid and the skin, respectively. None of the absorbed Salicylic Acid was metabolized in the diffusion cell studies. It was also not metabolized when incubated with hepatic and skin microsomal preparations.

Singh and Roberts (1993) determined the penetration of Salicylic Acid through the dermis of Wistar rat skin in vitro. Salicylic Acid in isotonic saline buffer, pH 7.4, was applied using diffusion cells mounted on the skin. Using three samples, the permeability coefficient was 0.013 cm/h.

The percutaneous absorption of Salicylic Acid through intact hairless mouse skin was determined in vitro using a glass flow-through diffusion cell system (Higo et al. 1995). A 0.95-cm^2 area of skin was exposed to 1% w/v Salicylic Acid, pH 4.0. A zero-order penetration pattern was observed. Approximately 14 μ mol Salicylic Acid penetrated after 10 h.

Salicylic Acid—In Vivo Animal Data

The percutaneous absorption of Salicylic Acid from four different vehicles was determined using groups of 10 New Zealand white rabbits (Stolar, Rossi, and Barr 1960). Salicylic Acid, 6%, was added to the oleaginous base petrolatum USP XV, the hydrophilic base petrolatum USP XV with water, the oil-in-water base (o/w) hydrophilic ointment USP XV, and the water-soluble base polyethylene glycol (PEG) ointment USP XV. The hair on the back of each animal was shaved, and 7.5 g of each ointment was applied to a 6.35 × 12.7-cm^2 area under an occlusive patch for 9 h. Blood samples were taken hourly. The greatest absorption was observed from the hydrophilic ointment; peak absorption was approximately 11.0 mg% at 5 h. The peak absorption concentrations with hydrophilic petrolatum with water and petrolatum were approximately 8.8 and 6.8 mg% at 6 and 4 h, respectively. Negligible absorption occurred with the PEG ointment.

Stelzer, Colaizzi, and Wurdack (1968) used New Zealand white rabbits to determine the absorption of Salicylic Acid from four vehicles, with and without dimethyl sulfoxide (DMSO). Fifteen percent DMSO was added to hydrophilic ointment USP XVII, hydrophilic petrolatum USP XVII, PEG ointment USP XVII, and a steareth-20 gel system, each of which contained 10% (w/w) Salicylic Acid. The ointments, with and without DMSO, were applied for 8 h under an occlusive patch to the shaved dorsal skin of four animals. Salicylate concentration was determined in blood samples that were drawn prior to dosing and at intervals for 8 h after application. Blood salicylate

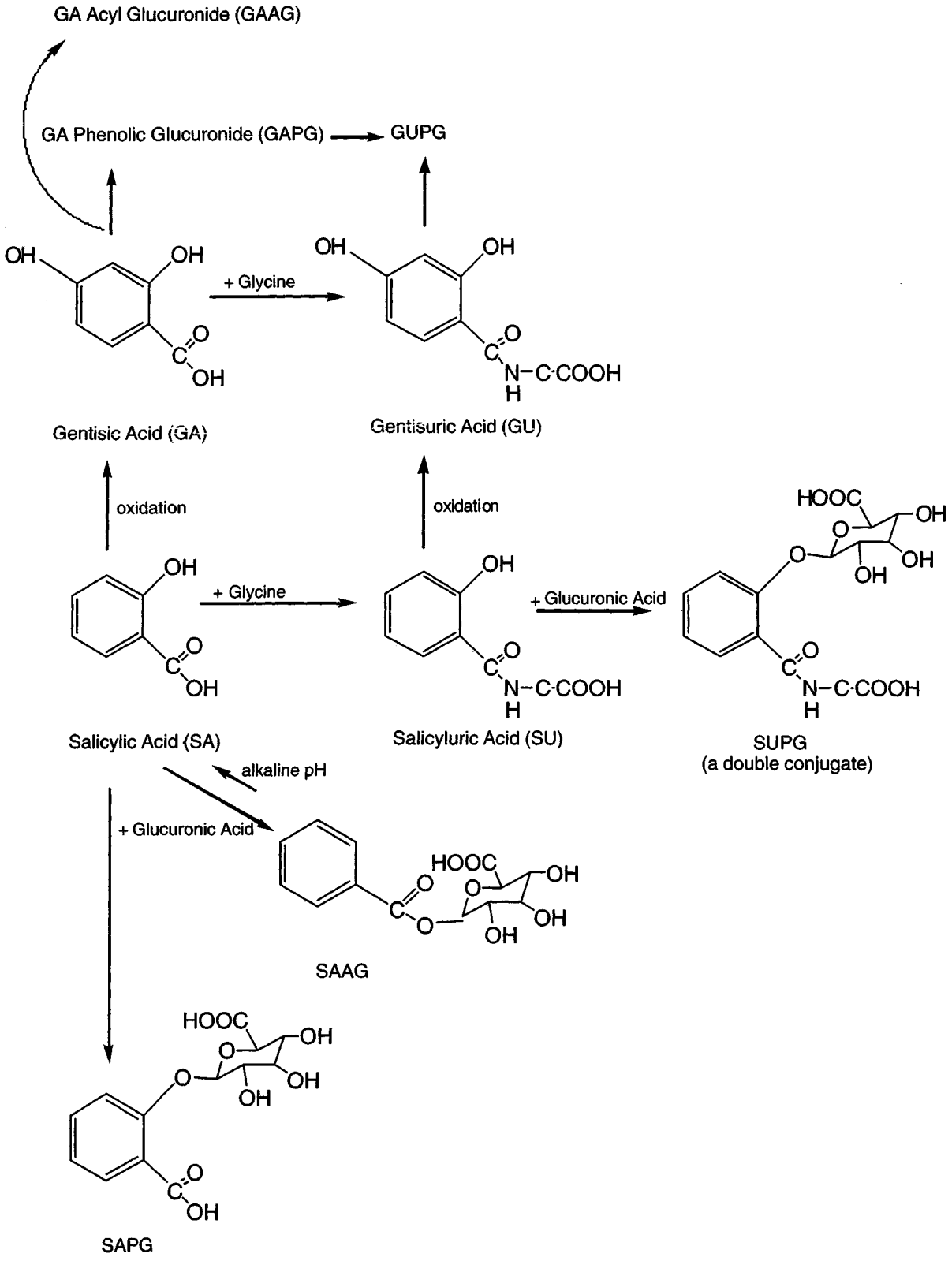


FIGURE 1

Metabolites of Salicylic Acid. SAPG, Salicylic Acid Phenolic Glucuronide; SAAG, Salicylic Acid Acyl Glucuronide; SUUG, Salicyluric Acid Phenolic Glucuronide (Vree et al. 1994b).

concentrations peaked at 5 h (5.81 mg%) without DMSO and at 2 h (10.44 mg%) with DMSO and hydrophilic ointment, at 8 h (3.82 mg%) without DMSO and at 3 h (9.68 mg%) with DMSO and hydrophilic petrolatum, at 8 h (0.94 mg%) without DMSO and at 6 h (1.14 mg%) with DMSO and PEG ointment, and at 3 h (2.50 mg%) without DMSO and at 4 h (2.66 mg%) with DMSO and steareth-20 gel.

The absorption of reagent-grade Salicylic Acid through abdominal guinea pig skin was examined by Arita et al. (1970). The abdominal area was shaved and a recirculation apparatus was applied to determine the rate of absorption. Salicylic Acid, pH 3.0, had a constant rate of absorption (approximately 4%) at concentrations of 250, 400, and 1000 μg/ml. Salicylic Acid at a concentration of 500 μg/ml was used to examine the absorption as a function of pH. The percent absorbed from 1 to 6 h was 6.1, 3.3, 0.6, and 0 at pH 2, 3, 4, and 5, respectively, and 0, 1.8, 8.0, and 15.5 at pH 7, 8, 9, and 10, respectively.

Marcus, Colaizzi, and Barry (1970) determined the effect of pH and DMSO on the percutaneous absorption of Salicylic Acid from hydrophilic ointment USP XVII using groups of four male New Zealand white rabbits. Salicylic Acid, 10% w/w, was added to ointments that had a pH of 2.97, 4.48, 6.80, 9.23, or 10.78; a set of Salicylic Acid–containing ointments containing 15% DMSO was prepared also at each pH. Application of the non–DMSO-containing and DMSO-containing ointments, which was made to a 6.35 × 12.70-cm^2 area, was varied so that two animals per pH group received the DMSO-containing and two received the non–DMSO-containing ointment. Occlusive patches were applied to the shaved dorsal area of each animal for 7.5 h. Blood samples were taken prior to dosing and at 1.5-h intervals; the last sample was taken at 7.5 h.

Without DMSO, the blood concentrations of Salicylic Acid increased at each time interval in the pH 2.97, 4.48, and 6.80 groups; the 1.5- and 7.5-h values for these groups were 5.75 and 14.07 mg%, 2.47 and 9.98 mg%, and 2.58 and 9.34 mg%, respectively. In the pH 9.23 group, the concentration peaked at 6.0 h, and the 1.5- and 6.0-h values were 5.49 and 11.96 mg%, respectively. In the pH 10.78 group, the blood salicylate concentration peaked at 4.5 h; the 1.5-h and 4.5-h values were 7.03 and 16.00 mg%, respectively. The blood salicylate concentrations were greater for all animals when DMSO was added to the ointment. With DMSO, the concentration peaked at 6.0 h in the pH 2.97 and 4.48 groups; the 1.5-h and 6.0-h values for these groups were 13.68 and 21.12 mg% and 8.31 and 12.73 mg%, respectively. The blood salicylate concentrations peaked at 4.5 h in the pH 6.80 and 9.23 groups; the 1.5- and 4.5-h values for these groups were 10.39 and 15.24 mg% and 8.67 and 16.70 mg%, respectively. In the pH 10.78 group, the salicylate concentration peaked at 3.0 h, and the 1.5- and 3.0-h values were 11.32 and 17.96 mg%, respectively (Marcus, Colaizzi, and Barry 1970).

Male Sprague-Dawley rats were used to determine the effect of pH on dermal absorption (Siddiqi and Ritschel 1972). The tails of the animals were immersed in a Salicylic Acid solution containing 5% ethanol that had a pH of 2, 3, 6, or 8. At pH 2, the total amount absorbed was 0.64 μg/mm^2/h and the k_a was 2.7725/h; the degree of ionization (α) was 9.678%. At pH 3, the total amount absorbed was 0.33 μg/mm^2/h and the k_a was 2.6157/h; the α was 51.726%. At pH 6 and 8, no Salicylic Acid was absorbed; the α values were 99.906% and 99.999%, respectively.

The absorption of 3% Salicylic Acid in water, 50% ethanol, and 75% ethanol was determined using guinea pigs (number per group not specified) (Yankell 1972). Fifty microliter of ^{14}C-Salicylic Acid (0.025 μCi/ml) in each vehicle was applied to a 1.5 × 1.5-cm^2 area of clipped skin on the back of each animal for 1 h. The animals were killed, and the skin was removed and tape-stripped 30 times. Absorption was greatest from the 75% ethanol vehicle, followed by 50% ethanol and then water; a total of 106.1%, 80.0%, and 56.9% of the applied dose, respectively, was recovered. Most of the radioactivity was found in tape strips 1 to 5; 89.3%, 76.3%, and 44.9% of the applied dose in 75% ethanol, 50% ethanol, and water, respectively, were recovered in these strippings.

This author then determined the distribution of Salicylic Acid in 75% ethanol. Three guinea pigs were each dosed with 0.2 ml 3% ^{14}C-Salicylic Acid (8.8 μCi) in 75% ethanol on a 3 × 2-cm site on the lower back. After 24 h, the animals were killed. Most of the radioactivity was recovered in the feces (401.3% to 808.5% applied dose/g × 10^3) followed by the treated back muscle (161.1% to 686.3% applied dose/g × 10^3). The amount recovered in the kidneys and the liver was 20.5% to 36.5% and 18.1% to 30.3% applied dose/g × 10^3, respectively (Yankell 1972).

The percutaneous absorption of Salicylic Acid through damaged guinea pig skin was studied using a recirculation apparatus (Washitake et al. 1973). After the abdominal skin of male guinea pigs was clipped and the stratum corneum removed, a glass vessel was attached and used for continuous recirculation and the amount of Salicylic Acid, 500 μg/ml and pH 3.0, absorbed was calculated from the concentration remaining in the solution. Also, concentrations of 250, 500, and 1000 μg/ml Salicylic Acid at pH 3.0 and 500 μg/ml at a pH of 2, 3, 4, 5, or 6 were used to determine the effect of concentration and pH, respectively, on absorption.

The absorption rate of 500 μg/ml Salicylic Acid from the recirculating solution was 79.4% for damaged skin; the disappearance of Salicylic Acid from the solution was linear from the start of exposure. (This was 10 times the rate through intact skin; disappearance from intact skin was linear 1 h after the start of exposure.) The rate of absorption from the recirculating solution was independent of concentration, but it did increase with an increasing fraction of un-ionized form.

The amount of drug retained in damaged guinea pig skin after various exposure times was then determined. The animals were exposed to 500 μg/ml Salicylic Acid, pH 3.0, for 0.5, 1.0, 3.0, 4.5, or 6.0 h, and then killed. The test area was wiped and the skin isolated to the corium. A peak in the amount of Salicylic Acid reserved in the skin was observed after 0.5 to 1 h. The researchers

attributed these results to an increase in percutaneous absorption and rapid decrease in concentration in the test solution due to removal of the stratum corneum and a rapid decrease in skin concentrations because of the decrease of Salicylic Acid in the solution. Varying the concentration of Salicylic Acid from 250 to 1000 μg/ml resulted in similar patterns of retention. Varying the pH from 3 to 6, the peak of the amount reserved became "lower and broader" with a decreasing fraction of unionized Salicylic Acid, and the time required to reach a peak had "a later trend."

The time course of the disappearance of Salicylic Acid from damaged guinea pig skin was also investigated. Animals were killed 0.5, 1, 2, 4, and 24 h after 1 h of recirculation of 500 μg/ml Salicylic Acid, pH 3.0. Again, the test area was washed and the skin isolated to the corium. The amount of Salicylic Acid reserved in damaged skin rapidly decreased in time; after 4 h, only trace amounts were found (Washitake et al. 1973).

Groups of eight rabbits were used to determine the dermal absorption of Salicylic Acid (Panse, Zeitler, and Sensch 1974). Patches containing 5 g of a Salicylic Acid salve (36.2 mmol/100 g) were applied for 6 h; urinary excretion of Salicylic Acid was measured. Approximately 5.50% and 11.08% of the dose was excreted in the urine after 24 and 48 h, respectively.

Washitake et al. (1975) examined the percutaneous absorption of Salicylic Acid from four vehicles using a recirculation apparatus attached to the shaved abdomen of guinea pigs. Doses of 500 μg/ml Salicylic Acid in hexadecyl alcohol, oleic acid, or isopropyl myristate and of 75, 150, and 300 μg/ml in liquid paraffin were used, as was intact skin and skin damaged by tape-stripping. The animals were killed at various intervals up to 6 h after recirculation, and the abdominal skin was removed and analyzed.

Using intact skin, 14.6%, 1.7%, 1.6%, and 1.5% of the Salicylic Acid was absorbed from liquid paraffin, isopropyl myristate, hexadecyl alcohol, and oleic acid, respectively, during 1 to 6 h. With damaged skin, the k_a during 1 to 6 h was approximately 10 times greater. The amount of Salicylic Acid retained in damaged skin was less than that retained in intact skin; however, with damaged skin, the amount of Salicylic Acid in the recirculating solution decreased with time (to 60% at 6 h) and this could be the reason for the decrease in retention. No saturation phenomenon was observed with absorption from liquid paraffin, suggesting that absorption was via simple passive transport. With all vehicles, the Salicylic Acid concentration of the recirculating solutions decreased, following first-order kinetics.

These authors also determined in vitro the adsorption of Salicylic Acid in each vehicle using excised guinea pig abdominal skin. The amount adsorbed from liquid paraffin, isopropyl myristate, hexadecyl alcohol, and oleic acid was 3.56, 2.26, 1.57, and 0.73 mg, respectively (Washitake et al. 1975).

New Zealand white rabbits were also used to determine the percutaneous absorption of Salicylic Acid with and without DMSO and with and without nonionic surfactants (Shen, Santi, and Bruscato 1976). Five grams of white petrolatum ointments consisting of 10% (w/w) Salicylic Acid, 10% Salicylic Acid and 10% (w/w) DMSO, or 10% Salicylic Acid, 10% DMSO, and 10% (w/w) of the surfactants were each applied to the shaved dorsal skin of two rabbits under an occlusive patch for 8 h. Blood samples, which were taken prior to and 30 min and hourly for 8 h after application, were analyzed for salicylate content. The approximate peak blood salicylate values and times are summarized in Table 4.

TABLE 4
Peak blood salicylate values in rabbits treated topically with Salicylic Acid in various formulations (Shen, Santi, and Bruscato 1976)

Test article	Peak value (mg%)	Time of peak value (h)
Salicylic Acid (SA)	3	7
SA + DMSO	5.5	2
SA + DMSO + Poloxamer 182	11.5	2
SA + DMSO + Poloxamer 184	7	3
SA + DMSO + Poloxamer 231	11.5	2
SA + DMSO + Oleth-2	12.5	2
SA + DMSO + Oleth-20	7.5	1
SA + DMSO + Laureth-4	12	3
SA + DMSO + Sorbitan Laurate	12.5	3
SA + DMSO + Sorbitan Palmitate	12	2
SA + DMSO + Sorbitan Trioleate	12.5	2
SA + DMSO + Polysorbate 20	6.5	2
SA + DMSO + Polysorbate 40	7	1
SA + DMSO + Polysorbate 60	7.5	1
SA + DMSO + PEG-8 Stearate	5.5	3

The effect of daily and weekly dermal applications, as well as the effect of concentration, on the absorption of Salicylic Acid was determined using female Wistar rats (Roberts and Horlock 1978). Salicylic Acid, 1%, 5%, or 10%, in hydrophilic ointment was applied for 7.5 h to a 3-cm^2 shaved area of the flank under an occlusive patch. Application was as a single dose, repeated daily for 5 days, or repeated weekly for 4 weeks. At least three animals were used per group. At the end of dosing, treated skin was excised, the appropriate ointment was applied to the epidermis, and it was placed in a diffusion cell. The penetration flux of Salicylic Acid through excised skin was compared to that of Salicylic Acid through dimethicone (an inert membrane).

With a single application, 1%, 5%, and 10% Salicylic Acid had a mean penetration flux of 0.014, 0.061, and 0.078 mg/cm^2/h, respectively. The ratio of the penetration fluxes of Salicylic Acid through skin versus through dimethicone decreased with increasing concentrations of Salicylic Acid. With weekly dosing, the penetration flux of 1% Salicylic Acid remained constant during weeks 1 to 4, but it decreased with 5% and 10% Salicylic Acid. Also with weekly dosing, a significant difference was observed in penetration fluxes with 5% and 10% Salicylic Acid. Repeated daily doses of 1%, 5%, or 10% Salicylic Acid resulted in signficant differences in penetration flux between all

concentrations. With 5% and 10% Salicylic Acid, an increase in the penetration flux was observed after 2 days; the flux decreased after day 3. With 1%, the flux increased slightly until day 4, and then decreased (Roberts and Horlock 1978).

Single and multiple dose studies were performed using four female Rhesus monkeys to determine the percutaneous absorption of Salicylic Acid (Bucks et al. 1990). In the single-dose study, 4 mg/cm^2 ^{14}C-Salicylic Acid (27 mCi/mM) was applied to the clipped abdomen of each animal. The test site was washed 24 h after application. Urine was collected for 7 days following dosing. In the multiple-dose study, 4 μg/cm^2 Salicylic Acid was applied to the same site daily for 14 days; radioactive Salicylic Acid was applied only on days 1 and 8. The test sites were not washed. The animals were restrained in metabolic chairs. The cumulative percentage of ^{14}C-Salicylic Acid absorbed was 59% following the single dose and 67% and 78% following the first and eighth doses, respectively, of the multiple-dose study. A significant difference in cumulative absorption was not observed with single versus multiple applications.

The effect of iontophoresis on absorption of Salicylic Acid was determined using male Wistar rats (Singh and Roberts 1993). Glass diffusion cells were attached to an area of depilated dorsal skin to apply 1 mM Salicylic Acid with 12 μCi ^{14}C-Salicylic Acid in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.4. Absorption was measured with and without iontophoresis. Also, the epidermis was removed and Salicylic Acid was applied to the dermis using diffusion cells. The absorption rate constant, clearance, and percent of dose applied was 0.0028/min, 0.50 ml/h, and 22.7%, respectively, with epidermal iontophoresis and 0.0032/min, 0.58 ml/h, and 34.3% with passive dermal absorption. The concentration of Salicylic Acid was greater in the skin, dermis, and subcutaneous (SC) tissue below the treated site than in the plasma. The researchers concluded that direct penetration of Salicylic Acid occured only to a depth of 3 to 4 mm.

In a study to determine whether Salicylic Acid can bypass dermal microcirculation to reach underlying tissues, anesthetized and dead male Wistar rats were used (Singh and Roberts 1994). On all rats, a 4-cm^2 area of the dorsum was clipped free of hair, and 80 μm of the epidermis was removed. Salicylic Acid was applied using a glass cell that was adhered to the exposed epidermis. The anesthetized animals were then killed, and the tissues below the treated site and similar tissues from the contralateral side were removed; these tissues were also removed from the dead animals. The dermal clearances for anesthetized and dead animals were 0.58 and 0.10 ml/h, respectively. The concentration of Salicylic Acid was greater in the underlying dermis and subcutaneous tissue compared with the concentration in the plasma and similar tissues on the contralateral side. The concentration in underlying fascia were comparable to that in the plasma. At greater tissue depths, the concentration in the underlying tissues was always less than that in the plasma but comparable to that in similar tissues from the contralateral side.

Salicylic Acid—In Vitro Human Data

The absorption of 5% Salicylic Acid from five different vehicles was determined in vitro using seven samples of human leg and/or breast skin (Flesch, Satanove, and Brown 1955). Positive spot tests in the corium were observed after 2 to 4 h. Penetration was greatest with lanolin, Plastibase®, or Hydrophilic Plastibase (Squibb) vehicle, was moderate from a carbowax vehicle, and was very slight from a petrolatum base.

In a study to evaluate percutaneous transport as a function of stratum corneum structure and lipid composition, Elias et al. (1981) measured the penetration of 5% ^{14}C-Salicylic Acid (0.4 μCi/mg) through abdominal (postmortem) and leg skin (amputation) obtained from human males. Tissue sheets were prepared and then frozen at −70°C. Samples were thawed and mounted in a diffusion cell. Radioactively labeled Salicylic Acid and unlabeled Salicylic Acid were combined to a final concentration of 5% in propylene glycol. For both abdominal and leg skin samples, the dermal penetration of Salicylic Acid steadily increased between 10 and 24 h, after an initial lag. Using eight samples, the mean penetration of Salicylic Acid through abdominal stratum corneum was 3.6 μmol/cm^2/24 h (range of 0.7 to 9.7 μmol/cm^2/24 h); these stratum corneum samples had an average thickness of 21.8 micron, an average of 20.6 cell layers, and a lipid content of 6.8%. Using six samples, the mean penetration of Salicylic Acid through leg stratum corneum was 5.7 μmol/cm^2/24 h (range of 1.9 to 8.7 μmol/cm^2/24 h); these stratum corneum samples had a mean thickness of 26.8 micron, an average of 22.4 cell layers, and a lipid content of only 3.0%. The difference in penetration across the two sites was not significant, although suggestive of a higher penetration in the leg sample with its lower lipid content.

The horny layer of excised human skin and a three-layer membrane system were used to determine the penetration of Salicylic Acid (Neubert et al. 1990). Ten milligrams of Salicylic Acid was applied to a 4.0-cm^2 area in both the experiments using the skin samples and the membrane system, and they were performed fourfold and sixfold, respectively. Using the human skin samples, 20 tape strippings of the horny layer were removed and assayed for Salicylic Acid content.

The amount of Salicylic Acid from an aqueous (5.0 g cholinsalicylate, 47.5 g PEG 1500, 47.5 g PEG 400) emulsion that penetrated the horny layer after 30 and 60 min was 20.5% and 20.7% of the dose, respectively, whereas the amount that remained in the emulsion was 12.7% and 10.9%, respectively. After 30 min, the Salicylic Acid content was greatest in tape strippings 1 to 5 (7 to 16 μg) and 5 to 10 (5 to 8 μg). The same trend was observed after 100 min (7 to 12 μg in strips 1 to 5 and 5 to 9 μg in strips 5 to 10).

Salicylic Acid in both vaseline and in the Aqueous emulsion were used with the membrane system. With vaseline as the base, 9.0%, 6.0%, and 5.0% of the dose penetrated into membrane layers 1, 2, and 3, respectively, after 30 min and 10.0%, 9.0%, and 9.2% penetrated into these layers, respectively, after 60 min. Using the aqueous emulsion, 21.3%, 12.9%, and 8.4%

of the dose penetrated into layers 1, 2, and 3, respectively, after 30 min and 17.8%, 15.8%, and 14.9% penetrated into these layers, respectively, after 60 min (Neubert et al. 1990).

The in vitro penetration of Salicylic Acid through human skin (obtained by surgical operation) was compared to that through rodent skin (Harada et al. 1993). Using Franz-type diffusion cells, Salicylic Acid in isotonic buffer, 500 μg/ml, was applied to a 0.785-cm^2 area of human skin from a number of sites and male Wistar and hairless rat and male nude mouse skin.

At pH 4.0, Salicylic Acid penetrated human skin in a "zero-order fashion following a lag time." The penetration rates (μg/h/cm^2) were approximately 18 through scrotum skin (one sample), 2.3 through the cheek (one sample), 2.0 through neck skin (one sample), 1.25 through inguinal area skin (two samples), 0.5 through thigh (three samples) and foot skin (one sample), and <0.5 through lower leg (one sample), breast (five samples), and back skin (one sample); penetration of Salicylic Acid was not detectable through the sole.

The effect of pH on penetration was determined using human breast and neck, hairless rat, Wistar rat, and nude mouse skin at pH 2.0 to 4.0. Penetration was always greatest at pH 2.0. At pH 2.0, the mean penetration rates through human breast and neck, hairless rat, Wistar rat, and nude mouse skin were 5.97, 10.29, 5.23, 12.41, and 9.77 μg/h/cm^2, respectively. At pH 4.0, these values were 0.37, 1.97, 0.66, 1.03, and 1.60 μg/h/cm^2, respectively. The researchers also examined the effect of age of the skin donor (five female donors, 38 to 74 years of age) on penetration using breast skin; no effect was observed. Overall, the researchers determined that the stratum corneum was the primary Salicylic Acid permeability barrier (Harada et al. 1993).

The dermis from human mid-abdominal skin was used to determine the in vitro absorption of Salicylic Acid in isotonic saline buffer (Singh and Roberts 1993). Diffusion cells mounted on the skin were used for application. Using four samples, the permeability coefficient was 0.017 cm/h.

Singh and Roberts (1994) examined the penetration of Salicylic Acid across human epidermis isolated from the mid-abdominal region. Penetration was determined at full and 50% ionization. The permeability coefficients at 100% and 50% ionization were 0.000331 and 0.0152 cm/h, respectively.

Salicylic Acid—In Vivo Human Data

A dose of 4 μg/cm^2 of ^{14}C-Salicylic Acid, 1 μCi, was applied in an open manner to a 13-cm^2 area of the ventral forearm of 17 subjects; urinary excretion was measured for 5 days (Feldmann and Maibach 1970). Total absorption of Salicylic Acid was 22.78% of the applied dose. The greatest absorption rate, 0.535%/h, was observed 12 to 24 h after dosing.

Treatment levels (not specified) of Salicylic Acid were applied to large areas of the body of 21 patients with dermatoses, and plasma salicylate concentrations were determined (Schuppli et al. 1972). The average plasma Salicylic Acid concentration was 5.4 mg%, with 15 mg% being the greatest value observed. The average plasma Salicylic Acid value for 22 untreated patients was 3.9 mg%.

Approximately 0.5 g of a salve containing Salicylic Acid was applied to the trunk and extremities of 10 male subjects, and urinary excretion was measured (Panse, Zeiller, and Sensch 1974). The mean amount applied was 9.10 mg/kg. Mean urinary excretion was 0.417%, 0.572%, and 1.060% of the dose after 12, 24, and 48 h, respectively.

Four patients with active psoriasis (>25% of the body surface involved) were used to determine the dermal absorption of 6% Salicylic Acid in a 60% propylene glycol/19.4% alcohol gel (Taylor and Halprin 1975). After showering, the patients applied the test material to their entire body surface below the neck, and the treated areas were occluded for 10 h. After the occlusive dressings were removed, the patients showered. This treatment was repeated for a total of 5 days. Blood samples were taken daily prior to application and 5 and 10 h after application. Twenty-four-hour urine collections were made for a total of 7 days.

The subjects applied 9.4 to 22.6 g of the gel daily. The four subjects had total absorptions of 64%, 82%, 63%, and 69%; the patient with the greatest absorption had the most widespread psoriasis, with >90% involvement. A total of 3708, 4998, 5898, and 4104 mg Salicylic Acid per patient was applied, and a total of 2370, 4072, 3740, and 2827 mg salicylate per patient, respectively, was excreted. The urinary metabolites were primarily SUA (41% to 65% on the days of dosing) and acyl and phenolic glucuronides of Salicylic Acid (32% to 57% on the days of dosing). The percentage of Salicylic Acid recovered in the urine ranged from 0% to 14% on the days of dosing. Salicylates were still excreted in the urine on days 6 and 7. The serum salicylate concentration was always <5 mg/100 ml, and the average peak serum concentration was 2.7 mg/100 ml. The serum salicylate concentration peaked within 5 h after application for three of the four patients; salicylate concentrations were low or undetectable 24 h after application. It did not appear that salicylate accumulated during dosing (Taylor and Halprin 1975).

The dermal absorption of Salicylic Acid through human skin from two different vehicles was determined (Birmingham, Greene, and Rhodes 1979). Salicylic Acid, 3% in an aqueous solution of 40% polyethylene glycol (PEG) 400 [PEG-8] USP, was applied by immersing the forearm of two subjects in the solution. A hydrophilic ointment containing 10% Salicylic Acid was "evenly spread" over the forearm of four subjects, and the site was occluded. In another two subjects, the skin on the forearm was stripped with adhesive tape prior to application of the ointment. The exposure time for the solution and the ointment was 3 h, after which time the forearms were washed and rinsed. Blood was collected prior to exposure and at hourly intervals for 8 h from an indwelling catheter placed in the opposite forearm.

Keratolysis was observed within 24 h on the arms of both subjects exposed to Salicylic Acid in PEG. Salicylic Acid in PEG resulted in minimal systemic absorption, with plasma Salicylic Acid concentrations of <1.0 mg/dl. The researchers concluded

that the poor systemic absorption could be attributable to the formation of a glycol-salicylate complex resulting in a molecule too large to pass the stratum corneum.

Application of the Salicylic Acid ointment to intact skin did not produce detectable salicylate in the blood, although absorption was observed in the two subjects whose arms were tape-stripped prior to application. In these subjects, the peak salicylate concentration was approximately 8 mg/dl; the calculated absorption rate constant (k_a), elimination rate constant (k_{el}), and $t_{1/2}$ were 0.189/h, 0.201/h, and 3.450 h, respectively. Using these data, and assuming the 10% Salicylic Acid ointment was applied every 12 h to 30% of a patient's total surface area, the authors calculated that plasma Salicylic Acid concentrations would exceed 20 mg/dl after the first application and a steady-state concentration of 30 mg/dl would be obtained after the third application (Birmingham, Greene, and Rhodes 1979).

The percutaneous absorption of Salicylic Acid in a bath was determined using 15 subjects (Pratzel, Schubert, and Muhanna 1990). The subjects took 20 min baths with 0.33 g/L Salicylic Acid. (The bath preparation contained 25.0 g Salicylic Acid, 5.0 g sodium huminate, and 0.5 g camphor.) Blood was taken at various times for 24 h, and urine was collected for 72 h. The mean plasma Salicylic Acid concentrations for 12 subjects were 10.80, 9.97, 10.47, 9.5, 10.12, and 9.72 ng/ml 1, 2, 4, 6, 8, and 24 h after the bath, respectively. The mean amount of Salicylic Acid excreted in the urine of 14 and 15 subjects was 0.086 and 0.078 mg at 0 to 24 and 24 to 48 h, respectively. The mean amount of salicyluric acid excreted in the urine of 15 subjects was 0.92 and 0.72 mg at 0 to 24 and 24 to 48 h, respectively. The elimination half-life ($t_{1/2}$) was 30 to 50 h. The calculated area under the curve (AUC) was 921 h $\times$ ng/ml.

The percutaneous absorption of Salicylic Acid from an ointment containing 3% Salicylic Acid and 0.1% diflucortolone-21-valerate (DFV) was determined using a group of six human subjects (Täuber, Weiss, and Matthes 1993). The subjects were treated twice daily for 8 days with 20 g of the test material; the ointment was applied to the trunk, upper arms, and thighs. The ointment was left in contact with the skin for 22 h/day, and the treated areas were covered with a cotton dressing. The concentration of Salicylic Acid in the plasma was determined from one day prior to until 4 days after dosing. The concentration of Salicylic Acid in the plasma increased during the day; 2 to 3 μg/ml were present in the morning and 4 to 7 μg/ml were present in the afternoon. The AUC (0 to 8 days) was calculated as 30 μg $\cdot$ day/ml.

The relative bioavailability of Salicylic Acid from two different vehicles after repeated dermal application was determined for female human subjects with various skin types (Davis et al. 1997). The test articles, which consisted of 2% Salicylic Acid in either a hydroalcoholic vehicle (63% water/35% ethanol) or a cream (80% water/18% cosmetic excipient mixture), were applied to the faces and necks of the subjects once daily for 16 days. Each application consisted of approximately 1.25 to 1.50 g of the test material (25 to 30 mg Salicylic Acid). Nine and 10 subjects with normal skin were dosed with Salicylic Acid in the hydroalcoholic and in the cream vehicle, respectively, 9 subjects with acnegic skin were dosed with Salicylic Acid in the hydroalcoholic vehicle, and 9 subjects with aged skin were dosed with Salicylic Acid in the cream vehicle. A reference control group of 10 subjects was given 81 mg of acetylsalicylic acid once daily. Blood samples were taken on days 0, 7, and 12, and at various intervals on day 15. Urine was collected for 24 h on day 15. One subject did not complete the study, and two subjects were excluded from data analysis because of suspected noncompliance (due to "abnormally high baseline concentrations" of salicylates or Salicylic Acid) regarding self-medication.

No skin irritation was observed and no adverse reactions were reported. Steady-state was reached by day 7. Peak plasma Salicylic Acid concentrations were significantly greater, and time to peak occurred earlier, in the groups that received Salicylic Acid in the hydroalcoholic vehicle as compared to those that received it in the cream. The Salicylic Acid terminal exponential $t_{1/2}$ was significantly shorter in subjects given acetylsalicylic acid orally compared to all groups given Salicylic Acid dermally. When comparing the terminal exponential $t_{1/2}$ among the subjects dosed with Salicylic Acid, skin type and/or vehicle did not have an effect. AUC Salicylic Acid values were significantly greater in the subjects given Salicylic Acid in the hydroalcoholic vehicle as compared to those given it in the cream. Skin type did not significantly affect any of the parameters (Davis et al. 1997).

Six subjects were used to determine the percutaneous absorption of Salicylic Acid (Wester, Noonan, and Maibach 1998). ^{14}C-Salicylic Acid (0.46 mCi/mg), 39.7 μg/cm^2, in ethanol was spread over a 10-cm^2 area of the ventral forearm for 24 h; the site was not covered. Starting the day of dosing, 24-h urine collections were made for 7 days. The test site was tape stripped 7 days after application. Percutaneous absorption was determined based on urinary ^{14}C excretion. The mean 7-day urinary excretion of Salicylic Acid was 5.8% $\pm$ 4.5% (range of 2.3% to 13.6%); 53.4% was recovered in the wash and only 0.22% was recovered with tape stripping. The researchers compared the results with those obtained using the isolated perfused porcine skin flap system (IPPSF). A 10-cm^2 area on five IPPSFs was dosed in a manner similar to the human skin. After 8 h, 7.1% and 0.43% of the dose was recovered in the skin and the perfusate, respectively; 16.6% and 48.2% of the dose was recovered in the tape strips and the wash, respectively.

The dermal penetration of Salicylic Acid was determined in normal and barrier-perturbed skin of 16 subjects, 9 males and 7 females, using microdialysis (Benfeldt, Serup, and Menné 1999). A Latin square design was used, and penetration was determined at the following four sites on the forearm of the subjects: normal skin; skin that had partial removal of the stratum corneum via tape-stripping; skin with irritant dermatitis induced by pretreatment with 1% or 2% sodium lauryl sulfate (SLS) for 24 h; and acetone-treated skin. An equilibration period of 1 h was allowed after insertion of the microdialysis probes, which were inserted 15 min after barrier perturbation. After equilibration,

5 ml of a 5% w/v solution of Salicylic Acid in ethanol was added to the chamber, and perfusion continued for 4 h. With some subjects, a fifth site was used as a control; ethanol was added to the chamber. Drug concentration controls were done by taking a sample of the test solution from each chamber at the start of the study; at the completion of each study, samples were also taken from the chambers of eight subjects and analyzed for Salicylic Acid. Skin thickness and probe depth were measured at the completion of the test using ultrasound scanning.

Salicylic Acid was detectable in all samples from areas to which it had been applied; the concentration increased rapidly up to 70 min. Comparing the AUC from 0 to 200 min, Salicylic Acid penetration increased 2.2-, 46-, 146-, and 157-fold in acetone-treated, 1% SLS pretreated, 2% SLS pretreated, and tape-stripped skin, respectively, as compared to normal skin. Transepidermal water loss, which was also measured at each site, was 4.3, 9.1, 19.5, 30.1, and 30.6 g/m^2/h at the normal and acetone-treated, 1% SLS-treated, 2% SLS-treated, and tape-stripped sites, respectively. Skin thickness at each of these sites was 1.72, 1.75, 1.85, 2.14, and 1.9 mm, respectively.

An intraregional variation in reactivity to barrier damage was observed; the most proximal location had higher reactivity scores. The sex of the subject had no effect on the penetration of Salicylic Acid. With the vehicle control using five subjects and 10 probes, occasional Salicylic Acid concentrations of 5 to 10 ng/ml were seen in 8 of the probes. In the drug concentration controls, the initial Salicylic Acid concentration of 48.9 mg/ml increased by a mean of 7% over the 4 h.

Ethylhexyl Salicylate—In Vitro Human Data

In the in vitro study by Treffel and Gabard (1996), skin samples from two women were dermatomed to a thickness of 600 μm. A dose of 2.26 and 2.52 mg/cm^2 of 3% Ethylhexyl (Octyl) Salicylate in the o/w emulsion gel and petroleum jelly, respectively, was applied for 2 min, 30 min, 2 h, or 6 h to a 1.76-cm^2 area of skin in a Franz cell. In the epidermis, 0.94%, 2.13%, 1.54%, and 7.29% of the dose from the o/w emulsion-gel vehicle and 1.81%, 0.60%, 1.97%, and 1.96% of the dose from petroleum jelly was recovered after 2 min, 30 min, 2 h, and 6 h, respectively. None to very little of the dose was recovered from the dermis at any time, and none was detected in the receptor fluid.

Ethylhexyl Salicylate—In Vivo Human Data

In the in vivo study by Treffel and Gabard (1996), 2 mg/cm^2 of 3% Ethylhexyl (Octyl) Salicylate in an o/w emulsion gel and in petroleum jelly were applied to a 100-cm^2 area of the back of four subjects. The sites were wiped 30 min and 2 and 6 h after application, and 15 tape strippings were performed. Sun protection factor (SPF) measurements were performed prior to and 30 min after application; a multiport solar UV simulator was used as the light source.

Maximal concentrations were reached 30 min after application. At this time, approximately 37% of the Ethylhexyl (Octyl) Salicylate in the o/w emulsion gel was found in the stratum corneum in tape strippings 1 to 5, as compared to approximately 10% of the Ethylhexyl (Octyl) Salicylate in petroleum jelly. The amount found in tape strippings 6 to 10 and 11 to 15 was approximately 9% and 4%, respectively, from the o/w emulsion gel and 3% and 1%, respectively, from petroleum jelly. Significantly more Ethylhexyl (Octyl) Salicylate was absorbed from the o/w emulsion gel vehicle as compared to the petroleum jelly vehicle. The SPF values prior to wiping were 14.2 $\pm$ 3.6 and 5.4 $\pm$ 1.3 for the emulsion gel and petroleum jelly, respectively. These values decreased by a factor of 2.2 after wiping. Again, the difference between vehicles was significant (Treffel and Gabard 1996).

Methyl Salicylate—In Vitro Animal Data

The in vitro percutaneous absorption of Methyl Salicylate was determined using whole skin from the external ears of Landrace pigs (Loveday 1961). At concentrations of 0.1 to 0.75 mg/ml, the penetration rate was approximately 0.125 to 0.6 mg/cm^2/24 h. At a concentration of 1 mg/ml, the pH of the buffer solution did not affect the rate of penetration.

Yano et al. (1991) performed an in vitro study using hairless mouse skin to determine the effect of menthol and camphor on the metabolism of Methyl Salicylate to Salicylic Acid. In vitro hydrolysis of Methyl Salicylate to Salicylic Acid was linear using skin, liver, and serum enzyme preparations. The formation of Salicylic Acid was inhibited by *l*-menthol and *dl*-camphor in a dose-dependent manner. Bis-*para*-nitrophenyl phosphate, an esterase inhibitor, produced 1000 times stronger inhibition than menthol and camphor.

The percutaneous absorption and metabolism of radioactive Methyl Salicylate was determined after application to viable and nonviable skin from male and female hairless guinea pigs in an in vitro study using flow-through diffusion cells (Boehnlein et al. 1994). Groups of three animals, and three to four repetitions per animal, were used. Methyl Salicylate in acetone was applied to the skin at a dose of 5 μg/cm^2. After 24 h, the surface was thoroughly washed to remove unabsorbed material. It was found that Methyl Salicylate did not spontaneously degrade in the receptor fluid, and that the metabolism that occurred took place during absorption through the skin and not as a result of contact with the receptor fluid.

No significant difference was observed in the percutaneous absorption of Methyl Salicylate through viable and nonviable skin from male or female hairless guinea pigs. The percutaneous absorption, as a percentage of the applied dose absorbed in 24 h, was 55% $\pm$ 6% and 56% $\pm$ 16% for male and females, respectively, through viable skin and 47% $\pm$ 2% and 50% $\pm$ 20% for males and females, respectively, through nonviable skin. Absorption was rapid, with greater than 75% of the absorbed compound found in the receptor fluid collected in the first 6 h.

Unlike absorption, the metabolism of Methyl Salicylate was significantly different in skin from males and females as well as in viable and nonviable skin. In viable skin, esterase activity was

observed, and Salicylic Acid was then metabolized by glycine conjugation to SUA. In nonviable skin, only esterase activity was observed. In viable skin of male animals, the metabolism of Methyl Salicylate, as a percentage of absorbed dose metabolized, was 36% ± 6% and 21% ± 5% to Salicylic Acid and SUA, respectively, for a total of 56% ± 5% of the absorbed dose metabolized. In viable skin of female animals, 12% ± 2% and 12% ± 4% was metabolized to Salicylic Acid and SUA, respectively, with a total of 25% ± 3% of the absorbed dose metabolized. In nonviable skin from male and female animals, 38% ± 5% and 13% ± 3% of the absorbed dose, respectively, was metabolized to Salicylic Acid. The formation of Salicylic Acid in the skin from males represented 34% of the absorbed radioisotope, as compared to 5% in skin from females. In examining the time course of metabolism, the extent of metabolism in male and female guinea pig skin was significantly different only at the 6-h interval (Boehnlein et al. 1994).

The percutaneous absorption of Methyl Salicylate through intact hairless mouse skin was determined in vitro using a glass flow-through diffusion cell system (Higo et al. 1995). A 0.95-cm^2 area of skin was exposed to 1% w/v Methyl Salicylate, pH 4.0. The penetration flux decreased 4 h after application. Approximately 17 μmol Salicylic Acid penetrated after 10 h. When a lower concentration was tested, a lower flux through the skin was observed, and more of the Methyl Salicylate was metabolized. Pretreatment of skin with *l*-menthol for 14 h prior to excision inhibited the metabolism of Methyl Salicylate to Salicylic Acid, but it did not significantly affect penetration.

Methyl Salicylate—In Vivo Animal Data

Male Sprague-Dawley rats were used to determine the effect of pH on dermal absorption of Methyl Salicylate (Siddiqi and Ritschel 1972). The tails of the animals were immersed in a Methyl Salicylate solution containing 5% ethanol that had a pH of 2, 3, 6, or 8. At pH 2, 3, 6, and 8, the total amount absorbed was 1.56, 0.76, 1.77, and 1.57 $\mu g/mm^2/h$, respectively, the k_a was 3.5439, 0.7421, 1.2059, and 2.2173/h, respectively, and the $\alpha(\%)$ was 0.645×10^{-6}, 0.645×10^{-5}, 0.645×10^{-2}, and 0.641, respectively.

The percutaneous absorption of ^{14}C-Methyl Salicylate from medicated plaster (8.47 μCi [3.54 mg] in 10 × 10-mm plaster) was determined using hairless HRS/J (hr) mice (Maruta et al. 1977). The plasters, which were covered, were applied for 1 to 48 h; the animals were killed at the termination of dosing. "High levels of radioactivity" were found in the skin at the test site 1 h after application; the amount peaked at 4 h and then declined. Very little radioactivity was seen at 48 h. "Slight radioactivity" was detected in skin adjacent to the test site at 2 and 4 h. Serum radioactivity peaked at 2 h at 15 μg/ml salicylates. Cumulative urinary excretion of the radioactivity was 27.2%, 33.5%, and 39.3% of the dose after 12, 24, and 48 h, respectively.

Female hairless HRS/J (hr) mice were used to determine the dermal penetration and metabolism of, and the effect of *l*-menthol and *dl*-camphor on, Methyl Salicylate (Yano et al. 1991). A 2 × 2-cm^2 plaster sheet containing 5.2 mg Methyl Salicylate, with or without 4.8 mg *l*-menthol and 1.0 mg *dl*-camphor, was applied to the dorsal skin of each animal for 1, 3, or 6 h. The animals were then killed and the skin removed, rinsed, and minced. In skin not exposed to menthol and camphor, the dermal concentrations of Methyl Salicylate and Salicylic Acid after 1 h were 0.64 and 0.49 μmol/g, respectively; these values decreased to 0.29 and 0.22 μmol/g after 6 h. Application of menthol and camphor increased the dermal concentrations of Methyl Salicylate and Salicylic Acid. After 1 h, these values were 1.79 and 0.39 μmol/g, respectively.

The depth of penetration following topical application of Methyl Salicylate was determined using male Wistar rats (Megwa, Benson, and Roberts 1995). Five preparations containing 10% to 28.3% Methyl Salicylate (Salicylic Acid equivalence of 18.9 mg/cm^2) were each applied to a 9.625-cm^2 area of depilated abdominal skin. The untreated contralateral side was used as the control. After 2 h, the formulations were removed using a spatula and blood samples were taken. The animals were then killed and tissue samples (skin, SC, top muscle, deep muscle, and fat) were sequentially removed from below the test and control sites.

Methyl Salicylate was primarily converted to Salicylic Acid during transport through the skin. The plasma Salicylic Acid concentration, which ranged from approximately 200 to 325 μg/g, was greater than the plasma Methyl Salicylate concentration, which ranged from approximately 25 to 50 μg/g, with application of all formulations. Salicylate appeared to directly penetrate to the SC tissue or top muscle underlying the treated area. Salicylate concentrations in the deeper tissues underlying the test site and on the contralateral side were similar, suggesting that the salicylate present in these tissues was due to systemic blood supply. Similar results were seen in preparations that contained 10% TEA-Salicylate in addition to Methyl Salicylate (Megwa, Benson, and Roberts 1995).

Methyl Salicylate—In Vivo Human Data

The dermal absorption of 20% Methyl Salicylate from three different vehicles was determined in male subjects by measuring urinary excretion (Beutner et al. 1943). The three ointments consisted of 20% Methyl Salicylate and 80% anhydrous lanolin (ointment 1), 60% anhydrous lanolin and 20% menthol (ointment 2), or 60% of a special aqueous base of 35% glycerin monostearate, 4.2% phenolic resin, 3.5% acacia, 28% water, 28% alcohol, and 1.3% glycerin and 20% menthol (ointment 3). Each subject applied and rubbed in a total of 10 g of ointment to the skin of the chest, abdomen, and thigh. Urine was collected.

A qualitative determination using eight subjects showed that salicylate was excreted within 2 h for 2 subjects, 12 h in five subjects, and >12 h in one subject. The mean salicylate excreted by the 5, 22, and 15 subjects who applied ointments 1, 2, and 3 was 41.6, 55.1, and 47.5 mg, respectively. Eight subjects who had "better cutaneous absorption than the average" ("dark-complexioned individuals apparently [had] a higher

absorption ability than blonds") were used to compare the excretion of salicylate following dermal inunction of ointments 1, 2, and 3, 64.6, 101.3, and 103.1 mg salicylate, respectively, were excreted. Menthol appeared to enhance absorption (Beutner et al. 1943).

Methyl Salicylate, was applied to the forearms of subjects under a 1 × 5 × 10-cm plastic cell using hydrous and anhydrous conditions (Wurster and Kramer 1961). For the hydrous condition, a 5 × 10-cm sponge was filled with 6 ml Methyl Salicylate and 3 ml distilled water, and for the anhydrous condition, the cell was filled with magnesium perchlorate and the sponge with 6 ml Methyl Salicylate without water. Urine samples were taken every 2 h during exposure. The cell was removed after 16 h, and the test site was washed. The urinary excretion rate for Methyl Salicylate was 8.6 and 2.7 mol/100 cm^2/h with hydrous and anhydrous exposure, respectively. In both cases, steady-state was reached at approximately 6 h.

These authors also determined the absorption of Methyl Salicylate through defatted and nondefatted skin. For the test using defatted skin, the arms of each subject were immersed in ethyl ether for 1 min. For both tests, "an excess of Methyl Salicylate" was applied on a 100-cm^2 area of the forearm for 2 h. The test area was washed. Urine was collected every 2 h until negative for salicylate. Defatting of the skin decreased total salicylate absorption by 27% (Wurster and Kramer 1961).

The dermal absorption of Methyl Salicylate from medicated plaster (containing 35.0 mg Methyl Salicylate/sheet) was determined using groups of six subjects (Maruta et al. 1977). One group received a single covered application to the back of 10 sheets of the plaster to the back, while the second group received repeated covered 12-h applications for 6 days of 10 sheets, with a 12-h nontreatment period in between. With the single application, blood samples were taken 4 to 48 h after application initiation and urine was collected for 48 h. With multiple applications, blood samples were taken immediately before the third and fifth application and 12 and 36 h after removal of the final application. With the single application, serum free Salicylic Acid peaked at 8 h after dose initiation at approximately 4 μg/ml; no free Salicylic Acid was determined in the serum at 48 h. Greatest total salicylate concentration, 12.5 μg/ml, occurred at 12 h. The cumulative total salicylates excreted in the urine was approximately 37% of the applied dose; 70% of this amount was excreted during the application period. With repeated applications, a trace to no free Salicylic Acid or total salicylates was found in the serum.

Five subjects were used to determine the dermal permeability and plasma uptake of five products containing 12% to 50% Methyl Salicylate (Roberts et al. 1982). Five grams of each product was applied to a 50-cm^2 area on the forearm of each subject in a Latin Square design; a small portion of the product was rubbed into the area and the remaining product was spread over the site. The test site was occluded for 10 h, after which time it was washed. There was a 1-week period between product applications. One of the products, which contained 25% Methyl Salicylate, was also applied to the abdomen, instep, heel, and plantar region of four subjects following the same protocol. Methyl Salicylate absorption and excretion was estimated from the total urinary excretion of salicylate. Urine was collected at various intervals for up to 48 h after application.

When applied to the forearm, skin permeability coefficients were similar for each product and ranged from 1.0 ± 0.4 to 1.9 ± 0.5 cm/h. The amount of salicylate absorbed from each product after application to the forearm ranged from 12% to 20%, and the estimated steady-state salicylate concentration ranged from 2.5 to 7.6 mg/L. The skin permeability coefficient, the percentage of salicylate absorbed, and the cumulative urinary salicylate recovery was greatest upon application to the abdomen, followed by the forearm, instep, heel, and the plantar area. Pain and redness were experienced by all test subjects at all sites of application, but the amount of pain associated with application to each site as reported by the subjects was greatest at those sites with the greatest absorption (Roberts et al. 1982).

The dermal penetration of a product containing 1% w/w Methyl Salicylate that was applied as a metered aerosol was determined in human subjects (Collins et al. 1984). The product also contained 5% w/w of each ethyl and 2-hydroxyethyl salicylate. The product was sprayed onto the forearms of the subjects, but was not massaged. Platelet aggregation and venous blood Ethyl and Methyl Salicylate concentrations were measured using six subjects, two males and four females, and venous blood oxygen was measured using two subjects.

Methyl Salicylate was absorbed faster than ethyl salicylate, but the concentration of ethyl salicylate in blood was greater. A relatively high salicylate concentration was found for up to 1 h after dosing in blood drawn from the treated arm; the amount of Methyl Salicylate found in the plasma peaked at approximately 20 min after dosing. A small amount of salicylates was detectable for only 20 min in blood drawn from the untreated arm. Platelets were resistant to arachidonic acid-induced clumping for approximately 15 min after application; this effect was not observed in blood drawn from the untreated arm. Venous blood oxygen increased, peaking between 30 to 40 min and then declining, in blood drawn from the treated arm; again, this effect was not seen in blood drawn from the untreated arm (Collins et al. 1984).

The effect of exercise and/or heat on the percutaneous absorption of Methyl Salicylate was determined using six male subjects (Danon, Ben-Shinon, and Ben-Zvi 1986). Five grams of Methyl Salicylate were applied to the back and chest, and the subjects were exposed to heat, exercise, or both for 6 h. Blood samples were taken at 0, 1, 2, 3, and 5 h and urine was collected hourly for 8 h. Exercise and/or heat increased plasma total salicylate concentrations and urinary SUA, indicating increased systemic salicylate availability. Plasma salicylate peaked at 2 h under all conditions; values at 1, 3, and 5 h were significantly increased with heat exposure compared to controls. The AUC_{0-5} was significantly increased under test compared to control conditions. The urinary metabolic profile was similar under all test and control conditions; SUA comprised 95% of the urinary metabolites.

However, heat and/or exercise resulted in an increase in the excretion of SUA; 2.6% of the applied dose was excreted following heat and exercise as compared to 1.0% under control conditions.

The percutaneous absorption of Methyl Salicylate in a bath was determined using 10 subjects (Pratzel, Schubert, and Muhanna 1990). The subjects took 20 min baths with 0.03 g/L Methyl Salicylate. (The bath preparation contained 15.0 g Methyl Salicylate, 1.5 g Siberian spruce-needle oil, 4.0 g thyme oil, and 3.0 g camphor.) Blood was taken at various times for 24 h, and urine was collected for 72 h. The mean plasma Salicylic Acid concentrations for 20 and 10 subjects were 452.6 and 116.6 ng/ml 1 and 6 h after the bath, respectively. For one subject, the 2-, 4-, 8-, and 24-h values were 308, 171, 63, and 41 ng/ml, respectively. The mean amount of salicyluric acid excreted in the urine was 5.08, 0.71, and 0.97 mg at 0 to 12, 12 to 24, and 24 to 48 h, respectively. The elimination $t_{1/2}$ was 2.4 to 4 h. The calculated AUC was 1000 to 3900 h × ng/ml.

Twelve subjects, six men and six women, were used to determine rate and extent of absorption following dermal application of an ointment containing 12.5% Methyl Salicylate (Morra et al. 1996). Five grams of the ointment (equivalent to 567 mg salicylate) was applied twice daily for 4 days to a 10-cm^2 area on the anterior aspect of the thigh under a nonocclusive patch. Blood samples were taken on days 1 and 4 just prior to dosing and at various intervals up to 24 h after the first daily application. Twenty-four-hour urine collections were made during the entire study. No unchanged Methyl Salicylate was detected in the serum samples. (The limit of detection was 0.3 mg/L.) Serum Salicylic Acid concentrations ranged from 0.3 to 0.9 mg/L within the 1 h of the first application and 2 to 6 mg/L on day 4. The mean serum pharmacokinetic values are summarized in Table 5.

Unchanged Methyl Salicylate was not detected in the urine. (The limit of detection was 1 mg/L.) Unchanged Salicylic Acid and SUA were detected in all urine samples at concentrations up to 15.6 and 491.9 mg/L, respectively. Glucuronides were also present. The total Salicylic Acid recovered on days 1, 2, 3, and 4 was 175.2, 249.0, 254.1, and 251.4 mg, respectively, and the percent recovered for these days was 15.5%, 22.0%, 22.4%, and 22.2%, respectively. The difference in recovery between day 1 and days 2, 3, and 4 was significant (Morra et al. 1996).

Sodium Salicylate—In Vivo Animal Data

The percutaneous absorption of Sodium Salicylate from four different vehicles was determined using groups of 10 New Zealand white rabbits (Stolar, Rossi, and Barr 1960). Sodium Salicylate, 6.95%, was added to the oleaginous base petrolatum USP XV, the hydrophilic base petrolatum USP XV with water, the o/w base hydrophilic ointment USP XV, and the water-soluble base PEG ointment USP XV. The hair on the back of each animal was shaved, and 7.5 g of each ointment was applied to a 6.35 × 12.7-cm^2 area under an occlusive patch for 9 h. Blood samples were taken hourly. The greatest absorption was observed from the hydrophilic ointment; peak absorption was approximately 4.6 mg% at 5 h. The peak absorption concentration with petrolatum and hydrophilic petrolatum with water were approximately 1.0 and 0.4 mg% at 6 and 5 h, respectively. Negligible absorption was seen with the PEG ointment.

New Zealand white rabbits were used to determine the absorption of 11.6% Sodium Salicylate from hydrophilic ointment and hydrophilic petrolatum bases, with and without DMSO (Stelzer, Colaizzi, and Wurdack 1968). (Protocol described previously.) Blood salicylate concentrations peaked at 8 h with all formulations. The peak values from hydrophilic ointment were 4.03 and 1.38 mg% without and with DMSO, respectively, and from hydrophilic petrolatum were 4.03 and 1.38 mg% without and with DMSO, respectively.

The percutaneous absorption of Sodium Salicylate, with and without DMSO and with and without nonionic surfactants, was determined using New Zealand white rabbits (Shen, Santi, and Bruscato 1976) using a protocol described previously. The

TABLE 5

Mean Salicylic Acid serum pharmacokinetic values after dermal application of Methyl Salicylate (Morra et al. 1996)

Pharmacokinetic parameters	Salicylic Acid concentration	
	Day 1	Day 4
Minimum concentration (C_{min}) (mg/L)		
0 h	0.00	2.0 ± 1.1
12 h	1.2 ± 0.7	1.7 ± 1.1*
24 h	1.5 ± 0.8	1.9 ± 1.0
Maximum concentration (C_{max}) (mg/L)	1.7 ± 0.7	3.9 ± 1.2*
Time to C_{max} (h)	6.0 ± 2.0	4.4 ± 1.3
AUC_{0-12} (mg · h/L)	15.3 ± 6.6	35.8 ± 11.8*
Apparent oral clearance (L/h)	3.89 ± 1.32	
Apparent oral volume of distribution (L)	20.28 ± 6.15	
k_{el} (/h)	0.1955 ± 0.0441	
k_a (/h)	0.1608 ± 0.0441	0.2803 ± 0.2489*

*Significantly different from day 1 value.

TABLE 6
Peak blood salicylate values with Sodium Salicylate in various formulations (Shen, Santi, and Bruscato 1976)

Test article	Peak value (mg%)	Time of peak value (h)
Salicylic Acid (SA)	2.5	8
SA + DMSO	1	8
SA + DMSO + Poloxamer 182	3.5	4
SA + DMSO + Poloxamer 184	2	5
SA + DMSO + Poloxamer 231	2.75	3
SA + DMSO + Oleth-2	2.75	5
SA + DMSO + Oleth-20	2	5
SA + DMSO + Laureth-4	3	8
SA + DMSO + Sorbitan Laurate	7	6
SA + DMSO + Sorbitan Palmitate	4	6
SA + DMSO + Sorbitan Trioleate	3	4
SA + DMSO + Polysorbate 20	2.25	7
SA + DMSO + Polysorbate 40	2	8
SA + DMSO + Polysorbate 60	2.25	8
SA + DMSO + PEG-8 Stearate	2	8

approximate peak blood salicylate values and times are summarized in Table 6.

Using guinea pigs, Yankell (1972) determined whether lateral diffusion occurred with the application of ^{14}C-Sodium Salicylate (equivalent to 3% Salicylic Acid) in water; absorption was compared to that of 3% ^{14}C-Salicylic Acid in 75% ethanol. Lateral diffusion did not occur; <2% of either applied dose was found in sites adjacent to the test site.

Sodium Salicylate—In Vitro Human Data

The absorption of 5% Sodium Salicylate from five different vehicles was determined in vitro using seven samples of human leg and/or breast skin (Flesch, Satanove, and Brown 1955). No penetration was observed with a petrolatum, carbowax, lanolin, Plastibase®, or Hydrophlic Plastibase® (Squibb) vehicle after 24 h of incubation.

The horny layer of excised human skin and a three-layer membrane system were used to determine the penetration of Sodium Salicylate (Neubert et al. 1990). (Protocol described previously.) The amount of Sodium Salicylate from an aqueous emulsion that penetrated the horny layer after 30 and 60 min was 19.0% and 23.2% of the dose, respectively, whereas the amount that remained in the emulsion was 26.6% and 24.1%, respectively. After 30 min, the Sodium Salicylate content was greatest in tape strippings 1 to 5 (5 to 27 μg). After 100 min, 5 to 28 μg was found in strips 1 to 7. Sodium Salicylate in the aqueous emulsion was used with the membrane system. After 30 min, 20.3%, 6.6%, and 3.1% of the dose penetrated into layers 1, 2, and 3, respectively, and after 60 min, 26.0%, 9.5%, and 5.5% of the dose penetrated into these layers, respectively.

TEA-Salicylate—In Vivo Animal Data

Groups of eight rabbits were used to determine the dermal absorption of TEA-Salicylate (Panse, Zeiller, and Sensch 1974). Patches containing 5 g of a TEA-Salicylate salve (36.2 mmol/100 g) were applied for 6 h; urinary excretion of Salicylic Acid was measured. Approximately 4.01% and 14.59% of the dose was excreted in the urine after 24 and 48 h, respectively.

Five male Beagle dogs were used in a study in which 10 g of a TEA ^{14}C-Salicylate cream (specific activity 140 dpm/μg of ^{14}C-salicylate, 2.77 mM = 24.3 μCi, specific activity 8.77 μCi/mM) was massaged into the shaved right knee of each animal, and dermal absorption was measured (Rabinowitz et al. 1982). Blood and urine samples were taken 30 or 60 min after application, and tissue samples were taken at the point of application. The ^{14}C-salicylate concentrations in skin at the application site, muscle, fascia, tendon, ligament, cartilage, bone marrow, bone, synovium, synovial fluid, blood, and urine after 60 min were 312.2, 38.20, 16.40, 3.00, 2.00, 1.62, 1.05, 1.00, 0.74, 0.80, 0.22, and 0.16 μg/ml, respectively. At 30 min, the concentration in the blood was 2.60 μg/ml.

The dermal penetration of TEA-Salicylate was determined using six female Yorkshire swine (Baldwin, Carrano, and Imondi 1984). A hydrophilic cream, 1.5 g, containing 10% (w/w) TEA-^{14}C-Salicylate (Salicylic Acid equivalence of 72 mg) was applied to a 100-cm^2 shaved area of the biceps femoris of each animal. In four animals, half the test site was washed 30 min after dosing and muscle and fat were removed. This procedure was repeated at 2 h. Blood samples were taken both times. In the remaining two animals, blood samples were taken from shallow incisions 10, 20, and 30 min after dosing.

One non–dose-related death occurred. Two hours after dosing, 7.9% of the dose remained on the skin and 9.3% remained in the skin tissue. At least 82% of the dose was absorbed in 2 h. Thirty minutes after dosing, 150.9 to 724.5 ng/g of muscle tissue (expressed as free Salicylic Acid equivalents) was recovered in the treated muscle and 31.6 to 56.4 ng/g of blood were recovered in the blood of four animals. At 2 h, 313.5 to 582.3 ng/g were recovered in the treated muscle and 38.7 to 84.5 ng/g were recovered in the blood of three animals. In the contralateral muscle (control), only 0 to 28.7 and 8.6 to 55.2 ng/g were recovered at 30 min and 2 h, respectively. A much greater amount of salicylate was found in the treated muscle as compared to the control. Little ^{14}C was excreted in the urine; 0.14% and 0.45% of the dose was excreted 30 min and 2 h after dosing, respectively. The ^{14}C recovered in blood from shallow incisions from two animals 10, 20, and 30 min after dosing was equivalent to 15.8, 6.2, and 5.3 μg salicylate/g (Baldwin, Carrano, and Imondi 1984).

Rabinowitz and Baker (1984) performed studies using male and female Beagle dogs in which radioactive TEA-Salicylate ointment was applied to the shaved knees of the animals, and the penetration was then determined. In one study using 5 male and 10 female animals, 10 g of radioactive 10% TEA-Salicylate ointment was massaged into a 100-cm^2 area. Tissue samples

were taken after 60 min. The average ^{14}C-salicylate concentration in the skin at the application site, fascia, muscle, cartilage, fat pad, tendon, synovial fluid, meniscus, ligament, and serum of the male animals was 50.898, 5.188, 1.847, 1.804, 0.833, 0.579, 0.536, 0.413, 0.253, and 0.004 μmol/g, respectively; for the female animals, these values were 32.644, 3.471, 0.644, 0.507, 0.398, 0.608, 1.434, 0.645, 0.224, and 0.013 μmol/g, respectively. No significant difference in absorption was observed between males and females.

These authors then examined the effect of varying the amount of radioactive TEA-Salicylate while keeping the weight applied constant; all tissues were examined 60 min after application. Creams containing 5, 1, and 0.1 g radioactive 10% TEA-Salicylate ointment, brought to a weight of 10 g using cold cream, were applied to the knees of 3, 2, and 1 animals and the results were compared with the combined averages of the 15 animals dosed with 10 g radioactive ointment. The amount of recovered ^{14}C-salicylate decreased proportionately.

They also applied ^{3}H-(G)-TEA 7-^{14}C-Salicylate to the shaved knees of dogs and determined the concentrations of each radioactive moiety. The concentrations of ^{3}H-TEA recovered in the skin at the application site, fascia, muscle, fat pad, synovial fluid, and serum were 23.695, 2.112, 0.528, 0.300, 0.039, and 0.001 μmol/g, respectively. The concentrations of ^{14}C-salicylate found at these sites were 34.427, 2.655, 1.199, 0.398, 0.061, and 0.002 μmol/g, respectively (Rabinowitz and Baker 1984).

TEA-Salicylate—In Vivo Human Data

Six male subjects with seropositive, adult-onset rheumatoid arthritis were used in a study in which 10 g of a TEA ^{14}C-Salicylate cream was massaged into a 25 to 30-cm^2 area of the skin over one knee, and dermal absorption was measured (Rabinowitz et al. 1982). Blood and urine samples were obtained prior to and 1 or 2 h after application. Synovial fluid aspiration was also performed. The ^{14}C-salicylate concentrations in synovial fluid, blood, and urine were 0.16, 0.03, and 0.02 μg/ml, respectively, at 1 h and 0.25, 0.08, and 0.18 μg/ml, respectively, at 2 h.

Ten hospitalized male patients with classical or definite rheumatoid arthritis, four of which were restricted to bedrest, participated in a study designed to determine the absorption of dermally applied radioactive 10% TEA-Salicylate ointment (Rabinowitz and Baker 1984). The ointment was massaged in for 60 min and the skin was then wiped. A synovial fluid aspiration procedure was performed at a site that was not in contact with the TEA-Salicylate ointment. The mean ^{14}C-salicylate concentrations in the synovial fluid, blood, and urine of the six patients not confined to bedrest were 0.0011, 0.0002, and 0.0001 μmol/g, respectively; in the four patients confined to bedrest, these values were 0.0014, 0.0011, and 0.0020 μmol/g. The blood and urine concentrations were significantly different for the two groups of patients.

Twelve subjects, six men and six women, were used to determine the rate and extent of absorption following dermal application of a cream containing 10% TEA-Salicylate (Morra et al. 1996). Five grams of the ointment (equivalent to 241 mg salicylate) was applied twice, with 12 h between applications, to a 10-cm^2 area on the anterior aspect of the thigh under a nonocclusive patch. Blood samples were taken just prior to dosing and at various intervals up to 24 h after the first application. Urine was collected for 24 h, starting just prior to the first application. No unchanged TEA-Salicylate or Salicylic Acid was detected in the serum. (The limit of detection was 0.3 mg/L.) The amount of unchanged Salicylic Acid and SUA found in the urine was 1.8 and 9.1 mg, respectively; the Salicylic Acid and SUA concentrations were often below the limit of detection (1 mg/L). The total Salicylic Acid and percent recovered in the urine were 6.9 mg and 1.4%, respectively.

The dermal absorption, distribution, metabolism, and excretion studies described above are summarized in Table 8a.

Oral Route of Administration

Salicylic Acid—Animal Data

Tanaka et al. (1973a) determined the Salicylic Acid concentration in maternal organs and in the fetuses. Five gravid Wistar rats were fed a diet containing 0.2% Salicylic Acid on days 8 to 14 of gestation. The animals were killed on the last day of dosing. The greatest concentration of Salicylic Acid was in the serum (115.96 μg/ml) and the lowest was in the brain (4.14 μg/g). All other examined organs, including the placenta, had similar concentrations (21.68 to 35.23 μg/g) with the exception of the kidneys, which had a relatively high concentration (60.89 μg/g). Fetal and amniotic fluid concentrations were relatively lower than those observed in maternal organs (13.86 μg/g in the fetus and 12.35 μg/ml in the amniotic fluid).

Tanaka et al. (1973b) determined the Salicylic Acid concentration in maternal organs and in fetuses. Five gravid Wistar rats were given 150 mg/kg Salicylic Acid orally once daily on days 8 to 14 of gestation or given one dose on day 14 of gestation. The animals were killed 3 h after the final dose. After both single and multiple doses, the greatest concentration of Salicylic Acid was in the serum (246.56 and 221.28 μg/ml, respectively) and the lowest was in the brain (23.82 and 24.86 μg/g, respectively). All other examined organs, including the placenta, had similar concentrations (63.13 to 88.5 μg/g with a single dose; 68.57 to 85.62 μg/g with multiple doses) with the exception of the kidneys, which had relatively high concentrations (121.18 and 128.47 μg/g with single and multiple doses, respectively). Fetal and amniotic fluid concentrations were relatively lower than those observed in maternal organs (55.83 and 62.48 μg/g in the fetus after single and multiple doses, respectively, and 39.41 and 62.29 μg/ml in the amniotic fluid after single and multiple doses, respectively).

The effect of age and dose on the metabolism and distribution of Salicylic Acid was determined using male Fischer 344 rats (McMahon, Diliberto, and Birnbaum 1990). A single dose of 5, 50, or 500 mg/kg 7-^{14}C-Salicylic Acid (10 μCi/kg) in corn

oil/ethanol (4:1) was given to 3-, 12-, and 25-month-old animals. Urine and feces were collected for 96 h, after which time the animals were killed. All of the 3-month-old animals dosed with 500 mg/kg Salicylic Acid died and two of the 25-month-old animals were killed at 48 h due to the toxic effects.

Almost all the radioactivity was excreted in the urine. At 5 mg/kg, urinary excretion was complete by 24 h in 3- and 25-month-old animals and by 48 h in 12-month-old animals. At 50 mg/kg, urinary excretion was complete in all groups at 48 h; excretion was significantly decreased in 25-month-old animals compared to 3-month-old animals at 6, 12, and 24 h. Within each age group, an increase in the dose resulted in an increase in the time for urinary elimination. Fecal excretion of radioactivity at 50 mg/kg was significantly decreased in the 25-month-old animals compared to the other groups; no difference was observed at 5 mg/kg. The urinary metabolite profile was affected by both age and dose (McMahon, Diliberto, and Birnbaum 1990).

The permeability of Salicylic Acid through the oral mucosa of male golden hamsters was also investigated by Kurosaki et al. (1991). A thin film dosage form of Salicylic Acid was prepared by drying a viscous solution of Salicylic Acid in ethanol, isotonic buffer solution (pH 3.0), and polyethylene glycol to yield an apparent content of Salicylic Acid of 43.3 μmol/cm^2. Aluminum foil was used as a backing for this film and one piece of the film (8.5 μmol Salicylic Acid/piece) was placed on each of four oral mucosae: sublingual mucosa, dorsum of the tongue, ventral surface of the tongue, and cheek pouch mucosa.

The plasma C_{max} occurred at 45, 60, 120 and 180 min in the sublingual mucosa, ventral surface of the tongue, cheek pouch mucosa, and the dorsum of the tongue, respectively. The C_{max} of the sublingual mucosa (~11.5 nmol/ml) was approximately 4.5 times greater than that in the dorsum of the tongue (~2.5 nmol/ml). Absorption was greatest where the stratum corneum was the thinnest. The authors stated that the finding that the AUC and stratum corneum thickness, but not whole epithelium, are inversely proportional suggests that the stratum corneum is the principal barrier (Kurosaki et al. 1991).

Salicylic Acid—Human Data

Four male patients (49, 60, 63, and 77 years of age) were orally given Salicylic Acid as either the free acid in capsules or as the sodium salt in aqueous solution, but the concentration and total dose were not given (Alpen et al. 1951). Urine was collected for 24 to 36 h. The urinary pH ranged from 5.0 to 6.7 for three of the patients and was 8.5 for the fourth patient. The amount of free Salicylic Acid recovered was 10% to 85%, of salicyluric acid was 0% to 50%, of gentisic acid was $\leq$1%, and of glycuronate conjugates was 12% to 30% (−OH conjugate) and 0% to 10% (−COOH conjugate). The total amount recovered in the urine was 85% to 95% of the dose.

Six female subjects ingested a tablet containing 66 μmol Salicylic Acid (Janssen et al. 1996). The mean recovery of salicylate in the urine over a 24-h period was 80%.

The presence of Salicylic Acid in semen following oral administration of acetylsalicylic acid (aspirin) was determined using seven male subjects (Kershaw et al. 1987). Fasted subjects were given three 325 mg tablets of aspirin. The t_{max} in plasma was 2.5 h and C_{max} was 49 μg/ml, the k_a and k_{el} were 0.64 and 0.27/h, respectively, and the AUC was 357 h · μg/ml. Elimination of salicylate became log-linear between 6 and 9 h when the amount of salicylate in the body was 200 to 400 mg (aspirin equivalence). The harmonic mean terminal $t_{1/2}$ of salicylate was 2.6 h. Equilibration of salicylate between plasma and semen was rapid and independent of Salicylic Acid concentration. The mean concentration ratio (×100) of Salicylic Acid (semen/plasma) was 14.6. The ratio of the salicylate concentration in plasma and semen was independent of the salicylate concentration in the plasma.

Magnesium Salicylate—Animal Data

The bioavailability of Magnesium Salicylate was determined using four female beagle-type mongrel dogs (Alam, Gregoriades, and Imodi 1981). Using a 4 × 4 Latin square design with a 1-week washout period, the animals were dosed with 650 mg Magnesium Salicylate in two different tablet forms, one with a gelatin binder (tablet A) and one with a pregelatinized starch binder (tablet B), or as an aqueous solution (concentration not stated), and 325 mg of aspirin. The fasted animals were given 200 ml water 30 min prior to dosing and 25 ml water immediately following dosing. Blood samples were taken at various intervals at 0 to 12 h.

No significant differences were observed between administration of Magnesium Salicylate in tablet or solution form. For tablet A: tablet B, the solution, and aspirin, the following pharmacokinetic parameters were determined: C_{max}: 119 ± 7.9, 119 ± 8.9, 117 ± 14.2, and 117 ± 0.2 μg/ml, respectively; t_{max}: 1.6 ± 0.4, 1.2 ± 0.2, 1.0 ± 0.2, and 2.9 ± 0.7 h, respectively; k_a: 3.7 ± 2.1, 3.4 ± 0.9, 4.5 ± 0.8, and 1.2 ± 0.5 h^{-1}, respectively; $t_{1/2}$: 8.4 ± 1.1, 6.5 ± 0.8, 8.0 ± 1.1, and 5.4 ± 1.8 h, respectively. The k_{el}, AUC_{0-12}, $AUC_{0-\infty}$, and bioavailability were not different for any of the four (Alam, Gregoriades, and Imodi 1981).

Magnesium Salicylate—Human Data

Eighteen fasted male subjects were given a single oral dose of Magnesium Salicylate (equivalent to 481 mg Salicylic Acid) with 240 ml of water, and blood and urine were collected at various intervals for 16 and 24 h after dosing (Mason 1980). Plasma salicylate and urine salicylurate concentrations were determined. The greatest plasma salicylate concentration, 36.5 μg/ml, was observed 1.5 h after dosing. The AUC_{16} and AUC_{∞} were 223 and 225 μg · h/ml, respectively, and the apparent elimination $t_{1/2}$ was 2.01 h. The plasma C_{max} and the plasma t_{max} were 39.1 μg/ml and 1.44 h, respectively. The greatest urine salicylurate concentration, 393 mg, occurred during the 0 to 12-h time interval, and the percentage of the dose of salicylate excreted as salicylurate in 24 h was 68.4%.

Methyl Salicylate—Animal Data

Groups of 10 rats were dosed orally with Methyl Salicylate in 2% methylcellulose (equivalent to 500 mg/kg Salicylic Acid), and the amount of total salicylate in the plasma and in brain homogenate was determined (Davison, Zimmerman, and Smith 1961). After 20 min, 217 and 8 mg/L free salicylate were found in the plasma and brain, respectively. After 60 min, these values were 278 and 42 mg/L, respectively. Methyl Salicylate values were negligible.

Methyl Salicylate—Human Data

Six fasted human subjects, four males and two females, ingested 0.42 ml Methyl Salicylate, and blood samples were taken after 15 and 90 min to determine plasma salicylate values (Davison, Zimmerman, and Smith 1961). After 15 min, the mean Methyl and free salicylate values were 4.9 and 7.9 mg/L, respectively. After 90 min, these values were 2.8 and 10.5 mg/L, respectively.

The oral absorption, distribution, metabolism and excretion studies described above are summarized in Table 8b.

Sodium Salicylate—Animal Data

Groups of 10 rats were dosed orally on day 11 of gestation with Sodium Salicylate in 2% methylcellulose (equivalent to 500 mg/kg Salicylic Acid), and the amount of total salicylate in the plasma and in brain homogenate was determined (Davison, Zimmerman, and Smith 1961). After 20 min, 296 and 38 mg/L free salicylate were found in the plasma and brain, respectively. After 60 min, these values were 316 and 52 mg/L, respectively.

Gravid Wistar rats were dosed orally with 500 mg/kg Sodium Salicylate; one group of animals was pretreated with 510 mg/kg benzoic acid 2 h before dosing (Kimmel, Wilson, and Schumacher 1971). Urine was collected, and three animals per group were killed 3, 6, or 12 h after dosing, while one animal per group was killed 24 h after dosing. Maternal and fetal free salicylate were determined. Without benzoic acid pretreatment, the greatest concentration of free salicylate was seen 3 h after dosing, after which time the concentration declined. Without pretreatment, the 3-h salicylate concentration was approximately 450 μg/ml in maternal serum and 0.25 μg/mg in the fetus. Salicylate concentrations were similar in pretreated and nonpretreated animals at 3 h. However, with pretreatment, the salicylate concentration in both maternal serum and the fetus was greater at 6 and 12 h after dosing than it was at 3 h. The maximum salicylate concentration was seen at 6 h in maternal serum and 12 h in the fetus; these values were approximately 475 μg/ml and 0.26 μg/mg, respectively.

To study the pharmacokinetics and excretion of Sodium Salicylate, four New Zealand white rabbits were given a single oral dose of 44 mg/kg Sodium Salicylate, and blood samples and urine were collected at various intervals for 36 and 96 h, respectively (Short et al. 1991). Plasma protein binding of Salicylic Acid was determined by adding radioactive Salicylic Acid to plasma to give final concentrations of 5, 50, and 500 μg/ml. Salicylic Acid was rapidly excreted in the urine, with slightly more than 50% of the dose eliminated as Salicylic Acid; 4% of the dose was excreted as SUA. Trace concentrations of sulfate conjugates were detected, and oxidative metabolites were not detected. Total recovery was 79.0%. SUA was only detectable in the plasma 30 h after dosing. The $t_{1/2}$ was 6.0 h. Plasma protein binding was concentration dependent.

Sodium Salicylate—Human Data

A fasted male subject was given an oral dose of 579.7 mg Sodium Salicylate (equivalent to 500 mg Salicylic Acid), and urine was collected at various intervals (Farid et al. 1975). Within 96 h, a total of 12.7% of the dose was excreted as Salicylic Acid; 6.9% and 4.9% was excreted as Salicylic Acid 0 to 12 and 12 to 24 h after dosing, respectively.

The metabolism and excretion of Sodium Salicylate was determined using 44 male and 78 female black subjects (Emudianughe et al. 1986). The subjects were given an oral dose of 1 g Salicylic Acid as the sodium salt and urine was collected for 12 h. The mean total Salicylic Acid, free Salicylic Acid, salicyluric acid, and Salicylic Acid glucuronide excreted by all subjects was 52.43%, 6.62%, 14.41%, and 31.35%, respectively. For male subjects, these values were 60.59%, 10.43%, 6.53%, and 43.63%, respectively, and for female subjects they were 47.8%, 4.5%, 18.94%, and 24.35%, respectively. The salicyluric acid/Salicylic Acid glucuronide ratio was 0.64 for all subjects and 0.164 and 0.814 for male and females, respectively. Females excreted significantly more of the dose as salicyluric acid, whereas males excreted significantly more as Salicylic Acid glucuronide.

The influence of gender on the metabolism and excretion of Salicylic Acid was examined using seven male and seven female black Nigerian subjects (Emudianughe 1988). The subjects were given an oral dose of 1 g Salicylic Acid as the sodium salt, and urine was collected hourly for 12 h. A mean of 48.72% and 53.63% of the total dose was excreted in 12 h by male and female subjects, respectively. Males excreted significantly less of the dose as free Salicylic Acid and SUA and significantly more as SAAG compared to females; males and females excreted 2.83% and 6.13% of the dose as free Salicylic Acid, respectively, 5.1% and 25.52% as SUA, and 40.48% and 21.96% as SAAG, respectively. The hourly male-to-female ratio for free Salicylic Acid was 1.3 to 4.1 and the hourly female-to-male ratios for SUA and SAAG were 2.1 to 10.8 and 0.30 to 1.30, respectively. The researchers stated that the results "suggest a possible genetic influence on the control of salicylic acid metabolism."

The pharmacokinetics of Salicylic Acid (as Sodium Salicylate) were determined using five male subjects (Shen et al. 1991). Each subject was given 3 g Salicylic Acid as Sodium Salicylate in 400 ml water prior to eating; food was allowed 2 h later. Blood and urine were collected at various intervals for 72 and 90 h, respectively. The mean total recovery was 98% of the dose; 13%, 48%, 20%, 12%, and 3.9% was Salicylic Acid, SUA, salicyl phenolic glucuronide, salicyl acyl glucuronide, and gentisuric

acid, respectively. The lowest urinary pH values for individual subjects corresponded to the lowest unbound renal clearance.

To determine sex differences in absorption kinetics, six male and six female fasted subjects were given an oral dose of 9 mg/kg Sodium Salicylate in 200 ml water on 5 separate days; the days corresponded to days 2, 7, 14, 20, and 25 of the females' menstrual cycle (Miaskiewicz, Shively, and Vesell 1982). Blood samples were obtained at various times 0 min to 10 h after dosing. Five months after the last dose, the subjects were given a 2 min intravenous (IV) infusion of Sodium Salicylate equivalent to the oral dose.

Mean kinetic values were similar for oral and IV administration. With the exception of plasma t_{max}, kinetic values were similar for males and females; t_{max} was less in males than females, i.e., 24 to 34 min for males and 37 to 60 min for females. The mean plasma C_{max} ranged from 65.8 to 71.0 for males and 55.2 to 63.7 for females. Throughout the month, no significant difference in salicylate distribution was seen. The mean oral and IV AUC, apparent $t_{1/2}$, apparent volume of distribution (aVd), and clearance were 331 and 333 mg/L · h, 4.4 and 5.0 h, 0.17 and 0.18 L/kg, and 27.5 and 27.4 ml/kg · h for males, respectively, and 304 and 334 mg/L · h, 4.1 and 4.6 h, 0.18 and 0.18 L/kg, and 29.9 and 27.0 ml/kg · h for females, respectively. When the study was expanded to include 20 males and 20 females dosed orally, similar results were observed. Using 25 male and 25 female subjects in an equilibrium dialysis study, no sex differences in Sodium Salicylate plasma-binding were observed (Miaskiewicz, Shively, and Vesell 1982).

Abdallah, Mayersohn, and Conrad (1991) examined the effect of age on the pharmacokinetics of Sodium Salicylate. Twenty-two fasted male subjects, 30 to 85 years of age, were given an oral dose of 600 mg Sodium Salicylate (equivalent to 517.5 mg Salicylic Acid), and blood and urine samples were taken at various intervals for 24 and 48 h, respectively.

Creatinine clearance ranged from 58.8 to 168.8 ml/min and decreased significantly with age. Salicylic Acid was detected in the plasma within 10 to 30 min in all subjects; no measurable Salicylic Acid was detected in 14 subjects at 24 h. SUA concentrations rose and declined slowly; no measurable SUA was recovered at 24 h. Urinary recovery of the dose was 95% at 48 h; most of the dose (80%) was excreted as SUA, whereas only a mean of 5% of the dose was excreted as unchanged Salicylic Acid.

The C_{max} of Salicylic Acid ranged from 41.6 to 81.1 μg/ml; the aVd ranged from 7 to 14 L and increased significantly with age. Renal clearance was "low and highly variable" (1.4 ml/min), whereas oral clearance was 28.6 ml/min; neither appeared to correlate with age. The terminal rate constant of Salicylic Acid was 0.193/h and the terminal $t_{1/2}$ was 2.5 to 5.2 h. The SUA C_{max}, which ranged from 1.6 to 4.8 μg/ml, increased significantly with age. Renal clearance of SUA decreased significantly with age and had a positive correlation with creatinine clearance. The researchers concluded that the data suggested that age has a minor influence on the disposition of salicylate in male subjects (Abdallah, Mayersohn, and Conrad 1991).

Four male subjects were given 650 mg salicylate in the form of two tablets four times daily for 3 days (Porat-Soldin and Soldin 1992). Blood and semen samples were obtained approximately 6 h after the last dose. The serum salicylate concentrations ranged from 21 to 170 mg/L and the semen salicylate concentrations ranged from 3 to 33 mg/L. Salicylate significantly reduced sperm motility.

A fasted male subject was given a single oral dose of 1 g Salicylic Acid, as the sodium salt (Vree et al. 1994a, 1994b). In one experiment, the urine was kept acidic by administration of 1.2 g ammonium chloride four times a day and in a second experiment, the urine was kept alkaline by the administration of 3 g sodium bicarbonate four times a day.

When the urine was kept acidic, Salicylic Acid and its metabolites had a terminal $t_{1/2}$ of 3 h. Approximately 85% of the dose was excreted in the urine, predominantly as SUA (68.7%) and the glucuronides SAPG (4.9%), SAAG (6.0%), and SUA phenolic glucuronide (SUPG) (5.2%). Only 0.6% of the dose was excreted as unconjugated Salicylic Acid. Salicylic Acid had a renal clearance of 0.16 ml/min. When the urine was kept basic, the terminal $t_{1/2}$ was 2.6 h. Approximately 91% of the dose was excreted in the urine, again predominantly as SUA (58.3%). The amount of the dose excreted as unconjugated Salicylic Acid was 22.2%. The amount excreted as glucuronides was 4.7% as SAPG, 2.3% as SAAG, and 3.9% as SUPG. Salicylic Acid had a renal clearance of 9.0 ml/min (Vree et al. 1994a, 1994b).

The oral absorption, distribution, metabolism, and excretion studies described above are summarized in Table 8b.

Oral Mucosal Route of Administration

Salicylic Acid—Animal Data

Tanaka et al. (1980) extended their work to evaluate the role of absorption of Salicylic Acid through the mucous membrane of the oral cavity. The absorption of Salicylic Acid from a number of different vehicles into the oral mucous membrane of the cheek was determined using male golden hamsters. One gram of each ointment containing 2% Salicylic Acid was placed on the inside cheek of the animals using a syringe; swallowing was prevented. Blood was collected for up to 5 h. The amount of Salicylic Acid in the tissue of the cheek pouch and the residual Salicylic Acid was determined.

The Salicylic Acid blood concentration peaked after 30 min at approximately 70 μg/ml with the hydrophilic base (25% white petrolatum, 22% stearyl alcohol, 12% propylene glycol, and 1.5% sodium lauryl sulfate [SLS]) and after 1 h at approximately 100 μg/ml with the "absorption ointment" (40% white petrolatum, 18% cetyl alcohol, and 5% sorbitan oleate). It did not peak until 3 h with the PEG ointment (49% each PEG-8 and PEG-90) and the white petrolatum ointment, and the peaks were at approximately 35 and 20 μg/ml, respectively. The k_a values for the hydrophilic, absorption, PEG, and petrolatum ointments were 5.13, 2.92, 0.36, and 0.56^{-1}, respectively, and the k_{el} values were 0.36, 0.37, 0.30, and 0.33 h, respectively. The AUC

values were 182, 235, 145, and 70 for the hydrophilic, absorption, PEG, and petrolatum ointments, and the total absorption concentration/distribution volumes were 81, 102, 74, and 34, respectively.

A base containing 35% petrolatum, 10% cetyl alcohol, and 5% of hexadecyl alcohol, lanolin, and sorbitan oleate had the greatest AUC and total absorption concentration/distribution volume, 459 and 216, respectively. In the cheek pouch, the change in Salicylic Acid concentration was greatest in the absorption and hydrophilic bases; the loss of Salicylic Acid from the bases was greater than the total quantity of Salicylic Acid recovered in the blood. A "relatively high concentration" of Salicylic Acid was detected in the tissue of the cheek pouch (Tanaka et al. 1980).

Kurosaki et al. (1988) examined the effect of surfactants on the absorption of Salicylic Acid from the keratinized mucosa of the cheek pouch of male golden hamsters. Absorption was measured at pH 3.0, 4.0, and 7.0 alone and with SLS, cetylpyridinium chloride, polysorbate-80, and sodium taurocholate. At 1 h, absorption of Salicylic Acid alone was 49.8% at pH 3.0 and 0.2% at pH 7.0. At pH 7.0, 20 mM SLS significantly increased absorption of Salicylic Acid to 8%; no effect was seen at the lower pHs. Cetylpyridinium chloride and polysorbate-80 decreased absorption of Salicylic Acid at the lower pHs. Sodium taurocholate did not affect absorption. The degree of Salicylic Acid-surfactant interaction was determined; only polysorbate-80 and cetylpyridinium chloride interacted with Salicylic Acid, and the interaction was strongest with the latter.

The effect of pretreating the cheek pouch with the surfactants was examined. Absorption of Salicylic Acid was significantly increased with pretreatment with 20 mM SLS and cetylpyridinium chloride. At pH 7.0, absorption at 1 h was 1.4% without pretreatment, 1.9%, 8.5%, and 19.0% after pretreatment with 1.0, 5.0, and 20.0 mM SLS, respectively, and 2.8%, 6.2%, and 10.3% after pretreatment with 1.0, 5.0, and 20.0 mM cetylpyridinium chloride, respectively. The difference in absorption after pretreatment with the surfactants was significant at 20 mM (Kurosaki et al. 1988).

The oral mucosal absorption, distribution, metabolism, and excretion studies described above are summarized in Table 8c.

Parenteral Route of Administration

Salicylic Acid—Animal Data

Dogs were dosed intravenously with 1 g ^{14}C-Salicylic Acid (containing 10 μCi) in sodium bicarbonate solution (Alpen et al. 1951). Urine was collected for 30 to 36 h. Urinary metabolite recovery from one animal, which was representative of all the dosed animals, was 50% unchanged Salicylic Acid, 25% glycuronates, 10% salicyluric acid, and 4% to 5% gentisic acid. Total recovery was >90% of the dose.

Koshakji and Schulert (1973) demonstrated that Salicylic Acid can readily penetrate into fetal circulation. Four gravid Sprague-Dawley rats were given an SC injection of 300 mg/kg Sodium Salicylate (177.4 mg/ml) containing 10 μCi/ml carboxyl-^{14}C-Salicylic Acid, and the animals were killed 1 h later. The percent of injected ^{14}C dose/g dry weight of fetal tissue was 4.06.

The transport of Salicylic Acid across the blood-testis barrier of male Charles River rats was determined by continuous IV infusion and measurement of Salicylic Acid concentration in rete testis fluid (Okumura, Lee, and Dixon 1975). The transfer rate of Salicylic Acid from plasma to rete testis fluid was stated to be 0.0041/min. Permeability across the blood-testis barrier correlated with partition coefficient; Salicylic Acid has a pK_a of 3.0.

Four pregnant, near-term (>137 days gestation) Suffolk or Suffolk-Dorset ewes were dosed intravenously at time 0 and 180 min with a bolus of ^{14}C-Salicylic Acid (56 to 187 μCi) and ^{3}H-acetylsalicylic acid (99 to 173 μCi) (Thiessen et al. 1984). An infusion of 42 μg/kg/min non-radioactive acetylsalicylic acid was started at 60 min. Blood samples were taken at various intervals from 0 to 240 min. Thin-layer chromatography was used to determine plasma drug concentrations. Both Salicylic Acid and acetylsalicylic acid crossed the placental barrier, and equilibrium was reached approximately 40 min after salicylate administration. The average equilibrium plasma fetal/maternal ratio was 0.4. The mean clearance in the ewe was 358 ml/min for Salicylic Acid and 764 ml/min for acetylsalicylic acid.

Groups of 3- and 25-month-old male Fischer 344 rats were dosed intravenously with 5 or 50 mg/kg ^{14}C-Salicylic Acid (25 μCi/kg) in an Emulphor:ethanol:water (1:1:4) solution at a volume of 1 ml/kg (McMahon et al. 1990). In both groups, plasma salicylate concentrations ranged from 17 to 28 μg/ml and 100 to 120 μg/ml with doses of 5 and 50 mg/kg, respectively. The $t_{1/2}$ values in 3-month-old animals were 4.08 and 30.1 h with doses of 5 and 50 mg/kg, respectively; these values were 21.3 and 21.9 h, respectively, in 25-month-old animals. No Salicylic Acid metabolites were detected in the plasma.

A perfused rat liver study was performed to determine whether the liver was a major site of metabolism of Salicylic Acid (Shetty, Badr, and Melethil 1994). Hepatic metabolism of Salicylic Acid was negligible during a single pass through the liver. The addition of glycine, glucose, or bovine serum albumin to the perfusate did not affect hepatic uptake or metabolism.

Salicylic Acid—Human Data

Human subjects (number not stated) were dosed intravenously with ^{14}C-Salicylic Acid (Feldmann and Maibach 1970). After 4 h, 89.8% of the radioactivity was recovered in the urine.

Sodium Salicylate—Animal Data

A gravid rabbit was given a single SC dose of 1 g/kg and another rabbit was given a dose of 1.5 g/kg Sodium Salicylate, both on day 30 of gestation, and blood was taken and the uterus removed from both animals 2 h after dosing (Jackson 1948). In the animal dosed with 1 g/kg, the maternal serum salicylate concentration was 0.58 mg/ml and the pooled fetal serum concentration was 0.37 mg/ml. In the animal dosed with 1.5 g/kg,

the maternal serum salicylate concentration was 0.75 mg/ml and fetal serum concentrations ranged from 0.45 to 0.62 mg/ml.

The distribution of Sodium Salicylate was investigated in non-gravid A/Jax mice and gravid A/Jax and CBA mice (Eriksson and Larsson 1971). Groups of three to five animals were dosed intramuscularly with radioactive Sodium Salicylate at a volume of 0.1 ml/20 g body weight, which corresponded to 1 μCi Salicylic-1-^{14}C-Acid in 10 mg Sodium Salicylate. Gravid animals were dosed either on day 14 of gestation and killed 30 or 240 min after dosing or on day 17 of gestation and killed 30, 60, 120, 240, or 480 min after dosing. Nongravid animals were killed 30, 120, or 240 min after dosing.

The amount of radioactivity in the blood was greater in nongravid than gravid animals. On day 14 of gestation, the blood radioactivity concentrations varied within the same strain of mouse at each time period; additionally, the concentrations were generally greater in the CBA than A/Jax mice. In animals dosed on day 17 of gestation, blood radioactivity was variable but a strain difference was not seen. The decrease in radioactivity in the blood of these animals was greatest 4 to 8 h after dosing. In nongravid mice, blood radioactivity concentrations decline linearly between 20 and 240 min to approximately 60% of the earlier value. In both gravid and nongravid mice, the hepatic radioactivity concentrations were relatively unchanged with time. For animals dosed on days 14 and 17 of gestation, the fetal radioactivity concentrations were initially greater in CBA mice but then decreased to those of A/Jax mice. For mice dosed on day 14 of gestation, average A/Jax fetal radioactivity per litter was 31 to 41 and 33 to 46 cpm/mg at 30 and 240 min, respectively, and average CBA fetal radioactivity per litter was 48 to 59 and 35 to 48 cpm/mg after 30 and 240 min, respectively. For mice dosed on day 17 of gestation, average radioactivity per litter in normal A/Jax fetuses was 32 to 37, 35 to 44, 32 to 44, and 36 to 45 cpm/mg after 30, 60, 120, and 240 min, respectively, and in normal CBA fetuses was 40 to 64, 43 to 50, 38 to 50, 32 to 44, and 16 to 19 cpm/mg after 30, 60, 120, 240, and 480 min, respectively.

Hemorrhages were observed in three A/Jax and three CBA fetuses after 240 min and in three A/Jax and at least seven CBA fetuses after 480 min. Radioactivity was 37 to 53 and 25 to 28 cpm/mg in A/Jax fetuses 240 and 480 min after dosing, respectively, and 35 to 55 and 16 to 26 cpm/mg in CBA fetuses 240 and 480 min after dosing, respectively. At least 13 A/Jax fetuses and six CBA fetuses from dams killed 480 min after dosing were dead. The radioactivity in these fetuses was 20 to 26 and 21 to 32 cpm/mg, respectively.

These authors also pretreated gravid A/Jax mice with nonradioactive Sodium Salicylate at a dose of 3 mg/20 g body weight on days 15 and 16 of gestation, and then dosed them with the radioactive solution on day 17 of gestation. The animals were killed 30, 60, 120, or 240 min after the last dose. Pretreatment with Sodium Salicylate increased the variability of the radioactivity in the blood between animals of the same groups. Maternal blood, maternal hepatic, and neonatal radioactivity concentrations were similar to those seen in the animals that were not pretreated (Eriksson and Larsson 1971).

Rabbits were dosed intravenously with 5 ml of a 4 g ^{14}C-Sodium Salicylate (200 μCi) in 110 ml distilled water solution, and blood samples were taken at various times from 15 to 360 min after dosing (Schuppli et al. 1972). The approximate $t_{1/2}$ was 1.5 to 4 h.

Six rats or six ferrets per group were used to determine the concentration of Sodium Salicylate in blood following a single SC dose of 125 or 400 mg/kg (Gulamhusein et al. 1980). Blood samples were taken 1, 2, 3, and 24 h after dosing. After 1 h, the blood salicylate concentration was 54 and 54.4 mg% in rats and ferrets, respectively, dosed with 400 mg/kg and 30 and 28 mg%, in rats and ferrets, respectively, dosed with 125 mg/kg. These values gradually decreased at 2 and 3 h, and were similar to blank samples at 24 h.

The pharmacokinetics of Sodium Salicylate were determined in male and female Sprague-Dawley rats of various ages (Varma and Yue 1984). The animals were given IV injections of 62 μmol/kg Sodium Salicylate. Older animals tended to have higher plasma concentrations of Salicylic Acid. The pharmacokinetics are summarized in Table 7. Following dosing, Salicylic Acid was the only compound found in the serum of the test animals.

TABLE 7

Pharmacokinetics of Sodium Salicylate in male and female rats of different ages given IV injections (Varma and Yue 1984)

	$t_{1/2}$ (h)		aV_d (ml/kg)		Plasma clearance (ml/kg/h)		Plasma Salicylic Acid concentration at 6 h (nmol/ml)	
Age (weeks)	Male	Female	Male	Female	Male	Female	Male	Female
1	13.9	12.0	433*	391*	22.0	25.0	125–150	100–150
3	2.5*	2.7*	149	201	44.0*	54.0*	50–113	50–81
8–9	6.6	7.3*	213**	144	23.2**	13.8	144–209	250–306
14–15	7.1**	11.9	186**	150	18.6**	8.9	125–250	159–388
56–60	10.4	15.7	165	175	13.5**	7.9	188–269	206–356

*Value significantly different from all other values in same column.

**Value significantly different from corresponding female animals.

Urinary excretion of Salicylic Acid and SUA was similar for male and female animals. In pregnant rats, the volume of distribution was greater than in adult female rats, while the other parameters were similar.

Salicylate distribution was compared in gravid and nongravid female (control) Wistar ST rats following IV administration (Yoshikawa et al. 1984). The gravid animals were given a single dose of 10 mg/kg Sodium Salicylate containing 5 μCi/kg ^{14}C-Sodium Salicylate on day 20 of gestation, and the controls were given the same dose. Blood samples were obtained at 0.5 and 1 to 8 h after dosing, and the animals were killed after 8 h. Approximately 10 fetuses were obtained from gravid animals. Serum protein binding was determined using serum obtained after 8 h.

Serum salicylate concentrations were significantly decreased in gravid animals compared to controls, although serum $t_{1/2}$ values were similar; this was attributed to an approximately 40% increase in the distribution volume of gravid animals. An increase in total body serum clearance was also observed for gravid animals. The fetal serum salicylate concentration was not significantly different from the maternal concentration. The average serum salicylate concentrations in control, gravid, and fetal animals were 34.3, 21.7, and 22.5 μg/ml, respectively; the unbound fractions were 0.137, 0.667, and 0.018, respectively. The serum unbound salicylate fraction in both control and fetal animals increased with increasing concentrations of Sodium Salicylate, whereas the value in the gravid animals was constant. In gravid animals, the serum protein binding of salicylate was greatly decreased, but the binding of salicylate to serum was linear over a wide concentration range. The researchers also determined the tissue-to-serum partition coefficients (K_p) for a number of tissues; the K_p values were significantly greater in all tissues except the liver in gravid rats compared to controls. The fetal K_p values were significantly increased in all tissues except the lungs and kidneys compared to maternal values.

These authors also determined the blood-to-plasma concentration ratio of Sodium Salicylate using blood pooled from five gravid and five nongravid female Wistar rats. The pooled blood was incubated with 100 nCi/ml ^{14}C-Sodium Salicylate and 20 to 160 μg/ml nonradioactive Sodium Salicylate for 20 min. The ratios were "almost constant" in this concentration range, and the average calculated ratios were 0.74 and 0.60 in the gravid and control animals, respectively (Yoshikawa et al. 1984).

Groups of three to nine gravid Sprague-Dawley rats were given a single IV injection of 15, 50, 100, 200, or 500 mg/kg Sodium Salicylate on day 8 of gestation (Gabrielsson et al. 1985). Blood samples were taken at various intervals at 1 min to 30 h after dosing. After 1 min, a dose of 500 mg/kg Sodium Salicylate resulted in a peak plasma concentration of 1800 μg/ml with a "typical non-linear" pharmacokinetic behavior. Doses of 100 and 200 mg/kg Sodium Salicylate resulted in the same but less pronounced pattern, with peak plasma concentrations at 1 min of 600 and 900 μg/ml, respectively. After 1 min, doses of 15 and 50 mg/kg resulted in peak plasma concentrations of 100 and 300 μg/ml, respectively, with a linear pharmacokinetic behavior.

These authors also gave 14 or 12 gravid animals constant infusions of 1 or 2 mg/h Sodium Salicylate, respectively, on days 6 to 13 of gestation, and blood samples were taken on the days of dosing. In the animals given 1 mg/h, the plasma concentration peaked on days 6 and 8 of dosing at approximately 110 μg/ml, and were in the range of 50 to 110 μg/ml during dosing. In the animals given 2 mg/h, plasma concentrations peaked on day 8 of gestation at approximately 240 μg/ml, and were in the range of 150 to 240 μg/ml during dosing (Gabrielsson et al. 1985).

Groups of five gravid hooded Wistar rats were given a single IV dose of Sodium Salicylate (equivalent to 50 mg/kg Salicylic Acid) on day 20 of gestation, and a control group of five nongravid animals were dosed in the same manner (Dean, Penglis, and Stock 1989). Blood samples were taken at various intervals and urine was collected for 24 h following dosing. Absolute total body clearance of salicylate was similar between the groups, but the absolute aVd was significantly increased in gravid animals as compared to controls. Normalized values (adjusting for increased body weight) indicated a significant reduction in total body clearance, with only a slight increase in the aVd. The $t_{1/2}$ of salicylate was significantly increased in the gravid animals. Urinalysis indicated that gravid animals excreted less of the administered dose than controls. Also, the metabolic profile was changed in gravid animals. Salicylate serum protein binding was decreased in gravid animals compared to controls; in both groups, binding decreased "in an essentially parallel fashion" with increased salicylate.

Gravid Sprague-Dawley rats were given a constant-rate IV infusion of 150 mg/kg/day Sodium Salicylate (at an infusion rate of 10 μl/h) on days 6 to 13 of gestation (Bergman et al. 1990). Blood salicylate concentrations were 112 to 141 μg/ml, with a mean of 120 μg/ml, on days 7 to 13 of gestation.

To study the pharmacokinetics and excretion of Sodium Salicylate, four New Zealand white rabbits were given a single IV dose of 44 mg/kg Sodium Salicylate, and blood samples and urine were collected at various intervals for 36 and 96 h, respectively (Short et al. 1991). Plasma protein binding of Salicylic Acid was determined by adding radioactive Salicylic Acid to plasma to give final concentrations of 5, 50, and 500 μg/ml. Salicylic Acid was rapidly excreted in the urine, with slightly more than 50% of the dose eliminated as Salicylic Acid; 4% of the dose was excreted as SUA. The sulfate conjugates of Salicylic Acid and SUA accounted for 7.25% and 2.3% of the excreted dose, respectively; the oxidative metabolites were not detected. Total recovery was 85.8%. SUA was only detectable for 120 h after dosing. The $t_{1/2}$ was 4.3 h. Plasma protein binding was concentration dependent.

Sodium Salicylate—Human Data

Human subjects (number not specified) were dosed intravenously with 250 mg Sodium Salicylate (equivalent to 215.7 mg

Salicylic Acid), and the absorption rate was determined using urinary excretion (Wurster and Kramer 1961). Urine was collected every 2 h for 24 h. The total amount of salicylate recovered in the urine varied from 83.8% to 94.5% of the dose.

The parenteral absorption, distribution, metabolism, and excretion studies described above are summarized in Table 8d.

Influence of Vehicles/Additives on Absorption

The majority of these studies have been described previously.

Salicylic Acid

In vitro, penetration of Salicylic Acid through human leg and breast skin was greatest from a lanolin, Plastibase®, and Hydrophilic Plastibase® (Squibb) vehicle, moderate from a carbowax base, and minimal from petrolatum (Flesch, Satanove, and Brown 1955).

Absorption of Salicylic Acid from four vehicles, the oleaginous base petrolatum USP XV, the hydrophilic base petrolatum USP XV with water, the o/w base hydrophilic ointment USP XV, and the water-soluble base PEG ointment USP XV, was compared using rabbits (Stolar, Rossi, and Barr 1960). The greatest absorption was observed with the hydrophilic base; negligible absorption was seen with the PEG ointment.

In a study using rabbits in which DMSO was added to four vehicles containing Salicylic Acid, DMSO increased the dermal absorption of Salicylic Acid from hydrophilic ointment USP XVII and hydrophilic petrolatum USP XVII when compared to absorption of Salicylic Acid from these bases without the addition of DMSO (Stelzer, Colaizzi, and Wurdack 1969). It did not affect absorption of Salicylic Acid when added to PEG ointment or steareth-20 gel. The 10% Salicylic Acid was completely solubilized by the DMSO and surfactant added to the ointment. Marcus, Colaizzi, and Barry (1970) also examined the effect of DMSO on absorption using rabbits. DMSO increased blood salicylate concentration when compared to hydrophilic ointment USP XVII without DMSO.

Yankell (1972) reported that, using guinea pigs, absorption of Salicylic Acid was greater from an ethanol vehicle than an aqueous vehicle.

In the study performed by Washitake et al. (1975) using a recirculation apparatus to examine the percutaneous absorption of Salicylic Acid from four "oily" vehicles, the amount of Salicylic Acid absorbed decreased as the affinity of drug to vehicle increased.

The effect of nonionic surfactants on the percutaneous absorption of Salicylic Acid was also examined using rabbits (Shen, Santi, and Bruscato 1976). Salicylic Acid was completely solubilized by the DMSO and surfactants. Percutaneous absorption was significantly increased with the addition of sorbitan palmitate, sorbitan trioleate, poloxamer 182, poloxamer 231, laureth-4, oleth-2, or PEG-8 stearate to ointment containing Salicylic Acid and DMSO. Mixed surfactants of varying hydrophilic-lipophilic balance (HLB) values resulted in a prolonged percutaneous absorption effect.

Salicylic Acid, 2% in PEG or an emulsified ointment, was applied in a thin layer, 0.5 mm, to a 60-cm^2 area on the inner forearm of human subjects (Zecchi et al. 1978). The residual Salicylic Acid concentration was measured at various times for up to 4 h. The permeability coefficient was 0.0917 and 2.53 $cm \cdot s \cdot 10^6$ with the PEG and emulsified vehicles, respectively.

In a clinical study in which Salicylic Acid was applied in a PEG-8 solution or a hydrophilic ointment, minimal systemic absorption occurred with the PEG solution; this was attributed to "the formation of a glycol-salicylate complex resulting in a molecule too large to pass the stratum corneum" (Birmingham, Greene, and Rhodes 1979). With the ointment, Salicylic Acid was not found in the blood but was found in tape-stripped skin.

In a study in which Salicylic Acid was applied to the oral mucous membrane of the hamster cheek pouch, the base affected absorption (Tanaka et al. 1980). More Salicylic Acid was absorbed from "absorption" and hydrophilic bases as compared to PEG and petrolatum bases.

The effect of polar lipids on the transport of lipophilic molecules through the human epidermis was examined (Cooper 1984). The addition of small amounts of fatty acids of alcohols to a formulation can increase the transport of Salicylic Acid by an order of magnitude.

In a study using a membrane system, a greater percentage of the Salicylic Acid dose penetrated the membrane layers from an aqueous emulsion compared to a vaseline base (Neubert et al. 1990).

Bioavailability of Salicylic Acid was determined in a clinical study using a hydroalcoholic vehicle (63% water/35% ethanol) or a cream (80% water/18% cosmetic excipient mixture) (Davis et al. 1997). C_{max} and t_{max} were greater and faster, respectively, with the hydroalcoholic vehicle.

Ethylhexyl Salicylate

In a clinical study, a greater amount of Ethylhexyl (Octyl) Salicylate absorbed into skin using an o/w emulsion gel compared to using petrolatum jelly (Treffel and Gabard 1996).

Methyl Salicylate

Dermal absorption of Methyl Salicylate from three different vehicles was compared in a clinical study (Beutner et al. 1943). Menthol seemed to enhance absorption. Yano et al. (1991) found that the addition of menthol and camphor to Methyl Salicylate increased absorption.

Sodium Salicylate

Absorption of Sodium Salicylate from four vehicles, the oleaginous base petrolatum USP XV, the hydrophilic base petrolatum USP XV with water, the o/w base hydrophilic ointment USP XV, and the water-soluble base PEG ointment USP XV, was compared using rabbits (Stolar, Rossi, and Barr 1960). The greatest absorption was observed with the hydrophilic base; negligible absorption was seen with the PEG ointment.

In a study using rabbits in which DMSO was added to two vehicles containing 11.6% Sodium Salicylate, DMSO significantly

TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate

Number/ species	Exposure concentration	Application site	Absorption	Reference
		Salicylic Acid (SA)		
Landrace pig skin	0.25–2.0 mg/ml, pH 2.2–7.5	Ear skin	Penetration was proportional to the concentration at, pH 2.2, ranging from 0.1–1.4 mg/cm^2/24 h with 0.25–2 mg/ml; at 1 mg/ml, penetration rates increased as a function of pH	Loveday 1961
Hairless mouse skin	Undiluted in 6 vehicles	Not stated	Permeability coefficients were 21.2, 21.0, 11.2, 4.8, 2.1, and 7.9 cm/h with oleic acid, isopropyl myristate, 1-octantol, 1-propanol, propylene glycol, and formamide, respectively	Sloan et al. 1986
Female fuzzy rats	5 μg/cm^2 in acetone	Back skin	Most radioactivity was found in the receptor fluid; 12.2 and 7.7% of the dose was found in the fluid and skin, respectively; none of the absorbed SA was metabolized	Bronaugh et al. 1989, 1989–90
Intact hairless mouse skin	1%, pH 4.0	Diffusion cell	A zero-order penetration pattern was observed; approximately 14 μmol penetrated after 10 h	Higo et al. 1995
10 NZW rabbits/group	6% in an oleaginous, hydrophilic, *o/w*, or water-soluble base	7.5 g was applied to the back under an occlusive patch for 9 h	Absorption was greatest from the *o/w* ointment, with peak absorption of 11.0 mg% at 5 h; peak absorption from the hydrophilic and oleaginous base was 8.8 and 6.8 mg% at 6 and 4 h; absorption from the water-soluble base was negligible	Stolar et al. 1960
4 NZW rabbits/group	10% in hydrophilic ointment, hydrophilic petrolatum, PEG ointment, or steareth-20 gel	Applied under an occlusive patch to shaved dorsal skin for 8 h without and with DMSO	Blood salicylate concentration peaked earlier in all vehicles with DMSO	Stelzer et al. 1968
Guinea pigs	200, 400, and 1000 μg/ml at pH 3; 500 μg/ml at pH 2, 3, 4, 5, 7, 8, 9, 10	Applied to abdominal skin using a recirculation device	At pH 3.0, rate of absorption was independant of concentration (approximately 4%); at 500 μg/ml, absorption decreased from 6.1% at pH 2 to 0% at pH 5 and 7 then up to 15.5% at pH 10	Arita et al. 1970
4 NZW rabbits/group	10% in hydrophilic ointment at pH 2.97, 4.48, 6.8, 9.23, and 10.78	Applied under an occlusive patch to shaved dorsal skin for 7.5 h without and with DMSO; blood taken at 1.5-h intervals	Without DMSO, SA blood concentration increased at each time interval with pH 2.97–6.8, peaked at 6.0 h w/pH 9.23, and at 4.5 h with pH 10.78; with DMSO, SA blood concentration peaked at 6.0 h with pH 2.97 and 4.48, at 4.5 h with pH 6.8 and 9.23, and at 3.0 h with pH 10.78; overall, SA blood concentration was greater with DMSO	Marcus et al. 1970
Male SD rats	Solution with 5% ethanol, pH 2, 3, 6, or 8	The tails were immersed in solution	The total amount absorbed at pH 2 and 3 was 0.64 and 0.33 μg/mm^2/h; no SA was absorbed at pH 6 or 8	Siddiqi and Ritschel 1972

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
Guinea pigs	3% in water, 50% ethanol, or 75% ethanol	Application to the back for 1 h; animals killed and the skin removed and tape-stripped	Absorption was greatest from the 75% ethanol and least from water; most of the radioactivity was found in tape strips 1–5	Yankell 1972
3 guinea pigs	3% in 75% ethanol	Application to the lower back for 24 h	Most of the radioactivity was recovered in the feces and the treated back muscle	Yankell 1972
Damaged guinea pig skin	250–1000 μg/ml, pH 2–6	Applied to abdominal skin in vitro using a recirculation device	At 500 μg/ml, pH 3, absorption rate of 79.4%; rate of absorption was independent of concentration, but increased with fraction of un-ionized form	Washitake et al. 1973
	500 μg/ml, pH 3		SA reserved in the skin peaked at 0.5–1 h, independent of concentration (250–1000 μg/ml); varying the pH from 3–6 resulting in a lower and broader peak that took longer to reach	
	500 μg/ml, pH 3		SA found in the skin decreased rapidly with time	
8 rabbits	36.2 mmol/100 g	Patches of 5 g of salve applied for 6 h	5.5% and 11.08% of the dose was excreted after 24 and 48 h, respectively	Panse et al. 1974
Guinea pig	500 μg/ml in hexadecyl alcohol, oleic acid, or isopropyl myristate; 75–300 μg/ml in liquid paraffin	A recirculation device was attached to the shaved abdomen; skin was intact or tape stripped	Intact skin: 14.6%, 1.7%, 1.6%, and 1.5% of the SA was absorbed from liquid paraffin, isopropyl myristate, hexadecyl alcohol, and oleic acid; less SA was retained in damaged skin than intact skin; with damaged skin, the amount of SA retained in solution decreased over time; no saturation phenomenon from liquid paraffin	Washitake et al. 1975
		Adsorption determined in vitro	3.56, 2.26, 1.57, and 0.73 mg SA adsorbed from liquid paraffin, isopropyl myristate, hexadecyl alcohol, and oleic acid, respectively	
NZW rabbits	10% in petrolatum alone, with 10% DMSO, or 10% DMSO and 10% surfactant	Ointments applied under occlusive patch to shaved dorsal skin for 8 h; blood samples taken hourly	Greatest peak blood salicylate concentration seen with SA, DMSO, and either oleth-2, sorbitan laurate, or sorbitan trioleate, 12.5 mg% at 2, 3, and 2 h, respectively; SA in petrolatum without DMSO and surfactant only had a peak value of 3 mg% at 7 h	Shen et al. 1976
≥3 Female Wistar rats/group	1%, 5%, or 10% in a hydrophilic ointment	Ointments were applied to a shaved flank under an occlusive patch daily for 5 days or weekly for 4 weeks; treated skin excised, the appropriate ointment applied, and the sample placed in a diffusion cell	A single application of 1%, 5%, and 10% had a mean penetration flux of 0.014, 0.061, and 0.078 mg/cm^2/h; with repeated daily doses, a significant difference in flux was seen between doses; the flux increased with 1% until day 4, whereas with 5% and 10% it decreased after day 3; weekly penetration flux of 1% remained constant, whereas that of 5% and 10% decreased; difference between 5% and 10% was significant	Roberts and Horlock 1978

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
4 female Rhesus monkeys	4 mg/cm^2	Applied to the abdomen as both a single dose and daily for 14 days	Single dose: 59% SA cumulative absorption; multiple dose: 67% and 78% cumulative absorption after 1st and 8th dose	Bucks et al. 1990
Male Wistar rats	1 mM in 20 mM HEPES buffer (pH 7.4)	Using glass diffusion cells, absorption was measured without and with iontophoresis in the epidermis and the dermis	The absorption rate constant, clearance, and percent dose applied were 0.0028/min, 0.50 ml/h, and 22.7% with epidermal iontophoresis and 0.0032/min, 0.58 ml/h, and 34.3% with passive dermal absorption	Singh and Roberts 1993
Wistar rat skin	1 mM in 20 mM HEPES buffer (pH 7.4)	Measured through the dermis in vitro using diffusion cells	Permeability coefficient was 0.013 cm/h	Singh and Roberts 1993
Male Wistar rats	Not given	Applied to the exposed epidermis of the dorsum of anesthetized rats in a glass cell and epidermis removed postmortem	Dermal clearances were 0.58 and 0.10 ml/h for epidermis in live rats and postmortem epidermis, respectively	Singh and Roberts 1994
Human skin	5% in 5 vehicles	Leg and/or breast skin in vitro in a corium spot test	Penetration was greatest with lanolin, Plastibase, and Hydrophilic Plastibase; moderate from carbowax; and minimal from petrolatum	Flesch et al. 1955
Human skin—male	5%	Abdominal and leg skin samples	Dermal penetration steadily increased between 10–20 h; mean penetration through abdominal and leg stratum corneum and was 3.6 and 5.7 μM/cm^2/24 h, respectively	Elias et al. 1981
Human skin	10 mg in an aqueous emulsion	Applied to a 4-cm^2 area of excised skin; 20 tape strippings were removed	20.5% and 20.7% of the dose penetrated the horny layer; after 30 and 60 min; 12.7% and 10.9% remained in the emulsion; after 30 min, the greatest SA content was in tape strippings 1–5 (7–16 μg) and 5–10 (5–8 μg); the same trend was observed after 100 min	Neubert et al. 1990
Human skin	10 mg in an aqueous emulsion or vaseline	Applied to a 4-cm^2 area using a 3-membrane system	In vaseline: 9%, 6%, and 5% (30 min) and 10%, 9%, and 9.2% (60 min) of the dose penetrated membranes 1, 2, and 3; in water: 21.3%, 12.9%, and 8.4% (30 min) and 17.8%, 15.8%, and 14.9% (60 min) of the dose penetrated layers 1, 2, and 3	Neubert et al. 1990
Human and rodent skin	500 μg/ml in vitro (pH 2–4)	Applied to a 0.785-cm^2 area of skin in a Franz cell	At pH 4, SA penetrated in a zero-order fashion following a lag time; penetration at pH 2 was always greater than pH 4; age did not affect penetration through human breast skin	Harada et al. 1993

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
Human dermis	1 mM in 20 mM HEPES buffer (pH 7.4)	Applied to excised midabdominal dermis in a diffusion cell	Dermal permeability coefficient was 0.017 cm/h	Singh and Roberts 1993
Human epidermis	Concentration not given; pH 7.4 (100% ionization) and at pH of 50% ionization	Applied to excised midabdominal epidermis at full and 50% ionization	Permeability coefficients were 0.000331 and 0.0152 cm/h at 100% and 50% ionization	Singh and Roberts 1994
17 humans	4 μg/cm^2	Open application to the ventral forearm	Total absorption was 22.78% over 5 days; the greatest absorption rate, 0.535%/h, was observed at 12–24 h	Feldmann and Maibach 1970
21 humans with dermatoses	Therapeutic levels	Applications were made to large areas of the body	Average plasma SA concentration was 5.4 mg%	Schuppli et al. 1972
10 male humans	9.10 mg/kg	0.5 g of salve applied to the trunk and extremities	Mean urinary excretion was 0.417%, 0.572%, and 1.060% after 12, 24, and 48 h, respectively	Panse et al. 1974
4 humans with active psoriasis	6% in 60% propylene glycol/19.4% alcohol gel	Occluded application to the entire body below the neck for 10 h; repeated for 5 days	Absorption ranged from 63%–82%; dose was excreted in the urine as 41%–65% SUA, 32%–57% acyl and phenolic glucuronides of SA, and 0%–14% SA	Taylor and Halprin 1975
2 humans/ group	3% in 40% PEG-8	Forearm was immersed for 3 h	Minimal systemic absorption; keratolysis was observed within 24 h after exposure	Birmingham et al. 1979
4 humans	10% in hydrophilic ointment	Forearm site was occluded for 3 h; in 2 subjects, the area was tape-stripped prior to application	Intact skin—SA was not detected in the blood; stripped skin—appreciable SA absorption; peak concentration—8 mg/dl; k_a—0.189/h; k_{el}—0.201/h; $t_{1/2}$—3.45 h	
15 humans	0.33 g/L	20-min bath	Mean plasma SA concentrations from 9.5–10.80 ng/ml over 24 h; 0.086 and 0.078 mg SA excreted, and 0.92 and 0.72 mg SUA excreted, in the urine at 0–24 and 24–48 h, respectively	Pratzel et al. 1990
6 humans	3% with 0.1% DFV	20 g twice daily for 22 h for 8 days to the trunk, upper arms, and thighs	Plasma SA concentration increased during the day from 2–3 to 4–7 μg/ml; AUC was 30 μg·day/ml	Täuber et al. 1993
9–10 human females with normal skin 9 with acnegic skin	2% in hydroalcoholic vehicle; 2% in cream	1.25–1.5 g to the face and neck for 16 days	Steady-state reached by day 7; peak plasma concentration reached earlier and AUC was greater with the hydroalcoholic vehicle; terminal $t_{1/2}$ was not affected by skin type or vehicle	Davis et al. 1997
6 humans	39.7 μg/cm^2 in ethanol	Open application to the ventral forearm for 24 h	Mean 7-day urinary excretion of SA 5.8%; 53.4% recovered in the wash and 0.22% recovered in tape strippings	Wester et al. 1998

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
9 male and 7 female humans	5% w/v SA solution	Microdialysis used; 5 ml added to the chambers using normal and damage skin	Absorption significantly increased in damaged skin compared to normal skin	Benfeldt et al. 1999
		Sodium Salicylate (SS)		
10 NZW rabbits/group	6.95% in an oleaginous, hydrophilic, o/w, and water-soluble base	7.5 g applied to the back under an occlusive patch for 9 h	Absorption greatest from the o/w ointment, with peak absorption of 4.6 mg% at 5 h; absorption from the oleaginous and hydrophilic bases were 1.0 and 0.4 mg% at 6 and 5 h; negligible absorption from the water-soluble base	Stolar et al. 1960
4 NZW rabbits/group	11.6% from hydrophilic and hydrophilic petrolatum bases	Application under an occlusive patch to shaved dorsal skin for 8 h ± DMSO	Blood salicylate concentration peaked at 8 h with all ointments; values were: 4.03 mg% from the both bases without DMSO and 1.38 mg% from both bases with DMSO	Stelzer et al. 1968
NZW rabbits	SS alone, with DMSO, or with DMSO and surfactants at 1, 2.75, 3.5 mg%	Application under an occlusive patch to shaved dorsal skin for 8 h; blood samples were taken hourly	Greatest blood salicylate concentration was seen with SS, DMSO, and poloxamer 182, 3.5 mg% at 4 h, followed by SS, DMSO, and either poloxamer 231 or oleth-2, 2.75 mg% at 3 or 5 h, respectively; least penetration was seen with SS and DMSO, 1 mg% at 8 h	Shen et al. 1976
Guinea pigs	In water; equivalent to 3% SA	Measured lateral diffusion	Lateral diffusion did not occur; <2% of the applied dose was found in sites adjacent to the test site	Yankell 1972
Human skin	5% in 5 vehicles	Leg and/or breast skin	No penetration was observed with petrolatum, carbowax, lanolin, Plastibase, or hydrophilic Plastibase vehicle after 24 h	Flesch et al. 1955
Human skin	In an aqueous emulsion	Horny layer of excised skin and a 3-layer membrane system	19.0% and 23.2% of the SS penetrated the horny layer after 30 and 60 min; 26.6% and 24.1% remained in the emulsion; after 30 min, the greatest SS was in tape strippings 1–5 (5–27 μg); with the membrane system, 20.3%, 6.6%, and 3.1% and 26.0%, 9.5%, and 5.5% of the dose penetrated into layers 1, 2, and 3 after 30 min and 60 min	Neubert et al. 1990
		TEA-Salicylate		
8 rabbits	36.2 mmol/100 g	Patches of 5 g salve applied for 6 h	4.01% and 14.59% of the dose excreted in the urine after 24 and 48 h	Panse et al. 1974
5 male Beagle dogs	10 g	Massaged into the shaved right knee	After 60 min, salicylate concentration in the application site, synovium, synovial fluid, blood, and urine were 321.2, 0.74, 0.80, 0.22, and 0.16 μg/ml	Rabinowitz et al. 1982

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
6 female Yorkshire swine	10%	Application of 1.5 g to a shaved 100-cm^2 area of the biceps femoris	After 2 h, ≥82% of the dose was absorbed, 7.9% remained on the skin, and 9.3% remained in skin tissue; 150.9–724.5 ng/g and 313.5–582.3 ng/g were recovered in the treated muscle after 30 min and 2 h	Baldwin et al. 1984
5 male and 10 female Beagle dogs	0.1, 1, 5, or 10 g of 10% ^{3}H-TEA-Salicylate to make 10 g cream	10 g massaged to a 100-cm^2 area of a shaved knee, measured at 60 min	At 10%, salicylate concentration in the application site, synovial fluid, and serum were 50.9, 0.54, and 0.004 μmol/g; proportionately less at the lower amounts; 23.7, 0.0039, and 0.001 μmol/g ^{3}H-TEA and 34.4, 0.061, and 0.002 μmol/g salicylate in the application site, synovial fluid, and serum	Rabinowitz and Baker 1984
6 arthritic human males	10 g cream	Massaged into a 25–30-cm^2 area over one knee	Synovial fluid, blood, and urine salicylate concentration were 0.16, 0.03, and 0.02 μg/ml after 1 h and 0.25, 0.08, and 0.18 μg/ml after 2 h	Rabinowitz et al. 1982
10 arthritic human males	10% cream	Massaged in for 60 min	Synovial fluid, blood, and urine salicylate concentration were 0.0011, 0.0002, and 0.0001 μmol/g for 6 patients not on bedrest and 0.0014, 0.0011, and 0.0020 μmol/g for 4 bedrest patients	Rabinowitz and Baker 1984
Humans/ 6 per sex	10%	2–5-g applications to a 10-cm^2 area of the anterior thigh	No unchanged TEA-Salicylate or SA in the serum; 1.8 and 9.1 mg unchanged SA and SUA in the urine; total SA recovered was 6.9 mg; 1.4% recovered in the urine	Morra et al. 1996
		Methyl Salicylate (MS)		
Landrace pig skin	0.1–0.75 mg/ml	Absorption measured using ear skin	Penetration rate was approximately 0.125–0.6 mg/cm^2/h for 0.1–0.75 mg/ml	Loveday 1961
Hairless mouse skin	5.2 mg in 2 cm^2 plaster	Plasters with the ingredient ± menthol and camphor	Linear hydrolysis of MS to SA; SA formation inhibited by menthol and camphor in a dose-dependent manner	Yano et al. 1991
Male and female guinea pig skin	5 μg/cm^2	Applied to viable and nonviable skin using flow-through diffusion cells	No significant difference in absorption through viable and nonviable skin; at 24 h, 55% and 56% and 47% and 50% of applied dose absorbed was through male and female viable and nonviable skin; metabolism was different in male and female and viable and nonviable skin	Boehnlein et al. 1994
Hairless mouse skin	1%, pH 4.0	0.95 cm^2 exposed using a glass flow-through diffusion cell system	Penetration flux decreased after 4 h; 17 μmol SA penetrated after 10 h; a lower concentration resulted in a lower flux and more metabolized MS	Higo et al. 1995

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
Male Wistar rats	10%–28.3%	Application was made for 2 h to 9.625 cm^2 of depilated abdominal skin	MS was primarily converted to SA during transport through the skin; plasma MS SA ranged from 25–50 and 200–325 $\mu g/g$	Megwa et al. 1995
Hairless mice	8.47 μCi (3.54 mg)	Applied to back	High levels of radioactivity found at the test site 1 h after application, peaking at 4 h; slight radioactivity seen at adjacent sites	Maruta et al. 1977
Female hairless HRS/J (hr) mice	5.2 mg in 2 × 2-cm plaster	Plaster sheet applied to dorsal skin ± menthol or camphor for 1, 3, or 6 h	1 h dermal concentration of MS and SA of 0.64 and 0.49 μmol/g, and the 6 h concentration of 0.29 and 0.22 μmol/g, respectively; menthol and camphor increased the 1 h values to 1.79 and 0.39 μmol/g	Yano et al. 1991
Male SD rats	Solution with 5% ethanol, pH 2, 3, 6, or 8	Tails immersed in solution	Total amount absorbed at pH 2, 3, 6, and 8 was 1.56, 0.76, 1.77, and 1.57 $\mu g/mm^2/h$	Siddiqi and Ritschel 1972
5–22 human males	20% in 80% anhydrous lanolin (1), 60% anhydrous lanolin and 20% menthol (2), or 60% of special aqueous base (3)	10 g was rubbed in to the skin of the chest, abdomen, and thigh	Mean salicylate excretion of ointments 1, 2, and 3 was 41.6, 55.1, and 47.5 mg; in 8 subjects with better cutaneous absorption than average (dark-complexioned subjects), 64.6, 101.3, and 103.1mg salicylate was excreted with ointment 1, 2, and 3	Beutner et al. 1943
Human	Hydrous—6 ml and 3 ml water Anhydrous—6 ml	Application to the forearm under a 1 × 5 × 10 cm plastic cell for 16 h; test article was applied with 5 × 10 cm sponge	Urinary excretion was 8.6 and 2.7 mol/100 cm^2/h with hydrous and anhydrous exposure; steady-state was reached at approximately 6 h	Wurster and Kramer 1961
		Application for 2 h to defatted and nondefatted skin on 100 cm^2 of forearm	Defatting of skin decreased total salicylate absorption by 27%	
6 humans/ group	35.0 mg/sheet	Single or 6 12-h applications of 10 sheets	Single application: serum free SA peaked at 8 h after dose initiation; greatest total SA concentration occured at 12 h; repeated applications: no free SA found 12 h after each application	Maruta et al. 1977
5 humans	12%–50%	Application of 5 g under occlusive patch for 10 h to a 50-cm^2 area of the forearm; a small portion was rubbed in and the rest was spread	Skin permeability coefficients ranged from 1.0–1.9 cm/h; the amount of salicylate absorbed from each product ranged from 12%–20%, and the estimated steady-state salicylate concentration ranged from 2.5–7.6 mg/l	Roberts et al. 1982

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TABLE 8a

Summary of dermal absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Sodium Salicylate, TEA-salicylate, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
4 humans	25%	Application also made to the abdomen, instep, heel, and plantar region	Cumulative urinary recovery was greatest from application to the abdomen, followed by the forearm, instep, heel, then plantar region	
2 male and 4 female humans	1% MS, with 5% each ethyl and 2-hydroxyethyl salicylate	Application as a metered aerosol to forearms	MS was absorbed faster than ethyl salicylate, but the blood concentration of ethyl salicylate was greater; MS in plasma peaked at 20 min	Collins et al. 1984
6 human males	5 g	Application was made to the back and chest and subjects were exposed to heat, exercise, or both for 6 h	Exercise and/or heat increased plasma total salicylate concentration and urinary SUA; plasma salicylate concentration peaked at 2 h under all conditions	Danon et al. 1986
10 humans	0.03 g/L	20-min bath	Mean plasma salicylate concentrations were 452.6 and 116.6 ng/ml after 1 and 6 h; 5.08, 0.71, and 0.97 mg SUA excreted at 0–12, 12–24, and 24–48 h	Pratzel et al. 1990
Humans/6 per sex	12.5%	5 g applied under a nonocclusive patch twice daily for 4 days to 10-cm^2 area of the anterior thigh	No unchanged MS was detected in serum or urine; serum SA ranged from 0.3–0.9 and 2–6 mg/L at 1 h and on day 4 and urinary maximum SA and SUA concentration were 15.6 and 491.9 mg/L; SA recovered on days 1, 2, 3, and 4 was 15.5%, 22.0%, 22.4%, and 22.2%	Morra et al. 1996
		Ethylhexyl (Octyl) Salicylate		
4 female human subjects	3% in *o/w* emulsion gel or petroleum jelly	Application made to a 100-cm^2 area of the back; sites wiped after 30 min and 2 and 6 h—15 tape strippings	Maximum concentration reached after 30 min; approximately 37% of Ethylhexyl (Octyl) Salicylate from the *o/w* gel and 10% from petrolatum jelly found in tape strips 1–5; before wiping, SPF values were 14.2 and 5.4 for the gel and the jelly; values decreased by a factor of 2.2 after wiping	Treffel and Gabard 1996
		Using a Franz cell, 2.26 and 2.52 mg/cm^2 in gel and jelly applied to a 1.76-cm^2 area for 2 or 30 min or 2 or 6 h	0.94%, 2.13%, 1.54%, and 7.29% of the dose from the gel and 1.81%, 0.60%, 1.97%, and 1.96% of the dose from the jelly was recovered after 2 min, 30 min, 2 h, and 6 h; no to very little was recovered in the dermis	

NZW, New Zealand white; SD, Sprague-Dawley.

TABLE 8b

Summary of oral absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Magnesium Salicylate, Sodium Salicylate, and Methyl Salicylate

Number/ species	Exposure concentration	Application site	Absorption	Reference
		Salicylic Acid (SA)		
5 Gravid Wistar rats	0.2%	Treated diet on days 8–14 on day of gestation	Greatest SA concentration was in the serum (115.96 μg/ml) and lowest in the brain (4.14 μg/g); fetal and amniotic concentration were 13.86 μg/g and 12.35 μg/ml	Tanaka et al. 1973a
5 Gravid Wistar rats	150 mg/kg	Dosed daily on days 8–14 or given one dose on day 14 of gestation and killed 3 h after final dose	After single and multiple doses, the greatest SA concentration was in the serum (246.56 and 221.28 μg/ml) and the lowest was in the brain (23.82 and 24.86 μg/g); after single and multiple doses, fetal concentration were 55.83 and 62.48 μg/g and amniotic fluid concentration were 39.41 and 62.29 μg/ml	Tanaka et al. 1973b
Male Fischer 344 rats	5, 50, or 500 mg/kg in corn oil/ ethanol (4:1)	3, 12, and 25 months old animals were given a single dose and killed after 96 h	All 3-months old animals died and two 25-months old animals were killed due to toxic effects of 500 mg/kg SA; almost all radioactivity was excreted in the urine; excretion was complete by 24 h in 3 and 25 mons and by 48 h in 12 months old animals in animals given 5 mg/kg and by 48 h in all animals given 50 mg/kg; the urinary metabolite profile was affected by age and dose	McMahon et al. 1990
4 Male humans		Given orally as the free acid or sodium salt	Urinary pH ranged from 5.0–8.5; amount of free SA, salicyluric acid, gentisic acid, and –OH and –COOH glycuronate conjugates recovered was 10%–85%, 0%–50%, $\leq$1%, and 12%–30% and 0%–10%; total recovery was 85%–95%	Alpen et al. 1951
6 Female humans	66 μmol	Ingested tablet	Mean urinary recovery was 80% in 24 h	Janssen et al. 1996
7 Male subjects	325 mg aspirin/ tablet	3 Tablets were ingested	Salicylate elimination became log-linear between 6–9 h; mean concentration ratio of SA (semen/plasma) was 14.6	Kershaw et al. 1987
		Magnesium Salicylate		
4 Female beagle-type mongrel dogs	In 2 tablet forms or as a solution	Animals were given 200 and 25 ml of water 30 min prior to and immediately following dosing	No difference between administration in tablet versus solution; ghe, C_{max} and t_{max} were 117–119 μg/ml and 1.0–1.6 h; bioavailability was 101 and 86% with tablets and 100% with solution	Alam et al. 1981
18 Human males	481 mg SA equivalent	Single oral dose given with 240 ml water	Greatest plasma SA concentration was seen after 1.5 h (36.5 μg/ml) and the greatest urine salicylurate concentration occurred at 0–12 h (393 mg); in 24 h, 68.4% of the dose was excreted as salicylurate; plasma C_{max} and t_{max} were 1.44 μg/ml and 1.44 h	Mason 1980

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TABLE 8b

Summary of oral absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Magnesium Salicylate, Sodium Salicylate, and Methyl Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
		Sodium Salicylate		
10 Gravid rats	500 mg/kg SA equivalence	Given on day 11 of gestation in 2% methylcellullose	After 20 and 60 min, 296 and 316 mg/L free salicylate was found in the plasma and 38 and 52 mg/L were found in the brain	Davison et al. 1961
Gravid Wistar rats	500 mg/kg	Some animals were pretreated with benzoic acid; animals were killed 3, 6, 12, or 24 h after dosing	Without pretreatment, the greatest free salicylate concentration was seen after 3 h; the 3 h salicylate concentration was 450 and 0.25 μg/ml in maternal serum and the fetus; with pre-treatment, the maximum, concentration in maternal serum was 475 μg/ml at 6 h and in the fetus was 0.26 μg/mg at 6 h	Kimmel et al. 1971
4 NZW rabbits	44 mg/kg	Single dose	SA was rapidly excreted in urine; more than 50% of the dose was SA and 4% was SUA; total recovery was 79%	Short et al. 1991
Human male	579.7 mg	Single dose	12.7% of the dose was excreted as SA within 96 h	Farid et al. 1975
44 male and 78 female black humans	1 g	Single oral dose	Males excreted more or the dose as SA glucuronide while females excreted more salicyluric acid	Emudianughe et al. 1986
Black Nigerian humans/7 per sex	1 g SA as SS	Single dose	Males and females excreted 48.72% and 53.63% of the total dose in 12 h and excreted 2.83% and 6.13% as free SA; compared to females, males excreted sig. less of the dose as free SA and SUA and significantly more as salicylic acid acyl glucuronide	Emudianughe 1988
5 human males	3 g SA as SS	Single dose in 400 ml water	Mean total recovery was 98% of the dose; 13%, 48%, 20%, 12%, and 3.9% was SA, SUA, salicyl phenolic glucuronide, and gentisuric acid	Shen et al. 1991
Humans/6 per sex	9 mg/kg	In 200 ml water on days corresponding to days 2, 7, 14, 20, and 25 of the menstrual cycle and a 2-min IV infusion 5 months after the last dose	Mean kinetic values were similar with oral and IV dosing; with exception of t_{max}, kinetic values were similar for males and females; t_{max} was 24–34 min for males and 37–60 min for females; plasma C_{max} was 65.8–71.0 for males and 55.2–63.7 for females; no significant difference in salicylate distribution was seen on the different days	Miaskiewicz et al. 1982
22 Human males, ages 30–85	600 mg	Single dose	Age had a minor influence on salicylate disposition; SA was detected in the plasma of all subjects at 10–30 min; none was detected in 14 subjects at 24 h; urinary recovery of the total dose was 95% at 48 h, with 80% of the dose excreted as SUA and only 5% as unchanged SA; SA and SUA C_{max} were 41.6–81.1 and 1.6–4.8 μg/ml; creatinine clearance was 58.8–168.8 ml/min and significant decrease with age	Abdallah et al. 1991

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TABLE 8b

Summary of oral absorption, distribution, metabolism, and excretion studies for Salicylic Acid, Magnesium Salicylate, Sodium Salicylate, and Methyl Salicylate *(Continued)*

Number/ species	Exposure concentration	Application site	Absorption	Reference
4 human males	650 mg	Given in 2 tablets 4×/day for 3 days	Serum and semen salicylate concentration were 21–170 and 3–33 mg/L 6 h after the last dose	Porat-Soldin and Soldin 1992
Human male	1 g SA as SS	Once with urine acidic	SA had a renal clearance of 0.16 ml/min and SA and its metabolites had terminal $t_{1/2}$ of 3 h; approximately 85% of the dose was excreted, primarily as salicyluric acid (68.7%); only 0.6% was unchanged SA	Vree et al. 1994a, 1994b
		Once with urine alkaline	SA had a renal clearance of 9.0 ml/min and a terminal $t_{1/2}$ of 2.6 h; approximately 91% of the dose was excreted, primarily as salicyluric acid (58.3%); 22.2% was unchanged SA	
		Methyl Salicylate		
10 rats	500 mg/kg SA equivalence	In 2% methylcellulose	After 20 and 60 min, 217 ad 278 mg/L free salicylate was found in the plasma and 8 and 42 mg/L were found in the brain	Davison et al. 1961
4 male and 2 female humans	0.42 ml	In 2% methylcellulose	Mean Methyl and free salicylate values were 4.9 and 7.9 mg/L after 15 min and 2.8 and 10.5 mg/L after 90 min	Davison et al. 1961

NZW, New Zealand white.

decreased the dermal absorption of Sodium Salicylate from hydrophilic ointment USP XVII when compared to absorption of Sodium Salicylate from the ointment without the addition of DMSO (Stelzer, Calaizzi, and Wurdack 1969). It did not affect absorption of Sodium Salicylate when added to hydrophilic petrolatum.

The effect of nonionic surfactants on the percutaneous absorption of 11.6% Sodium Salicylate was examined using New Zealand white rabbits (Shen, Santi, and Bruscato 1976). Approximately one third of the 11.6% Sodium Salicylate was solubilized by the DMSO and surfactant added to the ointment. Percutaneous absorption was significantly increased with the addition of sorbitan laurate, sorbitan palmitate, or poloxamer 182 to the ointment containing Sodium Salicylate and DMSO. Mixed surfactants of varying HLB values resulted in a prolonged percutaneous absorption effect.

TEA-Salicylate

The in vitro release of TEA-Salicylate from a hydrophilic and a "water-washable" base and from two commercial products was determined using a Franz diffusion cell assembly with 176 mm^2 surface area (Babar, Chickhale, and Plakogiannis 1991). The effect of 5% to 15% ethanol, propylene glycol, PEG-400, or DMSO, of 1% to 3% polysorbate-80, and of 2% to 5% urea on the release from each base was also determined. The ointments made with the hydrophilic and water-washable bases each contained 10% TEA-Salicylate. After 2.5 h, 7.4% and 12.6% TEA-Salicylate was released from the commercial products, and 13.3% and 15.3% was released from the hydrophilic and water-washable bases, respectively.

The greatest release from the hydrophilic base, 35.1%, was observed with the addition of 10% ethanol. With the addition of 5% DMSO, only 8.2% TEA-Salicylate was released in 2.5 h. The greatest release with the water-washable base, 20.3%, occurred with the addition of 3% polysorbate-80. With the addition of 10% ethanol, only 7.9% was released. With the hydrophilic base, the greatest first-order release-rate constant (Kr), diffusion coefficient (D), and permeability coefficient (P), and the lowest K_p, were observed with the addition of 10% ethanol. The lowest Kr, D, and P and the greatest K_p was observed, when compared to the hydrophilic ointment plus additives, with the commercial formulation. With the water-washable base, the greatest Kr was observed with the addition of 15% PEG-400 and the greatest D and P and the lowest K_p were observed with polysorbate-80. The lowest Kr, D, and P and the greatest K_p were seen with the addition of 15% ethanol to the water-washable ointment.

TABLE 8c

Summary of oral mucosal absorption, distribution, metabolism, and excretion studies for Salicylic Acid

Number/ species	Exposure concentration	Application site	Absorption	Reference
Male golden hamsters	2% in various vehicles	Ointment was placed on the inside cheek	Blood SA concentration peaked at 70 μg/ml after 30 min with a hydrophilic base, at 100 μg/ml after 1 h with an absorption ointment, at 35 μg/ml after 3 h with PEG ointment, and at 20 μg/ml after 3 h with white petrolatum	Tanaka et al. 1980
Male golden hamsters	PH 3, 4, and 7, ± pretreatment with sodium lauryl sulfate and other surfactants	Keratinized mucosa of the cheek pouch	After 1 h, absorption of SA alone was 49.8% and 0.2% at pH 3 and 7; sodium lauryl sulfate did not affect absorption at lower pH, but at pH 7, it significantly increased absorption of SA to 8%; cetylpyridinium chloride and polysorbate-80 decreased SA absorption at the lower pHs, whereas sodium taurocholate did not affect absorption	Kurosaki et al. 1988
Male golden hamsters	15 μmol/0.5 ml/kg, pH 3	Application was made to the oral mucosa using a cell system	Plasma C_{max} occurred at 45, 60, 120, and 180 min in the sublingual mucosa, ventral surface of the tongue, cheek pouch mucosa, and dorsum of the tongue; C_{max} of the sublingual mucosa was approximately 4.5 × greater than that in the dorsum of the tongue	Kurosaki et al. 1991

Penetration Enhancement

Salicylic Acid

In an in vitro study, a negative corium spot test was obtained with vitamin A alone after 24 h (Flesch, Satanove, and Brown 1955). However, with the addition of 10% Salicylic Acid, the test was positive after 3 h of incubation.

The penetration of 0.1% tritiated triamcinolone acetonide from 60% ethanolic solution alone and with 10% Salicylic Acid was compared in vitro using sheets of human epidermis obtained from abdominal skin (Polano and Ponec 1976). The penetration of 10% Salicylic Acid was also determined. Salicylic Acid "greatly increased" the penetration of triamcinolone acetonide. The penetration of Salicylic Acid peaked at ~4 h (>150 μg penetrated), whereas the penetration of triamcinolone acetonide peaked at ~25 h; however, enhanced penetration of triamcinolone acetonide persisted.

The influence of Salicylic Acid on the epidermis was then evaluated by incubating the epidermal sheets with water, 60% ethanol, and 10% Salicylic Acid in 60% ethanol for 2 h, and then determining the penetration of 1% methyl nicotinate (which penetrates the skin rapidly). There was no detectable change in the penetration of methyl nicotinate regardless of pretreatment.

Pascher (1978) stated that Salicylic Acid enhances the dermal permeation of ammoniated mercury.

Female rhesus monkeys were used to determine the effect of Salicylic Acid on the dermal penetration of hydrocortisone (Wester et al. 1978). The ventral forearm of each animal was lightly shaved and 13.3 μg/cm^2 ^{14}C-hydrocortisone (5 μCi) in either acetone (five animals) or a formulation of 60% ethanol, 5% propylene glycol, 5% glycerin, and 30% water (EPGW) (four animals) was applied to a 6-cm^2 area without occlusion. Hydrocortisone was also applied with Salicylic Acid (five animals/group); doses of 13.3 and 133.3 μg/cm^2 were used with acetone as the vehicle and of 133.3 μg/cm^2 with EPGW as the vehicle. (The concentrations used in the study were determined to be 0.8% hydrocortisone solution and 0.8% or 8% Salicylic Acid solution.) The animals were secured in metabolic chairs for the first 24 h; the test solutions were then washed from the skin and the animals were returned to metabolic cages. Urine was collected for 5 days. When hydrocortisone was applied in acetone alone or with 13.3 or 133.3 μg/cm^2 Salicylic Acid, 1.37% ± 0.97%, 1.19% ± 0.43%, and 1.14% ± 0.25% of the dose, respectively, was excreted in the urine. When applied in EPGW alone or with 133.3 μg/cm^2 Salicylic Acid, 1.27% ± 0.52% and 0.96% ± 0.41% of the dose, respectively, was excreted. There was no statistically significant difference in the percutaneous absorption of hydrocortisone with or without the addition of Salicylic Acid; Salicylic Acid seemed to slightly decrease hydrocortisone absorption.

TABLE 8d

Summary of parenteral absorption, distribution, metabolism, and excretion studies for Salicylic Acid and Sodium Salicylate

Number/species	Exposure concentration	Application site	Absorption	Reference
		Salicylic Acid (SA)		
Dogs	1 g	IV in sodium bicarbonate	>90% of the dose recovered in the urine; 50% as unchanged SA, 25% as glycuronates, 10% as salicyluric acid, and as 4%–5% gentisic acid	Alpen et al. 1951
Gravid SD rats	300 mg/kg	SC; animals were killed after 1 h	4.06% injected ^{14}C dose/dry weight fetal tissue	Koshakji and Schulert 1973
4 gravid Suffolk or Suffolk-Dorset ewes		IV at 0 and 180 min with SA and acetylsalicylic acid on day >137 of gestation	SA and acetylsalicylic acid crossed the placenta; equilibrium was reached at approximately 40 min; mean SA clearance was 358 ml/min	Thiessen et al. 1984
Male Fischer 344 rats	5 or 50 mg/kg	3 and 25 mos animals dosed IV in an Emulphor: ethanol:water (4:1:1) solution	At 5 and 50 mg/kg, plasma salicylate concentration were 17–28 and 100–120 μg/ml in both groups; $t_{1/2}$ values in 3-months olds were 4.08 and 30.1 h and in 25-month-olds were 21.3 and 21.9 h with 5 and 50 mg/kg; no SA metabolites were detected in the urine	McMahon et al. 1990
Perfused rat liver			Hepatic metabolism of SA was negligible during a single pass	Shetty et al. 1994
Humans		IV	89.9% recovered in the urine after 4 h	Feldmann and Maibach 1970
		Sodium Salicylate (SS)		
Gravid rabbits	1 or 1.5 g/kg	Single SC dose on day 30 of gestation	Maternal serum salicylate and pooled fetal serum concentration were 0.58 and 0.37 mg/ml in the animal given 1 g/kg and 0.75 and 0.45–0.62 mg/ml in the animal given 1.5 g/kg	Jackson 1948
3–5 nongravid A/Jax mice and gravid A/Jax and CBA mice/group	0.1 ml/20 g	Single IM dose on day 14 of gestation and animals killed after 30 or 240 min; or on day 17 of gestation and animals killed after 30–480 min	Blood radioactivity content was greater in nongravid than gravid animals; in gravid animals, the content was generally greater in CBA than A/Jax mice on day 14 of gestation; on day 17, a strain difference was not seen; on both days, fetal radioactivity was initially greater in CBA mice	Eriksson and Larsson 1971
Gravid A/Jax mice	3 mg/20 g	Dosed on days 15–16 of gestation with nonradioactive SS and with radioactive SS on day 17; killed 30–240 min after last dose	Pretreatment with nonradioactive SS increased the variability of radioactivity in the blood	

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TABLE 8d

Summary of parenteral absorption, distribution, metabolism, and excretion studies for Salicylic Acid and Sodium Salicylate *(Continued)*

Number/species	Exposure concentration	Application site	Absorption	Reference
Rabbits	4 g/110 ml water	Animals dosed IV	$t_{1/2}$ was 1.5–4 h	Schuppli et al. 1972
6 rats or 6 ferrets	125 or 400 mg/kg	Single SC dose	At 125 and 400 mg/kg, blood salicylate concentration were 30 and 54 mg% in rats and 28 and 54.4 mg% in ferrets after 1 h	Gulamhusein et al. 1980
Male and female SD rats (varying ages)	62 μmol/kg	Dosed IV	Only SA found in the serum of dosed animals; urinary excretion of SA and SUA similar for males and females, although at different times males were higher than females	Varma and Yue 1984
Non- and gravid Wistar ST rats	10 mg/kg	Dosed IV (on day 20 of gestation for gravid animals)	Serum salicylate concentration significantly decreased in gravid animals; serum $t_{1/2}$ values were similar; fetal serum salicylate concentration were similar to maternal values; average values were 34.3, 21.7, and 22.5 μg/ml for nongravid, gravid, and fetal animals; serum protein binding of salicylate was significantly decreased in gravid animals; blood-to-plasma concentration ratios of SS from pooled blood to which non- and radioactive SS was added were 0.74 and 0.60 for gravid and nongravid animals	Yoshikawa et al. 1984
3–9 gravid SD rats/group	15–500 mg/kg	Single IV dose on day 8 of gestation	After 1 min, 15, 50, 100, 200, and 500 mg/kg produced peak plasma concentration of 100, 300, 600, 900, and 1800 μg/ml; behavior of the 15 and 50 mg/kg doses was linear whereas that of 100–500 mg/kg was nonlinear	Gabrielsson et al. 1985
12–14 gravid SD rats	1 or 2 mg/h	Constant infusion on days 6–13 of gestation	Plasma concentration peaked on days 6 and 8 of at approximately 110 μg/ml (range of 50–110 μg/ml) in animals given 1 mg/h and on day 8 at approximately 240 μg/ml (range 150–240 μg/ml) in animals given 2 mg/h	Gabrielsson et al. 1985

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TABLE 8d

Summary of parenteral absorption, distribution, metabolism, and excretion studies for Salicylic Acid and Sodium Salicylate *(Continued)*

Number/species	Exposure concentration	Application site	Absorption	Reference
5 non- and 5 gravid hooded Wistar rats	50 mg/kg SA equivalence	Single IV dose (on day 20 of gestation for gravid animals)	Body clearance of salicylate was similar for both groups, but was significantly increased in gravid animals; normalized values indicated a significantly, decreased in clearance and a slight increased in aVd; $t_{1/2}$ was significantly increased in gravid animals; gravid animals excreted less of the given dose, and the metabolic profile was changed; salicylate serum protein binding was decreased in gravid animals	Dean et al. 1989
Gravid SD rats	150 mg/kg/day	Constant-rate IV infusion on days 6–13 of gestation	Blood salicylate concentration were 112–141 μg/ml (mean 120) on days 7–13 of gestation	Bergman et al. 1990
4 NZW rabbits	44 mg/kg	Single IV dose	SA was rapidly excreted in the urine, with slightly >50% of the dose as SA and 4% as SUA; total recovery was 85.8%	Short et al. 1991
Humans	250 mg	Dosed IV	83.8%–94.5% of the dose was recovered in the urine in 24 h	Wurster and Kramer 1961

NZW, New Zealand white; SD, Sprague-Dawley.

The effect of Salicylic Acid on the percutaneous absorption of DFV was determined using a group of six human subjects (Täuber, Weiss, and Matthes 1993). (Protocol described previously.) Compared to a formulation without Salicylic Acid, percutaneous absorption of DFV was not affected by the addition of Salicylic Acid. Also, it did not affect the concentration of cortisol in the urine or dehydroepiandrosterone in the plasma.

The effect of Salicylic Acid on the transdermal delivery of cyclosporin through abdominal skin of male hairless mice was determined in vitro (Wang et al. 1997). Salicylic Acid was added at concentrations of 0.1% to 5%. Salicylic Acid did not affect the transdermal absorption of cyclosporin through mouse skin.

Skin Effects

Salicylic Acid

The effect of Salicylic Acid on pathological epithelial proliferation was evaluated in an epidermal hyperplasia test using male Pirbright albino guinea pigs (Weirich, Longauer, and Kirkwood 1975). Hyperplasia was induced with hexadecane. A group of 15 animals were dosed dermally with 3% Salicylic Acid in 99% ethanol, one dose of 1 ml and then twice daily doses of 0.1 ml for 7 days, and a group of three animals were dosed with 1% w/w Salicylic Acid in a dimethylacetamide-acetone-ethanol mixture (DAE 244), 0.1 ml twice daily for 7 days; both test groups were dosed in conjunction with seven daily doses of 0.1 ml hexadecane. The site of application was a 5.31-cm^2 circular area on the back. Tissue biopsies were taken on day 11 with 3% and day 10 with 1% Salicylic Acid.

Salicylic Acid had an antihyperplastic effect. Salicylic Acid at 3% in ethanol reduced surface epithelial hyperplasia by 15%; it had no effect on proliferation of the deep epithelium. At 1% in DAE 244, Salicylic Acid reduced surface epithelial hyperplasia by 18% and also significantly reduced deep epithelial proliferation; the reduction in the deep epithelium was 10% compared to the effects seen with 3% Salicylic Acid. Total epithelial volume was reduced with 1% Salicylic Acid compared to DAE and hexadecane. The vehicle affected the keratolytic effect of Salicylic Acid.

Guinea pig skin was used to examine the keratolytic effect of Salicylic Acid (Huber and Christophers 1977). The external ears and soles of the feet of dead guinea pigs were removed, and a 50% solution of Salicylic Acid in ether was applied. The tissue specimens were then incubated in humidity chambers for 10 h, after which time they were rinsed with ether and cryostat sections were prepared.

No difference was seen microscopically in the horny layer of treated and untreated samples. Application of mechanical stress, applied by moving the cover slip, caused the treated stratum corneum to break. In the control samples, the cells became elongated and flattened, but no cellular separation occurred. Upon stretching the stratum corneum, intercellular separation was constantly observed with the test samples, but never observed with the controls.

Epidermoplasia tests were conducted to determine the effect of Salicylic Acid on normal guinea pig skin (Weirich, Longauer, and Kirkwood 1978). Salicylic Acid, 1% in acetone/ethanol (50:50 *w/w*), was applied to the skin of three animals 20 times within a 4-week period. The single dose volume was 200 μl/5.31 cm^2 and the single surface dose was 0.276 mg/cm^2. Skin biopsy samples were examined using epidermal pachometry and planimetry, and the mitosis rates in the basal epidermal layer were determined.

No irritation or degenerative changes were observed during the study. Salicylic Acid did not have an inhibitory effect on epidermopoiesis in normal guinea pig skin. It caused a significant thickening of the surface epithelium, a significant increase in the volume of deep and total epithelium, and a distinct but nonsignificant increase in the mitosis rate in the germinative zone of the epidermis. Some intracellular and interstitial edema or slight spongiosis was observed. No hyperkeratosis was seen, and most sections of the horny layer of the skin treated with Salicylic Acid were almost completely detached. A "definite increase" in the number of cells and cell layers was observed.

The effect of Salicylic Acid on the skin of female hairless rhinoceros mice was examined (Kligman and Kligman 1979). Using eight animals per group, 100 μl of 1%, 5%, or 10% Salicylic Acid in an ethanol vehicle was applied to the entire dorsal trunk of each animal twice daily, 5 days per week. Four animals per group were killed after 3 weeks and four after 6 weeks. Slight epidermal hyperplasia was observed with 10% Salicylic Acid. In all dose groups, a moderate reduction in the quantity of horny material within the psuedocomedones, which retained their shape, was seen, and the "core of the horny impaction often seemed empty as if the contents had been lost."

The effect of Salicylic Acid on the stratum corneum was determined by measuring turnover time using the dansyl chloride fluorescence method (Takahashi, Machida, and Marks 1987). Occlusive patches of 5% dansyl chloride in white soft paraffin were applied to depilated skin on the backs of five guinea pigs for 24 h. One test site was then treated daily with 0.1 ml/4 cm^2 of 6% Salicylic Acid. An untreated site was used as a control.

Salicylic Acid significantly increased the rate of stratum corneum exfoliation as compared to the control; the mean turnover rate was 2.9 and 11.0 days for the test and control sites, respectively. Skin thickness was not affected by Salicylic Acid. Because the epidermis was not thickened or irritated, the authors concluded that Salicylic Acid may act directly on the intercellular cement substance of the corneocytes.

Ototoxicity

Ototoxicity, manifesting as mild to moderate reversible hearing loss and tinnitus, is a reported side effect of salicylates (Jung et al. 1993). Salicylates rapidly enter the cochlea after systemic administration. Hearing loss is bilaterally symmetric and may be flat or in the high frequencies. Recovery usually occurs 24 to 72 h after cessation of salicylates. According to these authors, age increases the risk of salicylate toxicity even at lower salicylate doses.

Sodium Salicylate

In a study to track the distribution of ^3H-labeled Sodium Salicylate in the cochlea to better understand possible mechanisms of salicylate ototoxicity, Ishii, Bernstein, and Balogh (1967) injected five albino guinea pigs intravenously or intraperitoneally with a 50-mCi/27.5 mg solution. The animals were killed at intervals ranging from 15 min to 13 h and the cochleas examined (Ishii, Bernstein, and Balogh 1967). Fifteen min after IV dosing, the radioactivity was found primarily in the lumen of the vessels in the stria vascularis and spiral ligament. One hour after intraperitoneal (IP) injection, the radioactivity was still primarily in the stria vascularis and the spiral ligament, but some had diffused into the organ of Corti and Rosenthal's canal. Six and 13 h after IP injection, little and no radioactivity, respectively, was found. The authors concluded that the distribution was so rapid and widespread that the possibility that certain cells may be specifically susceptible to damage cannot be excluded.

Salicylate intoxication produced biochemical changes in endolymph and perilymph of the ears of cats (Silverstein, Bernstein, and Davies 1967). The electrical activity of the cochlea, recorded from the round window area, had an increase in threshold of 20 to 24 dB 2 to 3 h after IP injection of 350 mg/kg Sodium Salicylate.

In guinea pigs that were given a single SC dose of Sodium Salicylate, the salicylate interfered with the cochlea's ability to generate a nerve evoked potential; this effect was reversible (Mitchell et al. 1973). A corresponding change in the ability of the cochlea to generate the alternating current cochlear potential was not seen. The effect of Sodium Salicylate was dependent on the blood salicylate concentration and, more importantly, the perilymph concentration.

Five monoaural chinchillas were injected intramuscularly (IM) with 400 mg/kg Sodium Salicylate, and auditory thresholds were measured 2 h after dosing and at regular intervals for 16 days (Woodford, Henderson, and Hamernik 1978). Two hours after dosing, the median temporary threshold $shift_{max}$ (TSS_{max}) was 21 dB at 8 kHz. The range of threshold shifts at all frequencies was 0 to 28 dB, and the time of TSS_{max} was variable but generally occurred 2 to 6 h after dosing. There was a tendency toward larger TSS at higher frequencies. Noise in conjunction with dosing did not exaggerate the results.

Dunkin-Hartley guinea pigs were given a single SC dose of 500 mg/kg Sodium Salicylate and killed 4 and 24 h after dosing or were given daily SC doses of 375 mg/kg Sodium Salicylate

for 5 to 7 days and killed 24 h to 6 weeks after dosing, and the cochleas were examined (Douek, Dodson, and Bannister 1983). Effects were seen in the outer and inner hair cells after a single dose and after multiple doses.

Sodium Salicylate was added to cultures of postnatal cochlear explants to determine the ototoxic effect (Zheng and Gao 1996). Sodium Salicylate dose-dependently induced spiral ganglion neuron death and degeneration of their peripheral neurites. It did not affect hair cells. Neuronal degeneration could be prevented by the addition of neurotrophins.

Hemorrhagic Effects

Salicylic Acid

Five male Sprague-Dawley rats were fed a diet containing 1.67 mmol Salicylic Acid per 100 g diet and five male ICR mice were fed a diet containing 6.68 mmol Salicylic Acid for 1 week (Takahashi and Hiraga 1985). Control animals were given untreated feed. The mean daily intake of Salicylic Acid was 1.48 and 13.7 mmol/kg for rats and mice, respectively. The prothrombin (PT) and kaolin-activated partial thromboplastin time (K-PTT) indices were not significantly different between the treated and control rats due to large variances. However, two of the treated rats had abnormal PT (<30%) and K-PTT (<45%) indices. In mice, the K-PTT was slightly decreased. The relative liver weights of mice, but not rats, were significantly increased compared to controls.

Hemolytic Effects

Methyl Salicylate

Erythrocytes from adult males (HRBCs) and from sheep (SRBCs) were incubated with Methyl Salicylate to determine the hemolytic effect (Murugesh et al. 1981). The numbers of cells treated were not stated. Methyl Salicylate, 0.004 ml, induced hemolysis in both HRBCs and SRBCs. Hemolysis increased with concentration and duration of incubation. Methyl Salicylate caused extensive membrane damage, probably due to its ability to decrease the surface tension of saline.

Photoprotective Effects

Ethylhexyl Salicylate

The phototoxic protection factor of a formulation containing 5% Ethylhexyl (Octyl) Salicylate, 7% octyl dimethyl PABA (padimate O), and 3% benzophenone-3 was determined using subjects with type I or type II skin (Lowe et al. 1987). In phase 1 of the study, 2 μl/cm^2 0.1% 8-methoxypsoralen (8-MOP) in isopropyl alcohol was applied to all test sites and allowed to dry for 15 min; 2 μl/cm^2 was then applied to half the test sites. After 15 min, the sites were irradiated with 0.1 to 0.8 J/cm^2 UVA; after 72 h, erythema was graded at all sites. In phase 2, 8-MOP was again applied and allowed to dry. The formulation, at 2 μl/cm^2, was applied to one site; after 15 min the sites were irradiated with 3 to 8 times the minimal phototoxic dose, with a UVA dose range of 0.4 to 4.3 J/cm^2. The formulation containing 5% Ethylhexyl (Octyl) Salicylate had a mean phototoxic protection factor (ratio of the mean phototoxic dose for protected and unprotected skin) of 3.3; this was significantly increased compared to the vehicle.

Antimicrobial Activity

Salicylic Acid

The minimal inhibitory concentrations (MICs) of 0.5% Salicylic Acid against gram-positive bacteria, gram-negative bacteria, fungi and yeasts, and molds were 2000, 3000, 3000, and 5000 ppm, respectively (Kabara 1984). The author stated that only undissociated Salicylic Acid is active, and it should be used as an antimicrobial preservative in the pH range of 2 to 5. It was suggested that Salicylic Acid attacks the plasma membrane of bacteria and inhibits some enzyme systems.

Cytotoxicity

Salicylic Acid

The inhibitory effect of Salicylic Acid on HeLa cells, *Bacillus subtilis*, and *Escherichia coli* was examined (Sheu et al. 1975). The concentrations of Salicylic Acid needed for 50% growth inhibition were 1.8, 1, and 4 mM, respectively.

Kleinerman et al. (1981) reported Salicylic Acid enhanced spontaneous monocyte cytotoxicity.

Viljoen, van Aswegen, and du Plessis (1995) found that Salicylic Acid at concentration ranges from 10^{-10} M to 10^{-2} M had no effect on plating efficiency of human prostatic carcinoma DU-145 cells, but that cell growth was inhibited at concentrations $>10^{-8}$ M and completely inhibited at concentrations $>10^{-4}$ M. Salicylic Acid increased ^{3}H-thymidine (^{3}H-TdR) incorporation, with a decrease in DNA synthesis, and inhibited protein synthesis as detected by ^{3}H-glycine incorporation.

In a cytotoxicity study, Salicylic Acid had NI_{50} values (concentration required to induce a 50% inhibition of neutral red uptake) of 16.9, 7.1, 16.6, and 14.9 mmol/L in MDCK (dog distal renal tubular cells), LLC-PK1 (pig renal proximal tubular cell), NRK (normal rat kidney, indefinite origin), and HepG2 (human hepatoma) cells, respectively (Noble, Janssen, and Dierckx 1997). Salicylic Acid decreased the reduced glutathione (GSH) content in all renal cell lines, with the decrease in the NRK cells being concentration dependent, and it increased the GSH content in the HepG2 cells.

Methyl Salicylate

The inhibitory effect of Methyl Salicylate on HeLa cells and *B. subtilis* was examined (Sheu et al. 1975). The concentrations of Methyl Salicylate needed for 50% growth inhibition were 2.8 and 6.5 mM, respectively.

Sodium Salicylate

Hial et al. (1977) reported that low concentrations ($\leq$0.5 mM) of Sodium Salicylate stimulated protein and nucleic acid synthesis while high concentrations ($\geq$1 mM) inhibited growth and

protein and nucleic acid synthesis in human fibroblast and rat hepatoma cultures.

The effect of Sodium Salicylate on thiol content in the isolated liver was examined in order to determine toxicity (Nishihata et al. 1988). At concentrations <25 mM, Sodium Salicylate did not affect glucose release or thiol content. A slight but insignificant decrease in nonprotein and protein thiol was observed at 50 mM, and an increase in glucose release was observed at "an early stage after perfusion." Glucose release was then at control values.

The effect of Sodium Salicylate on inducible nitric oxide synthase (iNOS) expression and function was examined using murine macrophage cells (RAW 234.7) (Amin et al. 1995). Sodium Salicylate had no significant effect on nitrite production at pharmacological concentrations (3 mM), but it significantly inhibited nitrite production at suprapharmacological concentrations (5 mM). However, the IC_{50} for nitrite accumulation was 20 mM. Pharmacological concentrations of Sodium Salicylate had no effect on the activity of cyclooxygenase-2. Immunoblot analysis of iNOS expression in the presence of Sodium Salicylate showed variable inhibition (0% to 35%). Pharmacological concentrations of Sodium Salicylate did not affect iNOS mRNA expression. The researchers stated that lower concentrations of Sodium Salicylate interfere with enzyme synthesis, while greater concentrations inhibit catalytic activity of iNOS.

Cultured rat cardiac fibroblasts were used to determine the effect of Sodium Salicylate on the inhibition of the induction of iNOS (Farivar, Chobanian, and Brecher 1996). Sodium Salicylate inhibited cytokine-induced nitrite accumulation in a time- and dose-dependent manner, with an IC_{50} of 750 μmol/L. Sodium Salicylate was effective when added both before and after cytokine induction, and the effect was reversible. High-dose Sodium Salicylate pretreatment prevented cytokine-induced stimulation of prostaglandin E_2 (PGE_2). Sodium Salicylate inhibited cytokine-induced iNOS mRNA levels but not iNOS enzymatic activity.

Farivar and Brecher (1996) further investigated the effect of Sodium Salicylate on the inhibition of iNOS induction. It was again found that Sodium Salicylate inhibited of iNOS-2 mRNA accumulation in a dose-dependent manner, and inhibition occurred with addition of Sodium Salicylate both before and after the addition of cytokines. Sodium Salicylate was able to reduce mRNA following prolonged induction by cytokines. Sodium Salicylate did not affect iNOS-2 mRNA half-life, and it did not inhibit the induction of nuclear factor (NF)-κB or signal tranducers and activators of transcription-1 (STAT-1) by electrophoretic mobility shift assay (EMSA).

The ability of Sodium Salicylate to inhibit nitric oxide formation induced by interleukin (IL)-1β was evaluated using rat hepatocytes (Sakitani et al. 1997). Simultaneous addition of Sodium Salicylate with IL-1β inhibited nitrite production. Inhibition was also observed when Sodium Salicylate was added 1 to 3 h after IL-1β. Inhibition was dose dependent; maximal and half-maximal concentrations were 20 and 7 mmol/L, respectively. Sodium Salicylate did not affect NF-κB activation or iNOS mRNA expression induced by IL-1β. Sodium Salicylate abolished the synthesis of iNOS protein.

Schwenger et al. (1997) found that Sodium Salicylate treatment was cytotoxic to normal human diploid FS-4 fibroblasts. A p38 kinase inhibitor suppressed the Sodium Salicylate–induced apoptosis. In a cytotoxicity study, Noble, Janssen, and Dierckx (1997) reported that Sodium Salicylate had NI_{50} values of 20.6, 8.0, 21.2, and 9.0 mmol/L in MDCK, LLC-PK1, NRK, and HepG2 cells, respectively. Ekwall and Acosta (1982) found that Sodium Salicylate had an MIC of 1.0×10^4 μg/ml in HeLa cells, with an IC_{50} value of 3×10^{-2} mol/L with a 24 h incubation period. The concentration inducing lactate dehydrogenase (LDH)-release by 50% was 5×10^{-3} mol/L in primary rat hepatocyte cultures after 3 and 24 h incubation periods. Borel (1976) examined the effect of Sodium Salicylate on cell-mediated cytolysis in a system using C57BL/6 mouse spleen cells sensitized with allogeneic tumor cells. Sodium Salicylate did not inhibit, as compared to controls, the cytolytic interaction.

Immunologic Effects

Salicylic Acid

Female Sprague-Dawley rats were used to determine the anti-inflammatory effects of Salicylic Acid, Salicylic Acid and nicotinic acid, Salicylic Acid and pyridyl-3-methanol, and an ester of pyridyl-3-methanol and Salicylic Acid (S-2063) in a carrageenan-induced edema test (Cekanova et al. 1974). The test compounds were given orally as a dose of 2 ml/100 g body weight in a 1% solution of carboxymethyl cellulose 30 min prior to injection of the carrageenan. Salicylic Acid had "significant anti-inflammatory effects." The anti-inflammatory effects of Salicylic Acid and nicotinic acid were additive, the effects of Salicylic Acid and pyridyl-3-methanol were less than that of Salicylic Acid and nicotinic acid, and the effects of S-2063 were similar to that of Salicylic Acid and pyridyl-3-methanol.

A single oral dose of 100 mg/kg Salicylic Acid reduced paw swelling in the arachidonic acid–potentiated and carrageenan-induced edema tests 9% and 36%, respectively, compared to controls (Smith et al. 1979). In 9-h sponge exudates in the rat, a single oral dose of 200 mg/kg Salicylic Acid inhibited prostaglandin-like activity and total leukocytes 83% and 43%, respectively, compared to controls. However, direct administration of 0.5 mg Salicylic Acid to the sponge only inhibited prostaglandin-like activity and total leukocytes 16% and 0%, respectively.

Methyl Salicylate

A rodent ear assay was performed using Methyl Salicylate to assess the inflammation response using ear thickness as a determinant (Patrick, Maibach, and Burkhalter 1985; Patrick, Burkhalter, and Maibach 1987). A dose of 5 μl Methyl Salicylate was applied to the dorsal and ventral surfaces of the pinna of one ear of female ICR mice, and solvent was applied to the contralateral ear. Ear thickness was measured prior to application

and at various times following application. The components of the inflammatory response were determined; histological evaluation was made at the time of maximum ear thickness, i.e., 20 min; trypan blue was used, leakage of ^{125}I-bovine serum albumen (^{125}I-BSA) was measured, and differences in temperature of the treated and untreated external ears were determined.

Undiluted Methyl Salicylate produced a maximum response at 30 min; doses of 2.5 to 7.5 mg produced maximum responses at 15 min. The ears returned to normal thickness after termination of dosing. Microscopically within 20 min, Methyl Salicylate produced rapid dilation of blood vessels, prominent vessels of the margin of the external ear, and moderate edema. These results were confirmed with trypan blue and ^{125}I-BSA. The temperature of the external ears treated with Methyl Salicylate increased rapidly and returned to normal within 20 min.

Normal human epidermal keratinocytes from female breast skin were used to examine cytokine production due to Methyl Salicylate (Wilmer et al. 1994). Methyl Salicylate, 500 μg/ml, did not induce IL-8, tumor necrosis factor (TNF)-α, or granulocyte/macrophage colony-stimulating factor (GM-CSF).

Sodium Salicylate

Using male Swiss albino mice, SC injection of Sodium Salicylate at 100 mg/kg increased the serum concentration of interferon 210%, whereas a dose of 300 mg/kg decreased the serum concentration of interferon by 65% (Geber, Lefkowitz, and Hung 1975a, 1975b).

The effect of Sodium Salicylate on prostanoid synthesis and platelet aggregation was determined using female subjects (Rosenkranz et al. 1986). The subjects were given 52.6 mg/kg Sodium Salicylate daily for 8 days. Sodium Salicylate did not affect urinary excretion of PGE_2, PGE-M, or 2,3-dinor-6-keto-$PGF_{1\alpha}$. It also did not affect platelet aggregation or thromboxane formation.

Male Wistar rats were used to examine the effect of Sodium Salicylate on ex vivo mucosal eicosanoid release and on ethanol-induced gastric damage (Peskar et al. 1988). Some animals were given 6.25 to 400 mg/kg Sodium Salicylate in 0.25% w/v carboxymethylcellulose orally 30 min prior to gastric instillation of 1.5 mol ethanol; the animals were killed after 5 min. A second set of animals was given 25 to 400 mg/kg Sodium Salicylate in carboxymethylcellulose without ethanol instillation and killed 30 min later, and a third set of animals was given an SC injection of 400 mg/kg aqueous Sodium Salicylate and killed after 1 h.

Oral pretreatment with Sodium Salicylate prior to ethanol instillation dose-dependently inhibited the stimulatory action of ethanol on gastric leukotriene C_4 (LTC_4) release, but PGE_2 and thromboxane B_2 (TXB_2) release were not altered. Release of 6-oxo-$PGF_{1\alpha}$ was increased; the increase was significant with 100 mg/kg Sodium Salicylate. In animals not given ethanol, Sodium Salicylate did not affect LTC_4, PGE_2, 6-oxo-$PGF_{1\alpha}$, or TXB_2 release, and SC administration had no significant effect on ex vivo gastric mucosal eicosanoid release.

Raghoebar, Van den Berg, and Van Ginneken (1988) studied the association of Sodium Salicylate with leukocytes. The degree of cell association of salicylate with mononuclear leukocytes (MNLs) was approximately two times less than the amount of cells associated with polymorphonuclear leuocytes (PMNs). Association of Sodium Salicylate with PMNs is markedly enhanced when extracellular pH is decreased; the researchers stated that this suggests that passive nonionic diffusion is an important mechanism in cell association. Phorbol 12-myristate 13-acetate (PMA), 0.13 μM, increased the intracellular concentration of Sodium Salicylate at anti-inflammatory concentrations (1.5 to 2.1 mM Sodium Salicylate). Cell association of Sodium Salicylate with PMNs increased markedly in the presence of the metabolites SUA and gentisic acid.

The effect of Sodium Salicylate on neutrophil function was determined (Abramson et al. 1991). Sodium Salicylate inhibited neutrophil aggregation in response to stimuli that require signal transduction via a G protein, but it did not have an effect on stimuli that bypass receptor–G protein interaction. Sodium Salicylate inhibited the binding of $GTP_{\gamma S}$, a stable analog of GTP (guanosine triphosphate), to purified neutrophil membrane preparations. The researchers determined that Sodium Salicylate interacts with a G protein in the neutrophil plasmalemma and uncouples post-receptor signaling events.

The ability of Sodium Salicylate to inhibit stimulated neutrophil adhesion to epithelium was examined (Cronstein et al. 1994). Neutrophils were isolated from human whole blood, and human umbilical vein endothelial cells (HUVECs) were obtained by collagenase treatment fo fresh human umbilical cords. Sodium Salicylate, 5 mM, did not affect adhesion of unstimulated neutrophils, but it inhibited stimulated neutrophil adherence to epithelium (50% inhibition at 0.5 mM) in a concentration-dependent manner. In examining the effect of Sodium Salicylate on the ATP concentration in resting and stimulated neutrophils, 1 mM markedly decreased neutrophil ATP concentration with incubation less than 1 h. A concentration-dependent decrease in ATP concentration was observed with a 10-min incubation period (50% decrease at 0.6 mM). Adenosine deaminase reversed the effect of Sodium Salicylate on stimulated adhesion to the endothelium. Therefore, the researchers theorize that Sodium Salicylate promoted the release of adenosine from cells and that the released adenosine inhibited the adhesion of stimulated neutrophils to the endothelium.

The effect of Sodium Salicylate on the expression of monocyte chemotactic protein-1 (MCP-1/JE) and interferon inducible protein-10 kDa (IP-10) chemokines in stromal cells was determined as a function of concentration ranging from 0.5 to 40 mmol/L (Gautam et al. 1995). Sodium Salicylate inhibited induction of chemokine mRNA in bone marrow stromal cells, in a concentration-dependent manner, without affecting the viability of these cells. Maximum suppression of induction was seen at 40 mmol/L and moderate suppression at 20 mmol/L. The suppression of mRNA expression was not dependent on synthesis of new proteins. Sodium Salicylate did not affect mRNA

stability. Activation of transcription factor NF-κB was inhibited by Sodium Salicylate in a dose-dependent manner.

Pierce et al. (1996) studied the effect of Sodium Salicylate at concentrations ranging from 0 to 20 mM on the expression of adhesion molecules in HUVECs. Sodium Salicylate inhibited activation of NF-κB by preventing phosphorylation and subsequent degradation of the inhibitor IκB-α. Salicylate did not have an effect on TNF-α–induced phosphorylation of the transcription factor ATF-2. Sodium Salicylate inhibited the TNF-α–induced increase in mRNA concentrations of adhesion molecules and statistically significantly inhibited TNF-α–induced surface expression of the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) at concentrations above 5 mM and intercellular adhesion molecule-1 (ICAM-1) at concentrations above 10 mM. Sodium Salicylate inhibited neutrophil transmigration without affecting neutrophil adhesion.

Pretreatment of normal human diploid FS-4 fibroblasts with 20 mM Sodium Salicylate inhibited a TNF-mediated increase in tyrosine phosphorylation of p42/p44 mitogen-activated protein kinase (MAPK); significance of the inhibition increased with length of pretreatment (Schwenger, Skolnik, and Vilček 1996). Inhibition was correlated with an inhibition of the TNF-induced p42 MAPK mobility shift. The effect of Sodium Salicylate was specific for TNF; Sodium Salicylate did not block p42/p44 MAPK tyrosine phosphorylation in response to epidermal growth factor (EGF) stimulation or in response to platelet-derived growth factor. Inhibition was not due to toxicity.

Schwenger et al. (1997) found that 20 mM Sodium Salicylate also inhibited TNF-induced activation of the c-Jun N-terminal kinase (JNK)/stress-activated protein kinase in normal human diploid FS-4 fibroblasts. It was much less effective in reducing EGF- or IL-1–induced JNK activity. Sodium Salicylate inhibited c-*fos* mRNA induction by TNF. Tyrosine phosphorylation was enhanced by treatment with Sodium Salicylate, and Sodium Salicylate increased p38 kinase activity in COS cells.

The inhibitory effect of Sodium Salicylate on UVB-induced AP-1 activity was evaluated by incubating JB6 cells with Sodium Salicylate at concentrations ranging from 0 to 4 mM (Huang et al. 1997). The cells were incubated for 30 min and then sequentially exposed to 2 kJ/m^2 UVB. Sodium Salicylate inhibited UVB-induced AP-1 activity at concentrations above 0.25 mM, with complete inhibition at 2 and 4 mM. Sodium Salicylate was not cytotoxic to JB6 cells at these concentrations. Sodium Salicylate did not inhibit AP-1 activity when given after irradiation.

Pharmacologic Effects

Salicylic Acid

In a study of the interaction of Salicylic Acid and pyridyl-3-methanol in producing anti-inflammatory and teratogenic effects, Cekanova et al. (1974) also reported the effects of Salicylic Acid on lysosomal membrane stability in male and female Sprague-Dawley rats. β-Glucuronidase and acid phosphatase were used as marker enzymes. Salicylic Acid at a concentration of 1 mM increased lysosomal membrane stability, but concentrations of 10^{-1} and 10^{-2} mM did not.

The effect of topical application of an ointment containing 3% Salicylic Acid and 0.05% betamethasone dipropionate on plasma cortisol concentrations was evaluated using two male and three female subjects with extensive psoriasis and two male and one female subject with extensive eczema (Gip and Hamfelt 1976). The ointment was applied twice daily for 2 weeks. The treated area ranged from 8 to 18 dm^2, and the amount of ointment applied per day ranged from 10 to 15 g. Blood samples were taken twice a week prior to and during dosing. No effect on adrenal gland function, as determined by monitoring plasma cortisol concentrations, was observed. Salicylate was not detected in the plasma.

The effect of Salicylic Acid on isolated rat hepatocytes was determined (Walker, Change, and Martin 1989). Incubation with Salicylic Acid resulted in a dose-dependent decrease in alanine aminotransferase activity in the medium. A small increase in aspartate aminotransferase was also observed with 1.0 and 2.0 mg/ml salicylate.

Sodium Salicylate

Female albino rats were dosed intraperitoneally with 100 mg/ml Sodium Salicylate in sodium chloride, pH 7.0, to determine the hepatic effects (Bullock et al. 1970). Control animals were given sodium chloride only. Sodium Salicylate had no effect on total hepatic adenosine triphosphate (ATP) content, but Sodium Salicylate altered the distribution between the mitochondrial and supernatant fractions, increasing the proportion of ATP in the supernatant fraction. No changes in hepatic urate oxidase or catalase activities were observed, as were no changes in β-glucuronidase, acid phosphatase, or alanine aminotransferase (ALT). A very small change in the spectrum of cytochrome P_{450} was seen after the addition of 5 mM Sodium Salicylate to a microsomal suspension in vitro. The bile flow rate was "markedly increased" by Sodium Salicylate. Using light microscopy, no gross changes in the liver sections were observed. With electron microscopy, "large numbers" of peroxisomes were observed, and large numbers of multivesicular bodies near the Golgi apparatus were noted.

Dawkins, McArthur, and Smith (1970) demonstrated that Sodium Salicylate can displace long-chain fatty acids from human plasma proteins and bovine albumin in vitro.

The effect of Sodium Salicylate on blood pH was determined in four studies using Sprague-Dawley rats (Hill 1971). In the first study, 200 mg/kg Sodium Salicylate (expressed as Salicylic Acid) was infused into groups of five to eight anesthetized animals. After 1 h, the animals were treated with a 10% sodium bicarbonate infusion, saline (controls), or inhalation of a 20:80 carbon dioxide:oxygen mixture. The animals were killed after 30 min, and blood pH and plasma and tissue salicylate concentrations were determined. The study was repeated using rats with ligated kidneys.

In the rats with the intact kidneys, the blood pH range from 7.69 to 7.93, 7.45 to 7.58, and 6.68 to 6.93 after treatment with sodium bicarbonate, saline, and carbon dioxide, respectively. In the animals with ligated kidneys, the ranges were 7.68 to 7.90, 6.46 to 7.53, and 6.75 to 6.94, respectively. Following treatment with sodium bicarbonate, saline, and carbon dioxide, the muscle/plasma salicylate ratios were 0.4 and 0.36, 0.38 and 0.47, and 0.58 and 0.56 for animals with intact and ligated kidneys, respectively; the brain/plasma salicylate ratios were 0.27 and 0.23, 0.26 and 0.28, and 0.45 and 0.45 for animals with intact and ligated kidneys, respectively; and the liver/plasma ratios were 0.55 and 0.55, 0.55 and 0.72, and 0.93 and 0.98 for animals with intact and ligated kidneys, respectively.

In the second study, three rats were dosed by intraperitoneal injection with 400 mg/kg Sodium Salicylate. The animals were killed 3 h after dosing, and arterial blood pH and plasma and tissue salicylate concentrations were determined. The blood pH ranged from 7.40 to 7.51. The amount of salicylate found in the plasma, muscle, brain, and liver was 425, 174, 141, and 301 μg/g, respectively.

In the third study, six rats were killed with IP injections of 1380 to 1500 mg/kg Sodium Salicylate. Tissues were taken a few minutes after the animals died. Survival time was 20 to 34 min. The amount of salicylate found in the muscle, liver, and brain was 928, 1329, and 433 μg/g, respectively.

In the fourth study, two groups of three anesthetized rats were infused intravenously with 400 mg/kg Sodium Salicylate. After 30 min, the animals were given 0.1 ml/min infusions of 10% sodium bicarbonate or 0.9% sodium chloride. The animals were killed after 1 h and arterial blood pH and plasma and tissue salicylate concentrations were determined. In the animals given saline, the blood pH ranged from 7.47 to 7.50, and the amount of salicylate found in the plasma, muscle, brain, and liver was 570, 234, 181, and 367 μg/g, respectively. In the animals given sodium bicarbonate, the blood pH ranged from 7.79 to 7.85 and the amount of salicylate found in the plasma, muscle, brain, and liver was 459, 159, 109, and 214 μg/g, respectively (Hill 1971).

The protective effects of Sodium Salicylate against the gastric necrosis produced by ethanol and HCl, and against aspirin induced ulcers, was studied in Sprague-Dawley rats (Robert 1981). Oral doses of 5 to 50 mg/kg and SC doses of 150 and 300 mg/kg were used. Sodium Salicylate was dose-dependently protective against gastric necrosis. The concentration at which aspirin induced ulcers were reduced by 50% was 40 mg/kg orally and 100 mg/kg subcutaneously.

ANIMAL TOXICOLOGY

Acute Dermal Toxicity

Salicylic Acid

An occlusive patch containing 2 g/kg Salicylic Acid was applied to the clipped skin of five male and five female rats for 24 h (Bomhard 1996). The animals were observed for 14 days. None of the animals died. One hour after dosing, "poor general condition and piloerection" were observed; all animals were normal by day 2. At day 14 necropsy, "slightly swollen" livers were observed in two female animals. The dermal LD_{50} of Salicylic Acid was >2 g/kg for rats.

Butyloctyl Salicylate

Five male and five female Sprague-Dawley rats were used to determine the dermal LD_{50} of Butyloctyl Salicylate (Huntingdon Life Sciences 1998b). The hair was clipped from the back of each animal, and 2 g/kg Butyloctyl Salicylate was applied under an occlusive patch for 24 h. All animals surivived until study termination. Six animals had "slight red stains on the snout" on the day of dosing. The dermal LD_{50} of Butyloctyl Salicylate was >2 g/kg for rats.

Ethylhexyl Salicylate

The acute dermal LD_{50} of Ethylhexyl (Octyl) Salicylate was >5 g/kg for rabbits (Anonymous 1976).

Methyl Salicylate

The acute dermal LD_{50} of Methyl Salicylate was >5 g/kg for rabbits (Opdyke 1978).

In a limit test performed using rats following Organization for Economic Cooperation and Development (OECD) Test Guideline No. 402, the acute dermal LD_{50} of Ethylhexyl (Octyl) Salicylate was >2 g/kg (Haarmann and Reimer 1991).

Tridecyl Salicylate

Five male and five female CD rats, housed five per cage, were used to determine the acute dermal toxicity of Tridecyl Salicylate in a limit test (Biolab 1998a). The test material, at 2 g/kg, was applied undiluted for 24 h to a shaved area under an occlusive patch. The animals were observed for 14 days. None of the animals died. Body weights were normal, and no signs of toxicity were observed. The dermal LD_{50} of Tridecyl Salicylate was >2.0 g/kg for rats.

Acute Oral Toxicity

Salicylic Acid

Groups of five cats were dosed with Salicylic Acid (Bekemeier 1955). One animal given 1.0 g/kg and three given 0.35 to 0.45 g/kg died. All animals given 0.1 to 0.18 g/kg Salicylic Acid survived.

Sado (1973) examined the synergistic effect on the oral toxicity of Salicylic Acid in olive oil and 2% and pure furylfuramide using dd mice. Mixtures made either with equal quantities or according to LD_{50} ratios were not synergistic.

The oral LD_{50} of Salicylic Acid was 891 mg/kg for rats (Sax 1979) and 480 mg/kg for white mice (Prokopovich 1963).

A group of four to six rats was dosed orally with 0.5 ml of 100 mg/ml Salicylic Acid in PEG 400 (Strom and Jun 1974). The animals were killed 1 h after dosing, and their stomachs were removed. A "large amount of bleeding" and gastric lesions were observed.

The oral LD_{50} of Salicylic Acid was determined using groups of 10 fasted Wistar rats (Hasegawa et al. 1989). The oral LD_{50} of aq. Salicylic Acid in gum arabic was 1580 and 1250 mg/kg for male and female rats, respectively.

Groups of four male albino Wistar rats were given a single oral dose of 800 mg/kg Salicylic Acid in distilled water, pH 7.2 (Walker, Change, and Martin 1989). Hepatic and plasma parameters were determined after 4 h. Compared to controls, a significant increase in liver-to-body weight ratios and plasma ALT and a significant decrease in glutathione was observed.

Groups of 10 male Fischer 344 rats, 3 and 12 months old, were given orally 5 ml/kg of 500 mg/kg Salicylic Acid in corn oil/DMSO in a 5:1 ratio (McMahon et al. 1991). Control animals were given vehicle or were untreated. Urine samples were collected at various intervals up to 72 h after dosing, at which time the animals were killed. Two of the 3-month-old test animals were killed at 16 h due to moribund appearance, and two of the 12-month-old animals died between 16 and 24 h; the cause of death was not determined.

Urine output was significantly increased in both test groups from 8 to 72 h; the increase was significantly greater in the 12-month-old animals compared to the 3-month-old animals. Glucose and protein excretion were significantly increased in both groups at 8 to 24 h and 4 to 48 h, respectively; at 24 h, urinary glucose was significantly greater for the 12-month-old animals. In examining the effect on proximal tubular enzyme excretion, Salicylic Acid significantly increased excretion of *N*-acetyl-β-glucosaminidase (NAG) at 4 to 72 h, alkaline phosphatase (AP) at 4 and 16 h in both test groups and at 8 h in 3-month-old animals, and ALT in 3-month-old rats at 4 and 8 h and in 12-month-old rats at 24 to 72 h. Compared to 3-month-old animals, NAG was greater at 4, 24, and 48 h, AP was greater at 24 to 72 h, and ALT was greater at 24 h in 12-month-old animals. In examining the effect on distal tubule enzyme excretion, AST was significantly increased from 8 to 72 h and urinary LDH was increased at 4 to 48 h. Compared to 3-month-old animals, AST and LDH were significantly greater in 12-month-old animals at 24 h.

Microscopic evaluation showed proximal tubular regeneration in the renal cortex of 3- and 12-month-old animals at 72 h. Affected tubules were single or in small clusters occurring throughout the cortex, and the epithelium had hyperplasia, anisocytosis, anisokaryosis, and cytoplasmic hyperchromia. The lumens of many of the tubules contained eosinophilic stained granular material that was consistent with necrotic cellular debris (McMahon et al. 1991).

Butyloctyl Salicylate

The acute oral toxicity of Butyloctyl Salicylate was determined according to the methods described in the Federal Hazardous Substances Act (FHSA) (Leberco · Celsis Testing 1996a). The animals were dosed orally with 5 g/kg Butyloctyl Salicylate and observed for 14 days. All animals surivived until study termination. All animals had "yellow anogenital staining" on the days 1 and 2, and it was present for one female animal on day 3. The oral LD_{50} of Butyloctyl Salicylate was >5 g/kg.

Ethylhexyl Salicylate

The acute oral LD_{50} of Ethylhexyl (Octyl) Salicylate was >5 g/kg for rats (Anonymous 1976).

In a limit test performed using rats following OECD Test Guideline No. 401, the acute oral LD_{50} of Ethylhexyl (Octyl) Salicylate was >2 g/kg (Haarmann and Reimer 1991).

Isodecyl Salicylate

A group of 10 male Wistar albino rats was used to determine the acute oral toxicity of Isodecyl Salicylate (Vevy Europe 1973a). A single oral dose of 5.0 ml/kg (4830 mg/kg) was given at a concentration of 50% in peanut oil. The test volume was 0.01 ml/g. None of the animals died. "Symptoms of central nervous system depression lasting 2 days after treatment" were observed. The researchers concluded that Isodecyl Salicylate did not "produce significant acute systemic effects."

Methyl Salicylate

Rats were given an oral dose of 1–3 g/kg Methyl Salicylate in a 20% suspension in a gum syrup mixture (Giroux, Granger, and Monnier 1954). The LD_{50} was approximately 1.25 g/kg.

The oral LD_{50} of Methyl Salicylate in 2% methylcellulose (equivalent to 100 mg/kg Salicylic Acid) was 1110, 1250, and 1300 mg/kg for mice, rats, and rabbits, respectively (Davison, Zimmerman, and Smith 1961).

The oral LD_{50} of Methyl Salicylate was 887 mg/kg for rats and 1060 mg/kg for guinea pigs (Jenner et al. 1964). Groups of 10 fasted animals were used. After dosing, "depression" was observed in rats. In guinea pigs, convulsions were observed and Methyl Salicylate irritated the gastrointestinal tract. The rats died in 4 to 18 h and the guinea pigs died in 1 h to 3 days.

Four conscious (three males and one female) and three anesthetized (two males and one female) dogs were used to examine the toxicity of Methyl Salicylate (Lacroix and Ferragne 1964). In the conscious animals, one was dosed via gastric catheter with 1.7 g/kg and three (one per dose) were given intraduodenally 0.6, 1.8, or 4.7 g/kg Methyl Salicylate. Vomiting and changes in respiration were noted in all animals. The female animal dosed with 1.8 g/kg and a male animal dosed with 4.7 g/kg died. In the anesthetized animals, 0.6, 3.1, or 5 g/kg Methyl Salicylate were administered gastrically. An increase in respiratory amplitude was observed in all animals.

Mongrel dogs were given an intragastric dose of 700 mg/kg Methyl Salicylate, and blood samples were taken over a 4- to 5-h period (Ojiambo 1971a, 1971b, 1971c, 1972, 1975; Ojiambo et al. 1972). Arterial plasma salicylate concentrations and plasma flow increased for 4 h after dosing, peaking at 41.3 mg% and 9.6 ml/min/100 ml, respectively. An increase in creatine phosphokinase activity was observed in the coronary effluent and muscle bed of the hind limb, indicating myocardial cell damage. Total body oxygen consumption rose steadily and peaked

at 4 h, with a twofold increase over baseline values. Respiratory alkalosis was initially observed, and metabolic acidosis was seen after 3 h. Arterial potassium and lactate concentrations increased. A slight increase in Po_2 was reported. A net efflux of orthophosphate was observed after 2 h. A swelling of cardiac muscle cells, with separation of myofibrils and "bulging" of sarcolemma, was observed. A dilation of sarcoplasmic reticulum and abnormalities in the mitochondria were noted.

A group of four to six rats was dosed orally with 0.5 ml of Methyl Salicylate (Strom and Jun 1974). The animals were killed 1 h after dosing, and their stomachs were removed. Some slight redness and irritation of the stomach mucosa, but no bleeding or ulceration, was observed.

The oral LD_{50} was reported by Opdyke (1978) to be 700 mg/kg for guinea pigs and 2800 mg/kg for rabbits; it was noted that administration of 0.5 ml Methyl Salicylate by gavage caused slight redness and irritation of the gastric mucosa. Sax (1979) reported that the oral LD_{50} of Methyl Salicylate was 2100 mg/kg for dogs.

Based on the results of a short-term study (described later), the calculated oral LD_{50} of Methyl Salicylate was 1440 mg/kg for CD-1 mice (Research Triangle Institute 1984).

The oral LD_{50} of Methyl Salicylate was reported to be 2800, 700, 1220, 1060, and 580 mg/kg for rabbits, guinea pigs, male rats, female rats, and mice, respectively (Rumyantsev et al. 1992).

Sodium Salicylate

The oral LD_{50} values of Sodium Salicylate for the mouse, rat, and rabbit were 0.9, 1.6, and 1.7 g/kg, respectively (Hart 1947).

The oral LD_{50} of Sodium Salicylate in 2% methylcellulose (equivalent to 100 mg/kg Salicylic Acid) was 1070 mg/kg for mice (Davison, Zimmerman, and Smith 1961).

Six male albino rats were given a single oral dose of 300 mg/kg Sodium Salicylate, pH 6.1, and killed 1 h after administration (Wooles, Borzelleca, and Branham 1967). A negative-control group of five rats was given saline. Plasma free fatty acids were reduced 46% below control values, and plasma triglyceride concentrations were reduced 60%. The liver weights of treated animals were slightly but significantly decreased in test animals compared to controls. Hepatic triglyceride concentrations were similar to control values.

Using groups of 8 to 10 male Fischer 344 rats, the oral LD_{50} of Sodium Salicylate was 1126 mg/kg (Pryor et al. 1983).

The oral LD_{50} of Sodium Salicylate was determined using groups of 10 fasted Wistar rats (Hasegawa et al. 1989). The oral LD_{50} of aqueous Sodium Salicylate was 1050 and 930 mg/kg for male and female rats, respectively.

Tridecyl Salicylate

The acute oral toxicity of Tridecyl Salicylate was determined using 10 male albino Swiss mice (Vevy Europe 1973b). The animals were dosed by gavage with 5 ml/kg (4830 mg/kg) Tridecyl Salicylate in peanut oil at a concentration of 50%. The dose volume was 0.01 ml/g. The LD_{50} was >2.05 ml/kg.

Acute Inhalation Toxicity

Methyl Salicylate

Methyl Salicylate, heated to 80°C and given by inhalation for an unknown duration to white mice and rats was not lethal; the LC_{50} was >400 mg/m^3 (Rumyantsev et al. 1992).

In an acute inhalation study, again of unknown exposure duration, white rats were exposed to 18, 69, and 114 mg/m^3 Methyl Salicylate (Rumyantsev et al. 1992). The high exposure level caused a decrease in summation threshold indicator (STI), research activity (RA), and orientation reaction (OR) (nervous system functions) and in LDH acivity in the serum, an increase in ALT activity, and a decrease in the time to start of blood coagulation. Exposures of 18 and 69 mg/m^3 reportedly led to an unspecified change in the indicators of nervous system functioning.

Acute Parenteral Toxicity

Salicylic Acid

Sax (1979) reported that the subcutaneous LD_{50} of Salicylic Acid was 520 mg/kg for mice.

Ethylhexyl Salicylate

The lowest lethal IP dose of Ethylhexyl (Octyl) Salicylate for mice was 200 mg/kg (Anonymous 1976).

Isodecyl Salicylate

The acute IP toxicity of Isodecyl Salicylate was determined using a total of 40 male Wistar albino rats (Vevy Europe 1974b). The animals were given a single dose of 0.62, 1.25, 2.5, or 5.0 ml/kg (604, 1208, 2415, or 4830 mg/kg, respectively) with concentrations of 6.25%, 12.5%, 25%, and 50% (*v*/*v*) in peanut oil. None of the animals in the 0.62-ml/kg group died within 14 days of dosing. One, 4, and 10 animals in the 1.25-, 2.5-, and 5.0-ml/kg groups, respectively, died 2 to 7 days after dosing. "Symptoms of central nervous system depression lasting 2 days after treatment" were reported. The acute IP LD_{50} in rats was 2.5 ml/kg.

Methyl Salicylate

The minimum lethal dose was 1.5 g/kg in guinea pigs, and the lethal SC doses were 2.7 to 2.75, 4.25 to 4.35, and 2.25 g/kg for guinea pigs, rabbits, and dogs, respectively (Opdyke 1978).

Rats and guinea pigs were dosed with 0.5, 0.75, or 1 g/kg Methyl Salicylate in an alcohol suspension (Giroux, Granger, and Monnier 1954). The LD_{50} for rats and guinea pigs ranged from 0.75 to 1 g/kg.

Sodium Salicylate

One male mongrel dog was dosed intravenously with 0.3 and one with 0.6 g/kg Sodium Salicylate (Rapoport and Guest 1945). The animal dosed with 0.3 g/kg had moderate hyperventilation at 5 h. The increase in blood pH and decrease in CO_2 tension was greatest at 1.5 h after dosing. The animal dosed with 0.6 g/kg was "breathing very deeply" within 20 min of dosing

and died 3.5 h after dosing. A blood sample taken 45 min after dosing had an elevated pH, slightly decreased CO_2 content, and a markedly decreased CO_2 tension. The values were more normal at 3 h.

Groups of female A/Jax mice were given a single IM dose of 12 to 18 mg Sodium Salicylate/20 g body weight in 0.1 ml distilled water to determine the LD_{50} (Eriksson 1970). The IM LD_{50} for A/Jax mice was 15.2 mg /20 g body weight.

The IP LD_{50} doses for adult and 5-day-old Holtzman rats were 780 and 512 mg/kg, respectively (Goldenthal 1971).

Groups of 30 to 50 gravid and nongravid Konárovice mice were used to determine the IP LD_{50} of Sodium Salicylate (Nezádalová, Elis, and Rašková 1973). The gravid animals were dosed on days 7, 14, or 20 of gestation or on days 7 or 14 after parturition. The LD_{50} values were 760 mg/kg for control animals, 760, 535+, and 520+ mg/kg for animals dosed on days 7, 14, and 20 of gestation, respectively, and 700 and 780 mg/kg for animals dosed on days 7 and 14 after parturition, respectively The toxicity of Sodium Salicylate was increased in gravid mice.

Sax (1979) reported that the IV LD_{50} for mice was 780 mg/kg.

Male Sprague-Dawley rats, 3 and 12 months old, were given a single IP injection of 500 mg/kg Sodium ^{14}C-Salicylate (250 mCi/mmol) in saline (Kyle and Kocsis 1985). A control group was dosed with saline. The animals, which were placed in metabolism cages, were killed 1.5, 3, 6, 12, or 24 h after dosing; both kidneys were removed.

No changes were observed in control animals. In 3-month-old animals, dilation and vacuolization of proximal tubule cells occurred 6 h after dosing, and cytoplasmic eosinophilia was also observed. At 12 h, the kidneys were normal. In the 12-month-old animals, focal areas of proximal tubular necrosis and interstitial edema, characterized by extensive nuclear pyknosis and karyolysis and degeneration of the luminal membrane, were observed at 6 and 24 h. Regeneration of the tubular epithelium was observed at 24 h. Blood urea nitrogen (BUN) concentrations were significantly elevated in 3-month-old animals at 3 and 6 h; the values were normal at 12 h.

A more severe change (compared to younger animals) in BUN that was significantly different from control animals was observed in 12-month-old animals at 3, 6, 12, and 24 h. In 3-month-old animals, a significant increase in urinary protein was found at all time intervals, and small to moderate amounts of blood were found in the urine. In 12-month-old animals, a greater increase in urinary protein and blood was observed. Significant glucosuria was observed in 12-month-old animals at each time interval. Glucose was not detected in the urine of 3-month-old animals. No difference in excreted radioactivity was observed between the 3- and 12-month-old animals. In examining urinary metabolites, excretion of SUA and gentisuric acid was decreased 71% and 80%, respectively, in the older animals. Maximal covalent binding to mitochondrial protein occurred after 3 h in both groups. Mitrochondrial binding declined in 3-month-old animals but was steady in 12-month-old animals (Kyle and Kocsis 1985).

Tridecyl Salicylate

The acute IP toxicity of Tridecyl Salicylate was determined using 30 male Swiss albino mice (Vevy Europe 1973c). The animals were dosed with 1.25, 2.5, or 5.0 ml/kg (1208, 2415, 4830 mg//kg) Tridecyl Salicylate at a concentration of 12.5%, 25%, or 50% in peanut oil. The dose volume was 0.01 ml/g. Tridecyl Salicylate had an LD_{50} of >1.5 ml/kg.

Short-Term Oral Toxicity

Salicylic Acid

Groups of four male albino Wistar rats were dosed orally for 3 days with 500 mg/kg/day Salicylic Acid in distilled water, pH 7.2 (Walker, Change, and Martin 1989). Hepatic and plasma parameters were determined 18 h after the last dose. Compared to controls, a significant increase in aniline hydroxylase, glutathione, plasma aspartate aminotransferase (AST), and plasma ALT activities and a significant decrease in glucose-6-phosphatase activity were observed.

Butyloctyl Salicylate

Groups of five male and five female Sprague-Dawley CD rats were dosed orally with 15, 150, or 1000 mg/kg Butylactyl Salicylate in corn oil daily for 28 days, while a control group was given vehicle only (Huntingdon Life Sciences 1998c). The animals were observed for signs of toxicity, and body weights and feed consumption were determined periodically. Neurobehavioral studies were performed prior to and at the termination of dosing. Hematology and clinical chemistry evaluations were performed at study termination. The animals were killed at study termination. The tissues of animals of the 1000 mg/kg and control groups were examined microscopically.

Excessive salivation was observed in one female of the high-dose group during week 2 and in two males and two females of the high-dose group during week 3; one of the females also had "slight red stains on the snout" during week 3. Another female of the high-dose group had lacrimation during week 3. Mean prothrombin and activated partial thromboplastin times were increased in animals of the high-dose group. Body weights, feed consumption, motor activity, functional observational batteries, organ weights, and microscopic examinations were similar for all animals. The no-observable-effect level (NOEL) was 150 mg/kg/day (Huntingdon Life Sciences 1998c).

Methyl Salicylate

Groups of two dogs, one male and one female, were given 50 to 1200 mg/kg synthetic Methyl Salicylate (99% pure) orally in capsule form daily 6 days per week for up to 59 days (Webb and Hansen 1963). Clinical observations were recorded during the study. All animals dosed with ≥500 mg/kg Methyl Salicylate had weight loss and died or were killed due to moribund condition within 1-month of study initiation. One animal given 800 mg/kg and both given 1200 mg/kg Methyl Salicylate vomited for 3 to

4 h following each administration. Microscopically, moderate to marked fatty changes were observed in the liver of one animal given 800 mg/kg and both given 1200 mg/kg. Animals given 500 mg/kg had diarrhea and weakness during the last 3 to 4 days prior to death. No adverse effects were seen in animals given 50 to 250 mg/kg Methyl Salicylate.

LaWall and Harrisson Research Laboratories, Inc. (1964) conducted a series of studies of the effects of feeding Methyl Salicylate to rats for various durations.

In a study to determine the effect of Methyl Salicylate on bone, groups of 10 male rats were fed a diet containing 20,000 ppm Methyl Salicylate for 1, 2, 3, 4, or 5 days. Two animals per group were killed on days 2, 4, 6, 8, and 10 after discontinuation of the test diet. No bone lesions were observed.

Groups of 12 male and 12 female rats were fed a diet containing 6000, 9000, or 12,000 ppm Methyl Salicylate for 7 weeks, while a control group was fed untreated feed. X-rays were taken and two males and two females per group were killed weekly as of week 2. Bone lesions were observed for animals of the 12,000-ppm dose group, but they were not seen in the other dose groups. Mean body weights and feed consumption correlated inversely with dose. Serum salicylate concentrations correlated with dose.

Groups of 10 male and 10 female Sprague-Dawley rats were fed a 5% fat enriched diet containing 0.6%, 0.9%, 1.2%, or 2.0% Methyl Salicylate for 11 weeks. X-rays were taken of two animals per group weekly; the animals were killed 1 week after x-ray and the femurs of some animals were examined microscopically. Mean body weights were decreased in the 2.0% group after week 7. Positive bone lesions were seen at week 2 in animals of the 2.0% group, and unequivocal changes were seen at week 5 in the 1.2% group. Microscopic changes were seen at weeks 2 and 8 in these groups, respectively. No changes were seen in the other test groups.

Groups of five male rats were fed 20,000 ppm Methyl Salicylate and a "protein diet" (consisting of 75% basic feed and 25% casein) for 7 weeks, with one group given water and one given 40% dextrose; a control group was fed the protein diet only. Mean body weights were decreased in the test group given water. The animals in the test group given dextrose consumed less feed, reducing the Methyl Salicylate intake to 60% to 80% of that consumed by the group given water. An increase in cancellous bone was seen in the group given Methyl Salicylate with water.

Groups of 10 male and 10 female rats were fed 12,000 or 20,000 ppm Methyl Salicylate or 12,000 ppm Methyl Salicylate and given intraperitoneally 1 unit/day parathyroid extract and groups of five male and five female rats were fed 12,000 ppm Methyl Salicylate alone or with 1290 ppm $CaSO_4 \cdot 2H_2O$, 10 mg/day Ca^{2+} intraperitoneally, cod liver oil (equal to 100 units vitamin D/100 g feed), or 1000 mg/day vitamin C intraperitoneally; the animals were dosed for 8 weeks. Bone lesions were seen in all animals dosed with Methyl Salicylate only. Body weights were decreased in most test groups. No bone lesions were seen in the animals given parathyroid hormone, $CaSO_4 \cdot 2H_2O$, Ca^{2+}, cod liver oil, or vitamin C.

In an 11-week study, groups of five male and five female rats were fed 12,000 ppm Methyl Salicylate, alone, with 250, 750, or 1250 ppm calcium carbonate in feed, or with 45 mg/day Ca^{2+} given intraperitoneally. In the Methyl Salicylate–only group, bone lesions were seen at 4 weeks (the earliest time x-rays were taken). In the calcium carbonate groups, no bone lesions were observed; at week 11, survival was 50%, 60%, and 60% in the 250, 750, and 1250 ppm calcium carbonate groups, respectively. In the Ca^{2+} group, the animals were killed because calciphylactic lesions occurred at the site of injection (LaWall and Harrisson Research Laboratories, Inc. 1964).

The Research Triangle Institute (1984) dosed groups of eight male and eight female CD-1 mice orally with 50, 100, 250, 500, or 1000 mg/kg Methyl Salicylate in corn oil for 14 days. A control group was given vehicle only. Two females, one female and one male, and two females and three males of the 50-, 100-, and 1000-mg/kg groups, respectively, died during the study. Piloerection and dehydration were observed for these animals. The mortality rate was significantly increased in the 1000-mg/kg group compared to the other groups.

A study following the same protocol and using the same doses was performed by Environmental Health and Research Testing, Inc. (1984). In this study, one control and two 1000-mg/kg animals died due to pulmonary congestion or cardiac myodegeneration and tubular nephrosis.

Sodium Salicylate

Rats were given 400 to 600 mg/kg of 10% aqueous Sodium Salicylate for 4 to 21 days, and the effects on the liver and kidneys were examined (Barbour and Fisk 1933). The following were observed in the liver: no change (1 animal, 400 mg/kg—4 doses; 1 animal, 400 mg/kg—7 doses); marked congestion and vacuolization (1 animal, 400 mg/kg—4 doses; 1 animal, 400 mg/kg—7 doses); slight to moderate focal necrosis with moderate vacuolization (3 animals, 400 mg/kg—14 doses); marked necrosis and vacuolization (1 animal, 400 mg/kg—21 doses); moderate necrosis and vacuolization (3 animals, 400 mg/kg—21 doses; 4 animals, 500 mg/kg—21 doses); slight necrosis and vacuolization (1 animal, 400 mg/kg—21 doses); widespread necrosis and vacuolization (1 animal, 500 mg/kg—7 doses); and marked vacuolization with moderate focal necrosis (5 animals, 600 mg/kg—7 doses; 1 animal, 600 mg/kg—11 doses).

The following were observed in the kidneys: no change (1 animal, 400 mg/kg—7 doses); moderate necrosis and degeneration of the tubular cells (2 animals, 400 mg/kg—4 doses); marked necrosis and degeneration of tubular cells (1 animal, 400 mg/kg—7 doses); slight necrosis of tubular cells (3 animals, 400 mg/kg—14 doses; 1 animal, 400 mg/kg—21 doses); marked necrosis of tubular cells (3 animals, 400 mg/kg—14 doses; 1 animal, 600 mg/kg—11 doses); moderate necrosis of tubular cells (3 animals, 400 mg/kg—21 doses); moderate necrosis and degeneration of convoluted tubules (1 animal, 500 mg/kg—7 doses;

4 animals, 500 mg/kg—21 doses); and slight to moderate necrosis and degeneration of tubular cells (5 animals, 600 mg/kg—7 doses). The animals of the 600-mg/kg group died within 10 days. Half of the animals of the 500-mg/kg dose group died within 2 weeks.

These authors also gave four dogs 300 mg/kg of 10% aqueous Sodium Salicylate for 2 weeks, and examined the effect on liver function on alternate days using the Rosenthal bromsulphalein dye excretion test. No gastric or duodenal ulcers were seen. There was no marked dye retention. At microscopic examination, widespread vacuolization with moderate degrees of necrosis was seen in the centers of the lobules of the liver and widespread necrosis and degeneration of the tubules were seen in the kidneys. Only the glomeruli of the kidneys were intact (Barbour and Fisk 1933).

A group of six male and six female rats was fed a diet containing 21,020 ppm Sodium Salicylate for 11 weeks (LaWall and Harrisson Research Laboratories, Inc. 1964). Sodium Salicylate was introduced at 50% of the dose during weeks 1 to 2 and at 75% of the dose during weeks 3 to 4. A negative-control group of 3 males and 12 females was fed the basal diet. In the test animals, weight gain was inhibited and feed consumption was reduced compared to controls. A positive increase in cancellous bone was observed in the test animals. Mortality was significant as of week 3 and approached 100% by week 7.

Wooles, Borzelleca, and Branham (1967) dosed a group of six male albino rats orally with 300 mg/kg Sodium Salicylate, pH 6.1, for 7 days. The animals were killed 1 h after the last dose. A control group of five rats was given saline. No change in the plasma free fatty acids was observed, and hepatic and plasma triglyceride concentrations were similar for treated and control animals.

These authors also examined the effect of feeding standard chow or chow containing 1% cholesterol and 0.5% cholic acid to groups of 11 to 16 male albino rats that were dosed orally with 300 mg/kg Sodium Salicylate or given saline only for 7 days. The animals were given the appropriate diet immediately after the initial dose. The animals were fasted for 16 h after the last dose and then killed.

In the test animals fed the basic diet, hepatic triglyceride concentrations were not different from controls, while plasma triglyceride concentrations were increased 84%. In the animals fed the high-cholesterol diet, hepatic free and esterified cholesterol values were similar for test and control animals, whereas plasma total and free cholesterol values were increased 22% and 35%, respectively, in the treated animals as compared to controls. A mean increase in liver weight of 20% was observed in all animals given Sodium Salicylate (Wooles, Borzelleca, and Branham 1967).

Groups of 10 male Fischer 344 rats were dosed by gavage 5 days/week for 4 weeks with Sodium Salicylate in distilled water (Pryor et al. 1983). The 28-day LD_{50} was 646.5 mg/kg.

Short-Term Inhalation Toxicity

Methyl Salicylate

The inhalation toxicity of Methyl Salicylate was determined using a group of four female Alderley Park rats (Gage 1970). The animals were exposed to 20 7-h exposures at a concentration of 120 ppm in a saturated atmosphere at 700 mg/m^3. No toxicity was observed, and the organs appeared normal at necropsy.

Short-Term Parenteral Toxicity

Methyl Salicylate

LaWall and Harrisson Research Laboratories, Inc. (1964) performed several short-term studies. Groups of two rats were dosed subcutaneously with 400, 600, 900, or 1200 mg/kg/day Methyl Salicylate for 2 weeks. One animal of the 900- and one of the 1200-mg/kg dose groups died within 48 h; all others survived until the termination of dosing. No bone lesions were observed.

Rats, five males and five females per group, were dosed intraperitoneally with 0.025 mg/day Methyl Salicylate; one group was also fed a diet containing 3000 ppm calcium carbonate (LaWall and Harrisson Research Laboratories, Inc. 1964). No bone lesions were observed.

Ten male and 10 female rats were dosed intraperitoneally with 0.05, 0.1 (divided), and 0.2 ml/day Methyl Salicylate from weeks 0 to 5, 5 to 7, and 7 to 11, respectively, and were fed a diet containing 3300 ppm calcium carbonate. A control group was fed untreated feed. Body weights were similar for treated and control animals. Seven males, eight females, and six males were surviving at weeks 5, 7, and 8, respectively. No increase in cancellous bone was seen (LaWall and Harrisson Research Laboratories, Inc. 1964).

The maximum tolerated dose (MTD) (maximum single dose survived by all animals) of Methyl Salicylate was determined using groups of five A/He mice (Stoner et al. 1973). The animals were given six IP injections over a 2-week period. The MTD was 500 mg/kg Methyl Salicylate.

Sodium Salicylate

A Rhesus monkey was dosed intravenously with 1.1 g/kg Sodium Salicylate in five divided doses over a 30-h period (Rapoport and Guest 1945). Signs of toxicity included hyperpnea, flushed face, weakness, and profuse diuresis. The pH of blood drawn 1 h after the last dose was increased to 7.85; CO_2 content was "moderately" decreased and CO_2 tension was "greatly decreased."

A male mongrel dog was dosed intraperitoneally with 1.0 g Sodium Salicylate in divided doses over a 24-h period (Rapoport and Guest 1945). After the first dose (0.2 g/kg), hyperpnea was extreme and continued until the death of the animal 2 h after the last dose. Diuresis, weakness, and "hyper-reflexia" were also observed.

Groups of five male and five female rats were given IP doses of 200 mg/kg/day Sodium Salicylate for 8 weeks; one group was also given 3000 ppm calcium carbonate in feed (LaWall

and Harrisson Research Laboratories, Inc. 1964). No positive bone effects were observed in animals of either group.

Subchronic Dermal Toxicity

Methyl Salicylate

Groups of three rabbits were dosed dermally with synthetic Methyl Salicylate (99% pure) 5 days per week for up to 96 days (Webb and Hansen 1963). A dose of 0.5, 1.0, 2.0, or 4.0 ml/kg was applied to the clipped skin on the back of restrained animals for 6.5 h each day of dosing. The test sites were not wiped because the compound was absorbed. The three animals dosed with 4.0 ml/kg died after 6, 8, and 28 days of dosing; the animals had "anorexia, weight loss, and depression." A "slight sloughing of epidermal scales" was observed for two of the animals dosed with 2.0 ml/kg. No other observations were noted.

All but one animal of the high-dose group were examined microscopically. One high-dose animal had "several distinct lesions," including dilatation, desquamation, and formation of new atypical epithelium of the renal tubules; a moderate number of small foci of superficial necrosis and sloughing of the skin; foci of moderate necrosis and slight calcification of voluntary muscles; marked vacuolation of pancreatic acinar cells; slight myeloid hyperplasia and shift to the left of bone marrow; and slight hepatitis. These effects were not seen in the other examined high-dose animals, but an effect on the distal portion of the nephrons was indicated. Spontaneous nephritis and mild hepatitis, and slight to very slight dermatitis, was observed in the other animals (Webb and Hansen 1963).

Subchronic Oral Toxicity

Isodecyl Salicylate

Ten male and 10 female Wistar albino rats were fed 0.5% (~500 mg/kg/day) Isodecyl Salicylate in basal diet for 15 weeks (Vevy Europe 1975a). A control group of 10 males and 10 females was given untreated feed. The actual daily dose of Isodecyl Salicylate was 437 to 531 and 426 to 505 mg/kg/day for male and female animals, respectively. Body weight gain and feed intake were similar for test and control animals, and no treatment-related observations were reported. Oral administration of ~500 mg/kg/day Isodecyl Salicylate did not produce a significant toxic effect.

Methyl Salicylate

The effect of Methyl Salicylate on bone metabolism and growth was examined using groups of five male and five female Sprague-Dawley rats fed a diet containing 0.2%, 0.36%, 0.63%, 1.13%, or 2.0% Methyl Salicylate enriched with 5% hydrogenated fat for 12 weeks (Abbott and Harrisson, no date). The animals received 50% of the dose during weeks 1 to 2, 75% of the dose during weeks 3 to 4, and 100% of the dose thereafter. A negative-control group was given untreated feed. Males of the 0.63% group and all animals of the 1.13% and 2.0% groups had decreased body weight gains compared to controls. Radiographs taken at week 10 showed increased density at the metaphyses of the femur, humerus, tibia, and radius of animals of the 1.13% and 2.0% groups.

Groups of five male rats were fed a 5% fat enriched diet containing 0.6% or 2.0% Methyl Salicylate for 12 weeks (Abbott and Harrisson, no date). Mortality was 100% in the 2.0% group at week 6. None of the animals of the 0.6% group died during the study. Bone lesions were observed in the high-dose animals.

Five male and five female Sprague-Dawley rats were fed a 5% fat-enriched diet containing 1.2% Methyl Salicylate for 12 weeks, while 10 male and 10 female rats were fed the test diet with the addition of 0.3% calcium carbonate (Abbott and Harrisson, no date). Mean body weights were decreased in the Methyl Salicylate group. Mortality was 90% and 15% in the Methyl Salicylate groups without and with calcium carbonate, respectively. Bone lesions were not observed in the Methyl Salicylate plus calcium carbonate group.

Groups of 20 Osborne-Mendel rats, 10 males and 10 females per group, were given feed containing 0.1% or 1.0% synthetic Methyl Salicylate (99% pure) for 17 weeks (Webb and Hansen 1963). A control group was given basal diet. Body weights were measured weekly. Body weight gains for males and females of the high-dose group were significantly decreased compared to controls. No gross or microscopic findings were observed.

LaWall and Harrisson Research Laboratories, Inc. (1964) fed a group of 15 Sprague-Dawley rats diet enriched with 5% fat containing 2.0% Methyl Salicylate for 12 weeks; again, it was given as 50% of the dose during weeks 1 to 2 and 75% of the dose during weeks 3 to 4. A negative control was given untreated feed. Growth and feed consumption were decreased in the Methyl Salicylate group. Survival was 80% at study termination. Bone lesions were observed.

These authors also fed groups of 10 male Sprague-Dawley rats a 5% fat-enriched diet containing 0.6% or 2.0% Methyl Salicylate ad libitum or 0.6% Methyl Salicylate in a paired feeding at a ration equal to the mean daily amount of feed consumed by the 2.0% group fed ad libitum for 12 weeks. Body weights were decreased in the 2.0% ad libitum and the 0.6% paired feeding groups. Mortality was 50%, 80%, and 90% in the 2.0% ad libitum group and 60%, 70%, and 80% in the 0.6% paired feeding group at weeks 2, 3, and 4, respectively. Survival was 100% in the 0.6% ad libitum group.

In a 12-week study by these authors, groups of 10 male rats were fed a diet containing 4000 ppm or 20,000 ppm Methyl Salicylate and another group was fed a diet containing 20,000 ppm Methyl Salicylate and dosed intraperitoneally with a mixture containing 250 U vitamin A and 25 U vitamin D; the animals of the 20,000-ppm groups were fed a basal diet after 6 weeks. Body weights and feed consumption were decreased in both 20,000-ppm test groups during weeks 1 to 6. Bone lesions were observed in both 20,000-ppm groups.

These authors also dosed nine male and nine female rats daily with 12,000 ppm Methyl Salicylate and 1 mg cortisone (injected) for 12 weeks. A control group of three males and five females

was untreated. Body weights were decreased in the test group, and bone lesions were observed.

In another 12-week study, groups of 10 male and 10 female rats were fed 12,000 ppm Methyl Salicylate and 3000 ppm calcium carbonate or 1 mg cortisone (injected) and groups of 5 male and 5 female rats were fed 12,000 ppm Methyl Salicylate and 100 mg/day vitamin C (injected), a 50:50 water/milk mixture, 2 ml 10% calcium gluconate (IP), 12,000 ppm methyl p-OH benzoate, or 4700 calcium citrate.

In the group given calcium carbonate, body weight was not affected and no bone lesions were observed; survival was 85% at 12 weeks. In the group given cortisone, body weights were decreased and bone lesions were observed. In the group given vitamin C, the "bone condition was not as severe and constant as usually noted" with 12,000 ppm Methyl Salicylate. No bone lesions were observed with the water-milk mixture, but the intake of dry feed was decreased and the intake of fluid was increased.

The animals of the calcium gluconate group were killed at week 4 due to the development of calciphylactic lesions at the point of injection; body weights were decreased and bone lesions were not observed. In the animals given methyl *p*-OH benzoate or calcium citrate, body weights were not affected and bone lesions were not seen (LaWall and Harrisson Research Laboratories, Inc. 1964).

Sodium Salicylate

A group of six male and six female Sprague-Dawley rats was fed a 5% hydrogenated fat–enriched diet containing 2.1% Sodium Salicylate for 12 weeks (Abbott and Harrisson, no date). A control group was given untreated feed. The animals received 50% of the dose during weeks 1 to 2, 75% during weeks 3 to 4, and 100% thereafter. Growth and feed consumption was reduced in the test group. Mortality was 100% at week 11. Bone lesions were observed in the test group.

The neurotoxic potential of 138 to 550 mg/kg Sodium Salicylate was determined using groups of nine to 10 male Fischer 344 rats (Pryor et al. 1983). (The dose concentrations were based on proportions of the short-term LD_{20} [study described previously].) The animals were dosed 5 days per week for 15 weeks and were tested using a battery of neurobehavioral tests conducted prior to dosing, at 3-week intervals during dosing, and 3 and 6 weeks after the termination of dosing. A control group was dosed with vehicle only.

The LD_{50} during 15 weeks of dosing was estimated to be (via linear regression using the short-term LD_{50} value) 366.5 mg/kg. One animal, two animals, and one animal of the 218-, 346-, and 550-mg/kg dose groups, respectively, died during weeks 2 to 9 of the study; the deaths were not dose related. Weight gain of test animals was significantly decreased compared to controls as of week 3 for animals of the 218- and 550-mg/kg dose groups and as of week 6 for animals of the 346-mg/kg dose group; weight gains were still significantly decreased 6 weeks after the termination of dosing. After 15 weeks of dosing, the only behavioral change was a dose-related decrease in hindlimb grip strength; this was significant for all groups except the 138-mg/kg dose group. This effect also persisted after 6 weeks of recovery.

Groups of five male Sprague-Dawley rats were fed a 5% fat enriched diet containing 0.7% or 2.1% Sodium Salicylate for 12 weeks (LaWall and Harrisson Research Laboratories, Inc. 1964). A negative-control group of 10 animals was given basal feed. Mean body weights in the group fed 4000 ppm Sodium Salicylate was decreased compared to controls. Mortality was 100% in the low-dose group at week 7 and in the high-dose group at week 2. Bone lesions were observed with 2.1% Sodium Salicylate.

Tridecyl Salicylate

Ten male and 10 female Wistar albino rats were fed ~500 mg/kg/day Tridecyl Salicylate in basal diet for 15 weeks (Vevy Europe 1975b). A control group of 10 males and 10 females was given untreated feed. No treatment-related observations were seen. Oral administration of ~500 mg/kg/day Tridecyl Salicylate did not produce a significant toxic effect.

Subchronic Inhalation Toxicity

Methyl Salicylate

In an inhalation study, male white rats (number per group not specified) were exposed to 1.2, 8, or 40 mg/m^3 Methyl Salicylate for 4 h/day for 4 months (Rumyantsev et al. 1992). The high dose caused changes in nervous system functioning, a decrease in hemoglobin content and the number of erythrocytes, and a change in serum leucine aminopeptidase and LDH activities and urinary creatinine content. At microscopic examination, pulmonary focal hemorrhages and hyperplasia were observed in the peribronchial lymphoid tissue and the number of plasmatic cells in the lymphoid follicles was increased. In the kidneys, scaling of the epithelium of the convoluted tubules, focal infiltration, and focal hemorrhages were seen.

Chronic Oral Toxicity

Methyl Salicylate

Groups of 25 male and 25 female albino rats were fed a diet containing 700 or 2100 ppm (0.07% or 0.21%) Methyl Salicylate for 2 years (Packman et al. 1961). A control group was fed basal diet. No adverse effects were reported. Growth, feed consumption, general condition, mortality, and blood and urine chemistries were similar for test and control animals. No gross or microscopic findings were noted.

Webb and Hansen (1963) fed groups of 50 littermated Osborne-Mendel rats, 25 males and 25 females, diet containing 0.1%, 0.5%, 1.0%, or 2.0% Methyl Salicylate for 2 years. The group fed 2.0% consisted of 24 males and 26 females. A control group was given basal feed. Weights were measured weekly, and hematological examinations were done on 10 rats per group at 3, 11, 17, and 22 months. Organs of animals that died were not included in calculations. Tissues from 12, 6, and 5 animals of the control, 1.0%, and 2.0% groups, respectively,

were examined microscopically, and 10 leg bones and muscles of an additional 85 rats were examined.

In the high-dose group, half of the animals died by week 8 and all of the animals died by week 49 of the study. Animals of the 1.0% and 2.0% groups had statistically significant growth inhibition and developed rough hair coats. No hematological effects were observed. Average absolute organ weights were similar for all animals. However, relative organ to body weight ratios for the testes of male animals and for the heart and kidneys of the female animals of the 1.0% groups were significantly increased. Gross lesions of the pituitary gland were observed in 10 animals of the 0.5% group as compared to four animals in the control group. In the 2.0% group, 29 of the 50 animals had pneumonia.

The authors described the pneumonia as "more acute than that regularly seen." Microscopically, they stated that a "pronounced change" occurred in the bones of animals fed 2.0% Methyl Salicylate. Cancellous bone in the metaphysis was increased as compared to same-age controls; this was observed to a moderate degree in five and a marked degree in four of the nine bones examined from animals of the 2.0% group. Bone lesions were slight in 2 of 11 and 1 of 11 bones examined from animals of the 1.0% and 0.5% groups, respectively. The affected bones had fewer osteoclasts, and the number was inversely proportional to the degree of change.

An additional three male and three female Osborne-Mendel rats were fed 2.0% Methyl Salicylate, and the same number was fed basal diet, to assess the bone changes. Control animals were killed in conjunction with test animal death. One male test animal died on day 11, two males died on day 19, and females died on days 31, 40, and 71. Rough hair coat and growth inhibition was observed for all test animals, with some animals having labored respiration.

Upon gross observation, four of the six animals had slight to moderate pulmonary damage. Focal gastric hemorrhages were present in the glandular portion of three of the test animals. Bone growth was affected. A pathologist reported that "chondroclastic and especially osteoclastic activity [were] virtually completely blocked. Chondroblastic and osteoblastic activity [were] somewhat diminished."

These authors also gave groups of two male and two female Beagle dogs 50, 150, or 350 mg/kg synthetic Methyl Salicylate (99% pure) orally in capsule form 6 days per week for 2 years. A control group was given a placebo. The animals were weighed weekly, and hematologic evaluations were made at 2 weeks, 1, 3, and 6 months, and 1 and 2 years. No compound-related mortality was observed; one animal of the high-dose group died from hepatitis on day 33. No hematological effects were observed. Animals of the 150- and 350-mg/kg groups had retarded growth. Enlarged livers were observed in these animals, and the livers had larger hepatic cells than observed in control animals (Webb and Hansen 1963).

In a 30-week feeding study, groups of five male and five female rats were fed a diet containing 2000, 3550, 6300, 11,250, or 20,000 ppm Methyl Salicylate (LaWall and Harrisson Research Laboratories, Inc. 1964). During weeks 1 and 2, Methyl Salicylate was given at 50% and during weeks 3 and 4, it was given at 75% of the final dose. A negative-control group was given basal diet. Body weights and feed and water consumption were measured. Mean body weights were significantly decreased for animals of the 11,250- and 20,000-ppm groups; feed consumption was also decreased in these groups. At week 10, x-rays were taken; animals of the 11,250- and 20,000-ppm dose groups had positive increased bone density in the femur and tibia. This was not seen in the other groups.

The diets of several control and high-dose animals were exchanged after 11 weeks. The animals that had previously been given control feed and then given Methyl Salicylate lost weight, and the majority died. Those high-dose animals switched to control feed started to recover.

LaWall and Harrisson Research Laboratories, Inc. (1964) also gave groups of three male and three female beagles 150, 300, 500, or 800 mg/kg/day Methyl Salicylate in capsule form for 6.5 to 7.5 months; half the dose was administered following the morning and half following the afternoon feeding. A group of two males and four females served as the negative controls. All animals of the 150- and 300-mg/kg test groups and the negative-control group and two of the 500-mg/kg test group animals survived until study termination. Four animals of the 500-mg/kg group died between weeks 2 and 8. In the 800-mg/kg group, five animals died during week 1 and one died during week 2. Body weights of the animals of the 150- and 300-mg/kg dose groups were similar to control values. One of the two surviving animals of the 500-mg/kg group had "a slight loss in body weight." Hematology and clinical chemistry values were normal for animals of the 150- and 300-mg/kg dose groups.

Two animals of the 150-mg/kg and negative-control groups and three animals of the 300-mg/kg group were killed after 6.5 months. The remaining animals of the 150- and 500-mg/kg groups and the negative-control group were killed after 7.5 months. An increase in liver and kidney weights was observed in treated animals. The pathologist reported that 150- and 300-mg/kg Methyl Salicylate did not induce "lesions or other deleterious alterations" (LaWall and Harrisson Research Laboratories, Inc. 1964).

Groups of four male and four female dogs were given 50 or 100 mg/kg/day Methyl Salicylate and a group of six male and six female dogs was given 167 mg/kg/day Methyl Salicylate in capsule form for 6 months; half the dose was administered following the morning and half following the afternoon feeding (FDA 1966). A negative-control group of six male and six female dogs was used. All the animals of the low- and mid-dose groups and four males and four females of the high-dose and control groups were killed after 6 months; the remaining high-dose and control animals were killed after 8 months (following a 2-month nontreatment period).

All animals survived until study termination. During month 2 of the study, many test animals had dose-related seborrhea

oleosum and pyoderma; addition of lard to the diet alleviated this condition. After 6 months, hematological parameters were normal. At the 6-month necropsy, one animal from each test group had hyperemic foci of the pyloric mucosa. No test article-related hepatic or renal changes were found, and relative mean liver and kidney weights were within normal range.

Dermal Irritation

Butyloctyl Salicylate

The primary dermal irritation of Butyloctyl Salicylate was determined using rabbits according to FHSA methods (Leberco·Celsis Testing 1996b). Butyloctyl Salicylate produced very slight to well-defined erythema and edema. One animal had "blanched skin" at the test site and two had flaking skin. The primary irritation index (PII) was 2.12. According to the FHSA, Butyloctyl Salicylate was not a primary dermal irritant.

Ethylhexyl Salicylate

Ethylhexyl (Octyl) Salicylate applied undiluted to intact and abraded rabbit skin for 24 h was mildly irritating (Anonymous 1976).

A primary skin irritation study of undiluted and 1%, 5%, and 25% solutions of Ethylhexyl (Octyl) Salicylate was performed using groups of Six Rabbits following OECD Test Guideline No. 404 (Haarmann and Reimer 1991). The mean scores (24-, 48-, and 72-h readings) were 0.1, 0.1, 1.7, and 2.5 for erythema and 0.0, 0.0, 0.9, and 1.7 for edema with 1%, 5%, 25%, and 100% Ethylhexyl (Octyl) Salicylate, respectively. No erythema or edema was observed with the ethanol 96%/diethylphthalate (DEP) 1:1 w/w vehicle.

Isodecyl Salicylate

The dermal irritation potential of undiluted Isodecyl Salicylate was determined using six male New Zealand white rabbits (Vevy Europe 1974a). A volume of 0.5 ml containing 500 mg of the test material was applied (believed to be occlusively) for 4 h to both intact and abraded areas, 25 cm^2, on the dorsum of each animal. Four hours after application, very slight erythema and/or edema was reported at the abraded sites of four animals. One animal had very slight edema and one had very slight erythema 24 and 48 h after application, respectively, No reaction was observed 7 days after application. The average PII was 0.195. The researchers concluded that Isodecyl Salicylate was "not significantly irritant" to the skin of rabbits.

Methyl Salicylate

A modified Draize test was performed to determine the irritation potential of Methyl Salicylate in various vehicles (Yankell 1972). Methyl Salicylate, 1%, 3%, or 6%, in a water suspension, PEG 400, 70% ethanol, or 70% ethanol plus emollients was applied under an occlusive patch to the intact skin on the backs of three animals (species not specified). The test sites were scored for irritation at 24 and 72 h.

The irritation index was greatest with 70% ethanol; scores of 1.17, 4.17, and 4.00 were reported with 1%, 3%, and 6% Methyl Salicylate, respectively. Necrosis was seen in all three animals dosed with 3% and 6% Methyl Salicylate in 70% ethanol. With 70% ethanol plus emollients, scores of 2.17, 3.00, and 3.00 were reported with 1%, 3%, and 6%, respectively; necrosis and intradermal and SC hemorrhage were seen at all doses. The water suspension of 1%, 3%, and 6% Methyl Salicylate produced irritation indices of 0.0, 0.83, and 1.83, respectively, and with PEG 400, indices of 0.33, 0.50, and 0.50, respectively, were reported (Yankell 1972).

Although details were not provided, Opdyke (1978) reported that Methyl Salicylate was severely irritating to guinea pig skin and moderately irritating to intact and abraded rabbit skin when applied under an occlusive patch for 24 h.

Rumyantsev et al. (1992) reported that a single application of Methyl Salicylate to the skin of rabbits and guinea pigs did not cause irritation. However, repeated applications of Methyl Salicylate to guinea pigs caused scaling, dryness, and isolated and multiple infiltrates by days 4 to 6. Threshold changes were noted with application of a 50% oil solution. Concentrations of 10% and 25% did not cause any changes.

Tridecyl Salicylate

The dermal irritation potential of Tridecyl Salicylate was determined using six female Dunkin-Hartley albino guinea pigs (Vevy Europe 1973d). A dose of 500 mg/site was applied to intact and abraded dorsal skin on each animal. Tridecyl Salicylate was not irritating to guinea pig skin.

The dermal irritation potential was also determined using six male New Zealand white rabbits using the same dose and procedure (Vevy Europe 1973e). The average PII was 0.195; Tridecyl Salicylate was not irritating to rabbit skin.

Sensitization

Salicylic Acid

A local lymph node assay (LLNA) was performed in which groups of five CBA/J mice were dosed once daily for 4 consecutive days on each side of both external ears with 12.5 μl of 1%, 10%, or 20% Salicylic Acid in acetone (total of 25 μl/ear) (Gerberick et al. 1992). ^{3}H-TdR, 20 μCi, in phosphate-buffered saline (PBS) was injected intravenously 18 to 24 h after the fourth dose. The bilateral auricular lymph nodes were excised from each animal and pooled. Concentrations of 1%, 10%, and 20% Salicylic Acid produced 0.9-, 1.8-,and 7.2-fold increases; a positive response is a $\geq$2-fold increase that is significantly different than control values. (This was obtained with 20% Salicylic Acid.)

Boussiquet-Leroux et al. (1995) reported results of an LLNA performed using 5% to 20% Salicylic Acid dissolved 4:1 in acetone–olive oil (AOO). Groups of four female CD1 mice were dosed on the dorsum of the external ears with 25 μl of the test solution or the vehicle once daily on days 1 to 3. On day 5, the animals were given an IP injection of 100 mg/kg

bromodeoxyuridine (BrdU) and killed after 2 h. A test also was performed that involved a preexposure procedure. An occlusive patch of 5% to 20% Salicylic Acid or vehicle was applied to the flank of groups of four mice for 48 h. Topical application was made to the external ears on days 6, 7, and 8, and on day 9, the animals were given an IP injection of BrdU and killed after 5 h.

Significant T-cell proliferation was observed, with a maximum treated versus control (T/C) ratio of 1.74. No cortical lymphocyte proliferation was noted. Very slight paracortical hyperplasia was sometimes observed, but generally, no remarkable effects were seen in the cortex.

Butyloctyl Salicylate

A guinea pig maximization test was performed to determine the sensitization potential of Butyloctyl Salicylate (Huntingdon Life Sciences 1998d). Induction concentrations were 5% in propylene glycol given intradermally and 100% Butyloctyl Salicylate applied topically. The challenge was performed 14 days after the last induction dose. Patches of 50% and 100% Butyloctyl Salicylate were applied to two separate sites. Five male guinea pigs, which were used as an irritation control group, were treated concurrently during induction with propylene glycol and Freund's complete adjuvant/water emulsion and in the same manner as the test animals during challenge.

During induction, the test sites were evaluated 24 h after dosing and during challenge, the sites were evaluated 24 and 48 h after patch removal. None of the animals challenged with 100% Butyloctyl Salicylate had a sensitization response. The "severity indices" at 24 and 48 h were 0.4 and 0.2, respectively, for the test group and 0.6 and 0.3, respectively, for the irritation control group. One of 10 animals challenged with 50% Butyloctyl Salicylate had a clear dermal response. The "severity indices" at 24 and 48 h were 0.3 and 0.4, respectively, for the test group and 0.0 and 0.1, respectively, for the irritation control group (Huntingdon Life Sciences 1998d).

Ethylhexyl Salicylate

The sensitization potential of Ethylhexyl (Octyl) Salicylate was determined in a maximization test performed using guinea pigs following OECD Test Guideline No. 406 (Haarmann and Reimer 1991). Induction concentrations were 2.5% in arachis oil given intradermally and 50% in ethanol/DEP (1:1) applied topically. At challenge, a 25% solution in ethanol/DEP (1:1) was used. Ethylhexyl (Octyl) Salicylate was not a sensitizer in guinea pigs.

Methyl Salicylate

A modified Magnusson-Kligman guinea pig maximization test was performed using Dunkin-Hartley albino guinea pigs to evaluate the sensitization potential of Methyl Salicylate (Kimber et al. 1991). Ten animals were given a series of six intradermal injections of 2.5% Methyl Salicylate in 0.01% dodecyl benzene sulfonate/saline and Freund's complete adjuvant in the shoulder region. After 6 to 8 days, an occlusive patch containing undiluted Methyl Salicylate was applied to the injection site for 48 h. A group of four animals was treated with vehicle only. A challenge was performed 12 to 14 days later by applying an occlusive patch containing 10% Methyl Salicylate in acetone/PEG 400 (70:30) for 24 h to a previously untested site on the clipped flank of each animal. The sites were scored after 24 h. Methyl Salicylate was not a sensitizer.

An interlaboratory trial of the murine LLNA was performed with Methyl Salicylate in AOO (Kimber et al. 1991). Groups of four CBA/Ca mice were exposed on the dorsum of both ears to 25 μl of 1%, 2.5%, or 5% Methyl Salicylate or vehicle daily for 3 consecutive days. Four days after the initiation of treatment, the animals were given an IV injection of 20 μl PBS containing 20 μCi of ^{3}H-TdR (2 Ci/mmol). The animals were killed 5 h after the injection, and the draining auricular lymph nodes were excised and the data pooled for each group. No positive response was observed with Methyl Salicylate. (A positive response is a >3-fold increase [stimulation index of 3] in ^{3}H-TdR incorporation as compared to the vehicle.)

A guinea pig maximization study was performed to determine the sensitization potential of Methyl Salicylate (Basketter and Scholes 1992). Albino Dunkin-Hartley guinea pigs (approximately 350 g) were used. A series of six intradermal injections of 2.5% Methyl Salicylate (in 0.9% NaCl aided by acetone) were followed after 6 to 8 days with a 48-h occluded patch using Methyl Salicylate at 100% and then 12 to 14 days later with a challenge on one flank with a 24-h occluded patch at the maximum nonirritant concentration (10%) in acetone/PEG 400 (70:30). No responses were seen at challenge and Methyl Salicylate was not a sensitizer.

These authors also conducted an LLNA following the same procedure as Kimber et al. (1991), with the exception that the animals were injected with ^{3}H-TdR and killed 4 to 5 days after the first application of 5%, 10%, and 25% Methyl Salicylate in AOO. Methyl Salicylate was negative (Basketter and Scholes 1992).

An LLNA was performed in which groups of five CBA/J mice were dosed once daily for 4 consecutive days on each side of both external ears with 12.5 μl of 1%, 2.5%, or 5% Methyl Salicylate in acetone (total of 25 μl/ear) (Gerberick et al. 1992). ^{3}H-TdR, 20 μCi, in PBS was injected intravenously 18 to 24 h after the fourth dose. The bilateral auricular lymph nodes were excised and pooled for each animal. Doses of 1 to 5% Methyl Salicylate all resulted in a 0.8-fold increase, which is a negative response.

Another LLNA was performed following the same protocol, with the exception that the animals were injected with ^{3}H-TdR and killed 5 days after the first application (Basketter, Scholes, and Kimber 1994). Methyl Salicylate, tested at concentrations of 5%, 10%, and 25% in AOO, was negative.

Additional murine LLNA tests using female CBA/Ca or CBA/J mice were performed with Methyl Salicylate in AOO using standard and modified procedures in a number of laboratories (Kimber et al. 1995). One modification involved treatment

for 4 days, the lymph nodes were excised 4 days after the initiation of dosing, and lymph node analysis was pooled from individual animals. A second modification involved the use of ^{125}I-iododeoxyuridine (^{125}I-UdR) and the analysis of pooled data from individual animals. In the standard assay, 1% to 20% Methyl Salicylate produced no positive responses. Using the first modification, although a stimulation index of 3 was not observed, significant differences among individual mice were observed for test animals as compared to controls with 20% Methyl Salicylate.

Female Wistar and Brown Norway rats were used in an LLNA with 5% to 25% Methyl Salicylate (Arts et al. 1997). Serum immunoglobulin E (IgE) responses were also evaluated by applying 25% Methyl Salicylate to the shaved flank of Wistar and Brown Norway rats, followed by application of 12.5% to the dorsum of the external ear 7 days later. Methyl Salicylate did not cause a reaction in the LLNA; local lymph node weight and proliferation was actually decreased. Methyl Salicylate did not alter serum IgE response.

Photosensitization

Salicylic Acid

The contact photosensitization potential of Salicylic Acid was determined using groups of five female albino outbred ICR mice (Miyachi and Takigawa 1983). On days 0 and 1, 50 μl Salicylic Acid in acetone (believed to be at a concentration of 50%) was applied to the clipped abdominal skin of each animal, and the site was irradiated for 2.5 h at a distance of 15 cm. The irradiation source, a black light emitting UVA between 320 and 440 nm with a peak emission of 360 nm, consisted of three tubes in parallel arrangement with energy output at 15 cm of 2.7 mW/cm^2 at 360 nm and 0.17 mW/cm^2 at 305 nm (UVB); a glass filter was not used to limit UVB exposure. Control animals were dosed with vehicle and irradiated.

Prior to challenge, the ear thickness of all animals was measured. On day 5, the animals were challenged on both sides of the pinna with 20 μl Salicylic Acid in alcohol (believed to be at a concentration of 25%) followed by irradiation for 2.5 h at a distance of 15 cm. Ear thickness was measured at the peak time of ear swelling, i.e., 24 h after challenge. Some animals were pretreated by IP injection of 20, 100, or 200 mg/kg cyclophosphamide to enhance delayed-type hypersensitivity. Salicylic Acid was not a photosensitizer (Miyachi and Takigawa 1983).

Tridecyl Salicylate

Ten male Hartley albino guinea pigs were used to determine the phototoxic potential of Tridecyl Salicylate (Biolab 1998b). During induction, 0.5 ml of 2% Tridecyl Salicylate in dehydrated alcohol was applied to a shaved area of the back and massaged in three times daily for 2 weeks (Monday to Friday). The test sites were then irradiated with UVA + UVB, emission spectrum between 285 and 350 nm, for 15 min at a distance of approximately 30 cm. The challenge was performed 14 days after the last UV exposure. A dose of 0.5 ml of 0.1% Tridecyl Salicylate in dehydrated alcohol was applied once, and the site was irradiated. The test sites were examined 24, 48, and 72 h after the challenge. A control group of five animals was treated with dehydrated alcohol only. No erythema or edema was observed in test or control animals. Tridecyl Salicylate was not a photosensitizer.

Ocular Irritation

Butyloctyl Salicylate

The ocular irritation potential of Butyloctyl Salicylate was determined according to FHSA methods (Leberco-Celsis Testing 1996c). Butyloctyl Salicylate produced minimal conjunctival irritation in three of six animals; all eyes were normal by day 3. Butyloctyl Salicylate was not a primary ocular irritant according to the FHSA.

Ethylhexyl Salicylate

The ocular irritation of a 50% solution of Ethylhexyl (Octyl) Salicylate in DEP was studied using rabbits following OECD Test Guideline No. 405 (Haarmann and Reimer 1991). The 50% solution of Ethylhexyl (Octyl) Salicylate was nonirritating to rabbit eyes.

Isodecyl Salicylate

The ocular irritation potential of Isodecyl Salicylate was determined using six male New Zealand albino rabbits (Vevy Europe 1973f). A 0.1-ml dose of 10 mg of the test material (at a concentration of 10% [v/v] in liquid paraffin) was instilled into the conjunctival sac of each animal, and the eye was not rinsed. No irritation was observed at any time. The researchers concluded that Isodecyl Salicylate was not an ocular irritant at the dilution tested.

Methyl Salicylate

Methyl Salicylate was severely irritating to guinea pig eyes (Opdyke 1978). However, Rumyantsev et al. (1992) reported that Methyl Salicylate was not irritating to rabbit eyes.

Tridecyl Salicylate

A 0.1-ml dose of undiluted Tridecyl Salicylate was instilled into the conjunctival sac of the right eye of three male New Zealand white rabbits, and the ocular irritation was determined (Biolab 1998c). The contralateral eye served as a control. The eyes were examined 1, 24, 48, and 72 h and 7 days after instillation. In all animals 60 min after instillation, "congestion without chemosis" was observed; this lesion was not present in two animals 48 h after instillation nor in the remaining animal 7 days after instillation. No other effects were observed. The researchers concluded that Tridecyl Salicylate was nonirritating to rabbit eyes.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Salicylic Acid, produced when aspirin is rapidly hydrolyzed to Salicylic Acid after absorption from the gut, was reported to be the causative agent in aspirin teratogenesis in rats by Kimmel, Wilson, and Schumacher (1971).

In Vitro Studies

A number of in vitro teratogenicity studies have been performed on salicylates, all generally having positive results. Some were mechanistic and examined whether teratogenic effects were due to aspirin or Salicylic Acid (Yokoyama et al. 1984) or due to salicylate or its metabolites (Greenaway, Bark, and Juchau 1984).

The effect of Salicylic Acid on nervous system development was studied (Khera and Whalen 1988; Joschko, Dreosti, and Tulsi 1993), and the overall teratogenic potential of Salicylic Acid (Mummery et al. 1984) and Sodium Salicylate was examined (McGarrity et al. 1981; Greenaway et al. 1982; Flint, Ortin, and Ferguson 1984; Ebron-McCoy et al. 1988; Akita, Yokoyama, and Kuroda 1995).

The effect of Salicylic Acid on human spermatozoa was determined following incubation with 50, 100, or 200 mg/L salicylate for 2 to 48 h (Porat-Soldin and Soldin 1992). A dose-response effect was observed with significant inhibition of motility at all times, and the inhibition was significantly increased with time. The decrease was in sperm motility and not due to sperm death.

In Vivo Dermal Studies

Methyl Salicylate

Overman and White (1983) applied Methyl Salicylate (350 and 525 mg/100 g to the skin of the backs and delivered by oral intubation (1.75 g/kg) of timed-pregnant LVG hamsters (approximately 100 g body weight) at 7 days 9 h of gestation. The incorporated dose was measured using spectrophotometric analysis of blood salicylate concentrations. Blood levels reached a peak of 125 mg/100 ml at about 2 h after oral treatment. A peak salicylate level of 50 mg/100 ml was obtained 5 to 6 h after topical application of 350 mg/100 g and a peak of 120 mg/100 ml with the 525 mg/100 g topical treatment level.

The high topical dose level was not well tolerated and was discontinued. Most embryos were removed at 9 days of gestation. Of those that were allowed to develop, few survived beyond 12 days of gestation.

Malformations at 9 days of gestation were used as an indicator of teratogenic effect. Of 35 litters (fetuses per litter not given) in the oral treatment group, 72% of the fetuses had neural tube defects. Of 6 litters (number of fetuses per litter not given) produced by animals given the low topical dose, 6% of the fetuses had neural tube defects; and of 19 litters in the high-topical-dose group, there were 53% neural tube defects. The researchers stated that these results are consistent with the blood salicylate concentrations (Overman and White 1983).

The teratogenic potential of a petroleum-based grease manufactured using 3% Methyl Salicylate was determined using rats (Infurna et al. 1990). The test material was applied dermally to groups of 12 gravid animals at a dose of 1, 3, or 6 g/kg/day on days 6 to 15 of gestation. A positive-control group was dosed dermally with 2 g/kg/day undiluted Methyl Salicylate; the dose was reduced to 1 g/kg/day on days 10 to 15 of gestation because of maternal toxicity (i.e., 25% mortality and severe dermal irritation). A negative control group was also used.

In the test groups, no maternal toxicity was observed. No changes in reproductive parameters and no malformations or variation attributable to dosing were observed. Positive-control animals had 100% incidence of total resorptions. Urinalysis reported "very high concentrations" of Salicylic Acid in the urine of the positive-control animals and that a "significant proportion" of the available Methyl Salicylate was absorbed from the test material. However, the urinary concentrations of Salicylic Acid in the test animals were "far below the toxic levels" observed in the positive controls (Infurna et al. 1990).

In Vivo Oral Studies

Salicylic Acid

Groups of 20 gravid Wistar rats were fed a diet containing 0.06%, 0.1%, 0.2%, or 0.4% Salicylic Acid on days 8 to 14 of gestation (Tanaka et al. 1973a). The control group was given a basal diet. On day 20 of gestation, 15 of the animals of each group were killed; the remaining 5 were allowed to deliver. The offspring, which were weaned on day 21, were observed daily and weighed every 3 days. Offspring were killed and autopsied on the 56th day for examinations of visceral and skeletal abnormalities.

Maternal weight loss was marked for animals of the 0.4% group with initial dosing, but a gradual weight gain was observed after day 11. Maternal weight loss correlated with a decrease in feed and water consumption. Salivation and/or piloerection were observed in this group. All dams survived until study termination.

Uterine and placental weights were significantly decreased in animals of the 0.4% group as compared to controls; this groups had 71.2% neonatal mortality. The ratios of resorptions, placental remnants, and implantation sites to the number of implantations were 23.9%, 31.4%, and 15.9%, respectively. Litter size was significantly decreased in the 0.4% group; body weight, body length, and tail length of offspring were significantly decreased in the 0.2% and 0.4% groups, and tail length was significantly decreased in the 0.1% group. The effects seen in offspring (determined at autopsy on the 56th day) were 3.8% external anomalies, no internal organ anomalies, and 14.6% skeletal anomalies for the 0.2% group; and 29.6% external anomalies, 13.6 internal organ anomalies, and 46.8% skeletal anomalies for the 0.4% group.

In the 0.4% group, one dam gave birth to six live offspring, but all six offspring died within 1 day; none of the other dams in

this group gave birth to live offspring. Male and female offspring from animals of the 0.1% group had decreased body weights, body length, and tail length compared to controls.

In male offspring, again at autopsy on the 56th day, a significant increase in spleen weight was observed for the 0.06% group, a significant increase in carcass, heart, kidney, adrenal gland, and testis weights were observed for the 0.1% group, and a significant increase in kidney weights and a significant decrease in lung weights was observed for the 0.2% group compared to controls.

In female offspring at autopsy on the 56th day, a significant increase in carcass and ovary weights was observed for the 0.06% group and a significant increase in carcass, liver, adrenal gland, and ovary weights was observed for the 0.1% group compared to controls. The incidence of skeletal anomalies determined at the 56th day in offspring of the 0.2% group was 13.8%; skeletal anomalies were not observed in the other groups, and no external or internal organ anomalies were seen in the 0.06 to 0.2% groups (Tanaka et al. 1973a).

Tanaka et al. (1973b) dosed groups of 20 gravid Wistar rats orally with 75, 150, or 300 mg/kg Salicylic Acid in a 0.5% solution of sodium carboxymethylcellulose once daily on days 8 to 14 of gestation. The control group was given 5 ml/kg of vehicle. On day 20 of gestation, 15 of the animals of each group were killed; the remaining 5 were allowed to deliver. The offspring, which were weaned on day 21, were observed daily and weighed every 3 days. Offspring were killed and autopsied on the 56th day for examinations of visceral and skeletal abnormalities.

Maternal body weight gain was inhibited for animals of the 300-mg/kg group. Salivation and/or piloerection were observed in this group. Feed and water consumption decreased during the administation of 300 mg/kg Salicylic Acid. Three animals of this group died within a few days of the initiation of dosing. Decreased uterine weight was observed in animals of the 150- and 300-mg/kg dose groups as compared to controls; these groups had 25.7% and 100% fetal mortality, respectively. Litter size and neonatal body weight, body length, and tail length were significantly decreased in the 150-mg/kg dose group. The incidences of external, internal, and skeletal anomalies in offspring autopsied at the 56th day were 1.8%, 0%, and 2.5%, respectively, for the 75-mg/kg group and 27.8%, 12.7%, and 65.7%, respectively, for the 150-mg/kg group.

The offspring from animals of 150-mg/kg Salicylic Acid group had decreased body length and tail length compared to controls. The thyroid weight of male offspring from the 75-mg/kg group was significantly increased and the adrenal gland weight of male offspring from the 150-mg/kg group was significantly decreased compared to controls. The incidences of external organ, internal organ, and skeletal anomalies in offspring were 0%, 5.0%, and 0%, respectively, for the 75-mg/kg group and 13.7%, 17.2%, and 79.2%, respectively, for the 150-mg/kg group (Tanaka et al. 1973b).

Waltman et al. (1973) studied the effects of anti-inflammatory drugs on parturition parameters in the rat. Groups of 10 gravid Sprague-Dawley rats were orally given 10 mg/kg Salicylic Acid twice daily on days 20 and 21 of gestation (Waltman et al. 1973). Control groups were either untreated or given 2.0 ml distilled water on the same days. The animals were observed daily until day 20; after the first dose, the animals were observed every 2 h until delivering. Time of onset of parturition, duration of parturition, bleeding during parturition, and perinatal mortality were noted. Salicylic Acid significantly increased time of onset of parturition compared to controls. The duration of parturition was increased in only one animal. Bleeding at parturition was increased in four animals as compared to controls. None of the 106 pups born to these 10 animals were dead (compared to 4 of 109 pups in the control group).

Methyl Salicylate

In a reproduction study, groups of 25 male and 25 female mice (F_0 generation) were fed a diet containing 0.25% or 0.5% Methyl Salicylate for 30 days prior to mating (Abbott and Harrisson, no date). A negative-control group was fed untreated diet. The F_0 animals were mated twice to produce F_{1a} and F_{1b} litters. The F_{1a} litter was maintained through weaning, while 30 males and 30 females were chosen from the F_{1b} litter to parent the F_{2a} and F_{2b} litters. All animals were fed the appropriate diet from study initiation through weaning of the F_{2b} litters. All litters were culled to 10 neonates at day 5.

Results only included data from females in each generation that were available for two successive mating. No gross abnormalities were observed for neonates of any litter, and all surviving to weaning were normal in growth, appearance, and behavior. Conception rate, the number of unsuccessful matings for females, and the number of stillbirths were greater for the negative controls than the test groups. Litter size was slightly smaller in the test groups than the control, but the neonate death rate between birth and weaning was lower than controls. Viability, lactation, and reproduction indices of the test groups were comparable to greater than those of the controls.

These authors also performed a reproduction study using 25 male and 25 female Wistar rats following the same protocol, with the exception that the animals were fed a diet containing 0.25% or 0.5% Methyl Salicylate for 60 days prior to mating. No gross abnormalities were observed for neonates of any litter, and all surviving to weaning were normal in respect to growth, appearance, and behavior. The mating performance, reproduction indices, and viability indices were decreased in the 0.5% group compared to the other groups. Litter size was consistently decreased for the two test groups compared to controls, and the number of deaths between birth and day 5 was greater in the 0.5% group than in the control or 0.25% groups (Abbott and Harrisson, no date).

Groups of gravid CD rats were dosed (route not specified) with 0.05 or 0.1 ml Methyl Salicylate on days 10 and 11 of gestation, and either killed on day 21 of gestation or allowed to deliver (Woo and Hoar 1972). A control group was not treated. Test animals of the 0.1-ml group had decreased body weight

gain, fewer and smaller neonates, and more resorptions and malformed neonates. The kidneys of gestation day 21 fetuses and postnatal day 1, 6, 12, and 24 fetuses were examined. Fetal kidney weight was decreased in the treated groups compared to the controls. Methyl Salicylate inhibited lengthening of the renal papilla, and treated fetuses had a significant increase in the incidence of kidneys without papillae. There was little or no difference in neonatal kidney weights between test and control animals by postnatal day 6.

Groups of 24 to 27 Sprague-Dawley rats were fed a diet containing 4000 or 6000 ppm Methyl Salicylate and USP calcium carbonate for 60 days prior to mating (FDA 1966). The dams were fed the test diets until the neonates were weaned at day 20 or 21, and the procedure was repeated with a second mating. No abnormalities were observed in the offspring of test animals. Neonate survival at weaning was greater in the test groups than in the control group.

The reproductive effects of Methyl Salicylate were determined in a three-generation study using Osborne-Mendel rats (Collins, Hansen, and Keeler 1971). Concentrations of 500, 1500, 3000, and 5000 ppm synthetic Methyl Salicylate were mixed with chow and fed to groups of 10 males and 10 females ad libitum. Controls were given untreated chow. After 100 days of dosing, the animals (F_0 generation) were mated. Reproductive parameters were measured for the first litter (F_{1a}); these animals were killed at weaning. The F_0 parents were remated, and reproductive parameters were measured for the F_{1b} litter; 20 littermated pairs were selected to parent the next generation. The procedure was repeated for succeeding generations until the animals of the third generation were killed and necropsied. The effects on reproductive parameters are summarized in Table 9.

Gravid LVG hamsters were dosed orally with 175 mg/100 g at 7 days 9 h of gestation (Overman and White 1983). Controls were dosed with saline solution. Fetuses were recovered on day 9 of gestation. Plasma salicylate concentrations were determined. In 35 litters, 72% had neural tube defects. The plasma salicylate concentration peaked at 125 mg/100 ml 2 h after dosing and returned to control values within 8 to 10 h. Testing showed that salicylate was reaching the fetus.

Morrissey et al. (1989) reported on the results of 48 chemicals (including Methyl Salicylate) tested in the National Toxicology Program's Reproductive Assessment by Continuous Breeding (RACB) study using Swiss CD-1 mice. The study protocol begins with a 14-day dose ranging study (task 1), followed by the continuous breeding phase (task 2). In task 2, the animals were dosed for 7 days prior to mating and then during 98 days of mating and cohabitation. Task 3 is crossover mating, and task 4 is the second generation. In task 4, animal were reared by dams until weaning (postnatal day 21) and then dosed until mating at postnatal day 74. Task 2 used three treatment groups (20 animals per sex per group) and a control (40 animals per sex). Task 4 used the last litter in task 2 from the control and the high-dose group. Methyl Salicylate in corn oil was tested simultaneously in two laboratories. In one laboratory, doses were 25, 50, and 100 mg/kg/day by gavage (Research Triangle Institute 1984). In the other laboratory, the three doses were 100, 200, and 500 mg/kg/day (Environmental Health Research Testing, Inc. 1984). In both studies, corn oil alone served as the control. The RACB using 100 to 500 mg/kg/day found a decrease in live

TABLE 9

Effects of Methyl Salicylate on reproductive parameters in rats (Collins et al. 1971)

Reproductive parameter	Animals affected
Fertility index	No significant differences for any dose/1st generation; "appreciable decreases seen in 2nd and 3rd generations/5000 ppm
Average litter size/female	Significant decreases in 2nd generation/2nd mating/3000 ppm; significant decreases in 2nd generation/both matings/5000 ppm; decreases seen in 2nd generation/1500 ppm were not significant because of the large variation in progeny between females
Average no. liveborn pups/female	Significant decreases in 2nd generation/both matings/3000 and 5000 ppm
Viability index	"Possible loss of young through stillbirths" in 2 matings/5000 ppm
Average no. surviving progeny/female, day 4	Significant decreases in 2nd generation/both matings/3000 and 5000 ppm
Survival index, day 4	Adverse effect in 2nd generation/3000 & 5000 ppm and 3rd generation/1st mating/3000 and 5000 ppm
Average no. progeny weaned/female, day 21	Significant decrease in 2nd generation/1st mating/3000 ppm; significant decrease in 2nd generation/both matings/5000 ppm
Weaning index	"Appreciable decrease" in 2nd generation/2nd litter/5000 ppm
Average weanling weight, day 21	Consistent decreases/3000 and 5000 ppm
External examination	No grossly visible abnormalities
Necropsy—3rd generation weanlings	Negative findings, including microscopic examination of livers and kidneys of weanlings of control, 3000- and 5000-ppm groups

pups per litter, the percentage of live pups, and pup weight at 500 mg/kg/day. The RACB using 25 to 100 mg/kg/day found no effect on these parameters.

Lamb et al. (1997a, 1997b) further reported on these studies. In the RACB using 25 to 100 mg/kg/day (Lamb et al. 1997a), the last control group and 100-mg/kg/day group litters were dosed with Methyl Salicylate (100-mg/kg/day) until a task 4 mating as described above. There were no Methyl Salicylate related changes in the number of pups per litter, the percentage of live pups, or pup weight. The F_1 adults were necropsied and no effects were found on body or organ weights, and the motility, density, and morphological endpoints for sperm were normal.

Lamb et al. (1997b) further described results from the 100 to 500-mg/kg/day study, confirming the findings reported in Morrissey et al. (1989), but also indicating a 3% reduced pup weight for the 250-mg/kg/day dose group not reported in Morrissey et al. (1989). In an attempt to define the affected sex that led to positive reproductive toxicity findings in this study, a task 3 crossover mating was done using the control and 500-mg/kg/day dose group. There were no discernable effects.

Sodium Salicylate

Groups of two CFE rats were given a single oral dose of 500 mg/kg Sodium Salicylate in 0.9% saline on day 8 of gestation or daily doses of 100 mg/kg Sodium Salicylate on days 7 to 11 of gestation; the animals were killed on day 15 or 19 (Lansdown et al. 1970). Controls were given vehicle only. A single dose of 500 mg/kg resulted in 50% maternal toxicity. With this dose, the incidence of resorptions and dead fetuses was 53% and the incidence of malformations was 13%. None of the animals dosed over 5 days died. With this dosing regimen, the incidence of resorptions and dead fetuses was 15%; no malformations were seen. Aberrations in skeletal preosseous cartilage, particularly the cartilage matrix, were observed. The authors concluded that the findings suggest an inhibition of mucopolysaccharide synthesis during skeletal development.

Eriksson (1971) gave 25 gravid A/Jax mice a single oral dose of 66.6 mg/ml Sodium Salicylate in 1% sodium carboxymethylcellulose at a volume of 0.2 ml/20 g body weight on day 17 of gestation, and a control group was dosed with vehicle only. Five of the animals were killed 4 h after dosing and at least five fetuses per litter were used for hepatic glycogen determination. The remaining 20 dams were killed 24 h after dosing; one of the 20 delivered prior to being killed. In the animals killed 24 h after dosing, fetal mortality was 47% and the incidence of superficial, hepatic, and gastric hemorrhage was 6%, 1%, and 2%, respectively. Carboxymethylcellulose significantly decreased fetal hepatic glycogen, and dosing with Sodium Salicylate further decreased glycogen in a significant manner.

As described earlier, Waltman et al. (1973) studied the effects of anti-inflammatory drugs on parturition parameters in the rat. The results below were obtained in rats given 10 mg/kg Sodium Salicylate orally twice daily on days 20 and 21 of gestation. Time of onset of parturition, duration of parturition, bleeding during parturition, and perinatal mortality were noted. Sodium Salicylate had no significant effect on the time of onset of parturation compared to controls. The duration of parturition was increased in only five animals compared to controls. Bleeding at parturition was increased in five animals as compared to controls. Thirteen of the 121 pups born to these 10 animals were dead (compared to 4 of 109 pups in the control group).

Two groups of 21 gravid albino rats were dosed orally with 200 mg/kg Sodium Salicylate once daily on days 6 to 15 of gestation (Keplinger et al. 1974). Three control groups of 16 to 21 gravid rats were given 1.5% aqueous methylcellulose (vehicle) only. All animals were killed on day 20 of gestation.

In the second test group, a significant increase was observed in the number of resorption sites and the total number of females with one or more resorption sites (71.4%); the number of viable fetuses was significantly decreased in this group. No significant reproductive effects were observed in the first test group. Regarding fetal development, the number of fetuses with skeletal abnormalities was significantly increased in both test groups (67.8% and 75.9% in test groups 1 and 2, respectively) as compared to controls; the number of fetuses with external and internal abnormalities was significantly increased in the second test group (6.2% and 45.3%, respectively) but not in the first test group (Keplinger et al. 1974).

A group of 22 gravid CD-1 mice was dosed orally with 800 mg/kg once daily on days 8 to 12 of gestation (Chernoff and Kavlock 1982, 1983). A control group of 21 gravid mice was dosed with water only. All animals were allowed to deliver. Average neonatal weight, measured on days 1 and 3 of parturition, was decreased in test neonates as compared to controls.

Groups of gravid Sprague-Dawley and Long-Evans rats were dosed orally with 125 or 175 mg/kg Sodium Salicylate on days 8 to 10 of gestation; a control group was dosed with distilled water (Buelke-Sam et al. 1984). The litters were culled to eight neonates on postnatal day 1 and weaned on day 21. Locomotor activity was tested for 30 min in the dark on days 12, 16, 20, 24, 30, 60, 90, and 120 using both clean bedding and homecage bedding. Dosing did not affect maternal weight gain or length of gestation.

No malformations were noted in the neonates, and there were no significant differences in body weights on day 1. Male offspring had more salicylate-related activity changes compared to female offspring. Male Long-Evans test rats were less active than controls on test days 30+. Regarding dose and age interaction, the activity level was significantly decreased on days 20, 30, and 60 in high-dose male Long-Evans rats tested over homecage bedding, increased on day 12 and decreased on day 30 in Long-Evans female rats tested over clean bedding, increased on days 20 and 24 in low-dose male Sprague-Dawley rats tested over clean bedding, and increased on days 24, 30, and 90 in high-dose male Sprague-Dawley rats tested over clean bedding. The researchers concluded that the alterations in activity were the result of a complex interaction among dose, strain, offspring, sex, and bedding condition during testing (Buelke-Sam et al. 1984).

Gravid New Zealand White rabbits were dosed orally with 100 mg/kg Sodium Salicylate in water on days 4 to 7 of gestation (Fabro, McLachlan, and Dames 1984). The animals were killed on either day 8 or 28 of gestation, and the number of implantations and corpora lutea or the incidence of malformations was determined, respectively. Sodium Salicylate did not affect the preimplantation ratio in animals killed at 8 or 28 days, and it did not affect the average litter size of viable offspring or induce teratogenic effects in animals killed at 28 days.

Groups of 12 to 15 gravid albino rats were orally given Sodium Salicylate in tap water at a volume of 1 ml/100 g body weight at a dose of 25, 75, or 150 mg/kg on days 15 to 20 of gestation or at a dose of 4.2, 12.5, or 25 mg/kg on days 20 to 21 of gestation (Fritz and Suter 1985). The surviving neonates were weighed at various times through day 35, and behavioral tests were performed. The dams were killed after weaning and the neonates were killed on day 42.

Maternal body weight gain was comparable for all groups, and no signs of toxicity were observed. Parturition was delayed in one female of the control and 25-mg/kg group and two females of the 150-mg/kg group. Litter size and male-to-female ratios were similar for all groups. The neonatal mortality rate in the 150-mg/kg group dosed on days 15 to 20 and in the 12.5- and 25-mg/kg groups dosed on days 20 to 21 was increased in a dose-dependent manner. Body weight gains were similar between groups. No development abnormalities were observed (Fritz and Suter 1985).

Gravid CD-1 mice were dosed orally with Sodium Salicylate in distilled water at a volume of 0.5 ml on day 8 of gestation; 19 animals were given 2000 mg/kg and 37 were given 2600 mg/kg Sodium Salicylate (Kavlock, Chernoff, and Rogers 1985). A control group of 15 gravid animals was dosed with the vehicle. The animals were killed on day 18 of gestation. Maternal weight gain was significantly reduced in both test groups, and maternal mortality was 11% and 24% in the 2000- and 2600-mg/kg dose groups, respectively. Fetal weight was not affected.

The incidence of viable litters was 71% and 79% for the low- and high-dose groups, respectively. In one dam given 2600 mg/kg Sodium Salicylate, the whole litter was resorbed. The incidence of fetal mortality was 14% and 7% in the low- and high-dose groups, respectively, and 7% in the control group. The incidence of supernumerary ribs was significantly increased in fetuses of both test groups. However, in the 2000-mg/kg group, the number of fetal sternal ossifications was significantly decreased compared to controls. In the low-dose group, 7% of the fetuses and 17% of the litters had malformations; in the high-dose group, these values were 3% and 9%, respectively (Kavlock, Chernoff, and Rogers 1985).

Beyer and Chernoff (1986) dosed groups of gravid CD-1 mice and Sprague-Dawley rats orally with 1500 and 300 mg/kg Sodium Salicylate in distilled water, respectively, on day 7, 8, 9, 10, or 11 of gestation; controls were dosed with vehicle. The mice were killed on day 18 and the rats on day 21 of gestation.

Some of the mice died as a result of dosing; maternal toxicity was not seen in the surviving animals. Fetal weight gain was not affected, but fetal mortality was significantly increased with dosing on day 10. The number of extra ribs was significantly increased with dosing on days 8 and 9, and the combined effect of extra ribs and ossification sites was greater on day 9 than day 8. None of the rats died on study, and maternal toxicity was not seen. Fetal weight gain and the number of implantation sites was not affected by dosing. Extra ribs were induced significantly more with dosing on day 10 than any other day, and ossification sites were also seen more frequently. Cervical ribs were significantly increased with dosing on day 8 (Beyer and Chernoff 1986).

A group of 30 gravid ICR/SIM mice were dosed orally with 1600 mg/kg Sodium Salicylate in distilled water on days 8 to 12 of gestation, and a control group of 30 gravid mice was given vehicle only (Seidenberg, Anderson, and Becker 1986). All animals were allowed to deliver. Seven animals died as a result of dosing. Maternal weight gain was significantly decreased compared to controls. Percent neonate survival (96%) was significantly decreased compared to controls. The average number of viable neonates per litter was significantly decreased on days 1 and 3 of parturition and the number of dead neonates per litter was significantly increased on day 1. Average neonatal weight was similar to controls.

Groups of 17 to 19 gravid Sprague-Dawley rats were dosed orally with 30, 90, or 180 mg/kg Sodium Salicylate in distilled water on days 6 to 15 of gestation; a control group was dosed with vehicle only (Fritz and Giese 1990). The dose volume was 1 ml/100 g. The animals were killed on day 21 of gestation. Some reduction in feed consumption was observed in the 180-mg/kg dose group. As indicated by decreased fetal body weight and retarded skeletal maturation, growth was dose-dependently decreased in the 90- and 180-mg/kg groups. Teratogenicity occurred at a rate of 0.7% and 30% in these groups, respectively. The most prominent malformation in the high-dose group was cranio(rachi)schisis. In the mid-dose group, no embryotoxicity or maternal toxicity was observed. In the high-dose group, marked embryotoxicity and low maternal toxicity were observed.

Davis et al. (1996) dosed gravid Sprague-Dawley rats by gavage twice daily on days 15 to 21 of gestation with Sodium Salicylate to determine the effect on reproduction. Groups of 25 animals received 20 or 80 mg/kg/day and a group of 16 animals received 200 mg/kg/day Sodium Salicylate in 0.5% aqueous methyl cellulose. One-half of the dose was administered in the morning and the other half was given 6 to 8 h later; dose volume was 10 ml/kg/ "dosing occasion." A group of 16 gravid rats was dosed orally with 260 mg/kg/day acetylsalicylic acid (ASA) in methyl cellulose twice daily on days 15 to 21 of gestation. The animals were observed twice daily until day 21 of gestation; as of day 21, the animals were observed hourly for the onset of labor. Surviving F_0 dams and F_1 neonates were killed on day 1 of lactation.

The onset and the duration of labor were increased in animals dosed with 200 mg/kg Salicylic Acid and those dosed with ASA; the delay in the onset of labor in the Sodium Salicylate group was not statistically significant. A nonstatistically significant increase in fetotoxicity and peripartum mortality was also observed for the 200-mg/kg Sodium Salicylate group; a significant increase in neonates born dead and peirpartum death was seen with ASA.

The researchers stated that "it is likely that the observed increased peripartum death of fetuses in these dose groups is associated with complications of prolonged labor, since those pups that did not survive delivery had no visible abnormalities or signs of overt toxicity. However, the pups were not examined for the development of hemorrhage." A statistically significant increase in maternal perinatal death was reported in animals of the 200-mg/kg Sodium Salicylate and ASA groups. A statistically significant decrease in gestational index was also observed as a result of increased maternal death (Davis et al. 1996).

In Vivo Parenteral Studies

Salicylic Acid

A group of 17 gravid Sprague-Dawley rats was given an SC injection of 380 mg/kg Salicylic Acid on day 9 of gestation; the dose was injected in two equally divided doses 2 h apart (Koshakji and Schulert 1973). A group of 15 controls was given deionized water. Immediately following the second dose, mineral isotopes of ^{54}Mn, ^{65}Zn (both carrier free), and ^{59}Fe (27 mCi/mg) were given subcutaneously. Urine was collected and assayed for mineral isotopes. The animals were killed on day 20 of gestation.

Effects of dosing included "loss of appetite, complete relaxation, weakness, drowsiness, muscular limpness, inactivity, accelerated respiration rate, and occasionally elevated water intake and urinary excretion." Marked maternal weight loss and death of one test animal were observed after dosing. In the test animals, mean fetal weight was significantly decreased compared to controls. Administration of Salicylic Acid resulted in 46.6% resorptions and in 5.3% of the viable fetuses being malformed. The researchers stated that "none of the other metabolites, derivatives or analogs of salicylic acid [that were also tested] resulted in fetal anomalies." Salicylic Acid did not affect the urinary excretion of ^{54}Mn, ^{59}Fe, or ^{65}Zn.

These authors also gave groups of three or four gravid Sprague-Dawley rats an SC injection of 300 or 380 mg/kg ^{14}C-Salicylic Acid in two equal doses 2 h apart on day 16 of gestation followed by SC injection of ^{54}Mn, ^{59}Fe, and ^{65}Zn. The animals were killed 6 or 24 h after dosing. Salicylic Acid did not affect the maternal-fetal uptake of the minerals. An increased ^{65}Zn content in the liver 6 h after administration was the only difference observed compared to control values. Administration of 380 mg/kg Salicylic Acid on day 16 of gestation resulted in three incidents of hematuria, a "high rate" of fetal mortality, and superficial hemorrhage which was occasionally observed along the brain and spine (Koshakji and Schulert 1973).

Methyl Salicylate

Gravid female rats were given a single SC dose of 0.1 to 0.5 cc Methyl Salicylate on day 9, 10, or 11 of gestation (Warkany and Takacs 1959). Twenty-six dams died and 47 resorbed their fetuses. The remaining 43 dams were killed on day 21 of gestation. External abnormalities were seen in 45 of 298 fetuses. Skeletal anomalies were seen in 75 of 253 fetuses which appeared externally normal. No information was provided as a function of dose.

The effect of fetal growth retardation on organ differentiation was examined using groups of five gravid CD rats (Kavlock et al. 1982). The animals were dosed intraperitoneally with 200 or 400 mg/kg Methyl Salicylate on days 8 to 9 and killed on day 20 of gestation. A negative-control group was used.

Embryotoxicity was observed in the high-dose group. Average fetal mortality was 2% and 50% for the 200- and 400-mg/kg groups, respectively. In the 200-mg/kg group, one fetus had a diaphragmatic hernia and two had encephalocele. In the 400-mg/kg group, one incidence each of cleft palate and hydrocephaly and two incidences each of encephalocele, gastroschisis, and spina bifida were observed. The fetal body weight index was significantly reduced in the 400-mg/kg group, with a delay of 0.96 day. Dose-related reductions were observed in brain weight, lung growth, hepatic growth, and renal weight. The reduction in kidney development was related to growth retardation.

In a study to determine the effect of Methyl Salicylate on renal function, groups of 5 to 16 gravid Sprague-Dawley rats were dosed intraperitoneally with 250 to 450 mg/kg on day 11, 200 to 300 mg/kg on days 10 to 11, 300 to 375 mg/kg on days 11 to 12, or 200 to 300 mg/kg Methyl Salicylate on days 11 to 13 of gestation (Daston et al. 1988). A control group was dosed with 5 ml/kg 0.85% saline on days 10 to 13 of gestation. All animals were killed on day 20 of gestation. Maternal toxicity was observed in many of the dose groups, and a few, non–dose-related, maternal deaths occurred.

Malformations were observed in fetuses of groups dosed with $\geq$350 mg/kg on day 11 of gestation or with $\geq$300 mg/kg on more than 1 day. The incidence of resorptions was significant in the 400-mg/kg group dosed on day 11 of gestation. Fetal weight was significantly reduced in a dose-related manner. Methyl Salicylate did affect kidney development, but there was no relationship between the incidence of dilated renal pelvis and Methyl Salicylate.

These authors also dosed gravid Sprague-Dawley rats intraperitoneally with 200, 250, or 300 mg/kg Methyl Salicylate on days 10 to 13 of gestation. A high incidence of maternal mortality was seen in the 300-mg/kg dose group. During postnatal days 1 to 2, neonate mortality was increased in the 250- and 300-mg/kg groups; no external abnormalities were seen in the surviving pups, and weights were similar to control values. No effect on average litter size or birth weight was observed in the 200 mg/kg group. Relative kidney weights were significantly increased in all test groups on postnatal day 15; however, no difference was seen at week 4. Renal defects were "rarely

observed." Neonatal urinary parameters were not affected by prenatal dosing with Methyl Salicylate, but some effect on the urine concentrating ability was seen in young neonates (Daston et al. 1988).

Sodium Salicylate

Jackson (1948) examined the effect of Sodium Salicylate on gravid rats and rabbits. Groups of one to five rats were given a single SC dose of 0.20 to 0.75 g/kg Sodium Salicylate during the last week of pregnancy, and the animals were killed 2 days later. The maternal death rate was 2/5 and 3/3 animals of the 0.50- and 0.75-g/kg dose groups, respectively. In the surviving animals, all the fetuses survived. Using gravid rabbits, four were dosed subcutaneously with 0.5 and two with 1.0 g/kg Sodium Salicylate as a single dose during the last week of pregnancy, and again the animals were killed 2 days later. All of the animals of the 1.0-g/kg and one of the animals of the 0.5-g/kg dose group died. Of a total of 23 fetuses in the surviving rabbits, 19 survived.

Gravid female rats were given a single SC dose of 60 to 180 mg Sodium Salicylate on day 9, 10, or 11 of gestation (Warkany and Takacs 1959). Six dams dosed with >120 mg died (no other information given as a function of dose); 24 animals resorbed their fetuses. Thirteen surviving animals were pregnant on day 21 of gestation, at which time the animals were killed. External abnormalities were seen in 15 of 100 fetuses, and skeletal anomalies were seen in 11 of the 85 fetuses that appeared externally normal.

Groups of 9 to 42 gravid Sprague-Dawley rats, housed 3 per cage, were dosed subcutaneously with 200 to 500 mg/kg Sodium Salicylate on day 10 and killed on day 20 of gestation (Goldman and Yakovac 1963). "Significant numbers" of anomalies were observed in fetuses of the 400- and 500-mg/kg dose groups.

Gravid A/Jax mice were given a single IM dose of 10 mg Sodium Salicylate in 0.1 ml distilled water on day 7, 8, 9, 10, 11 , 12, or 13 of gestation, and the animals were killed on day 18 (Larsson, Boström, and Ericson 1963). "A high incidence of external anomalies" was observed in fetuses of animals dosed on day 12 or 13 of gestation. The "appearance of reddish-brown spots on the nose, chin, and paws," which was "a large mass of blood enclosed in a thin-walled capsule," was observed in a number of fetuses. A "high incidence of deformities of ribs and vertebrae" was observed in all dose groups.

Gravid A/Jax and CBA mice were given a single IM injection of 10 mg Sodium Salicylate in 0.1 ml distilled water on either day 9 or 12 of gestation, and the animals were killed on day 18 of gestation (Larsson and Boström 1965). Untreated animals were used as controls. The incidence of resorption was 18.1% and 41.3% in A/Jax mice dosed on days 9 and 12, respectively; the incidence in controls was 10.6%. The incidence of resorption in CBA mice dosed on days 9 and 12 of gestation, 5.9% and 4.4% respectively, was less than that observed in control animals (9.6%). The incidence of vessel anomalies in animals dosed on day 12 of gestation was 11.9% and 0% for A/Jax and CBA mice, respectively; no vessel anomalies were seen in any of the controls.

In animals dosed on day 9 of gestation, the incidence of rib anomalies was 52.2% and 16.7% for A/Jax and CBA mice, respectively, as compared to 1.4% and 0% in controls, respectively. The incidence of vertebral anomalies was 33.3% and 4.0% in A/Jax and CBA mice, respectively, as compared to 1.9% and 0% in the respective controls (Larsson and Boström 1965).

Larsson and Eriksson (1966) gave groups of five to eight gravid A/Jax and CBA mice a single IM dose of Sodium Salicylate, 10 mg/20 g body weight in 0.1 ml distilled water, on either day 9, 11, 13, 15, or 17 of gestation. Animals were mated within and across strains. The animals were killed on day 18 of gestation.

Seven A/Jax mice, five of which were mated to CBA males, delivered prior to being killed on day 18; all seven had been given Sodium Salicylate on day 17 of gestation. In A/Jax females, the incidence of resorption generally increased the later Sodium Salicylate was administered. In A/Jax females mated with A/Jax males, the incidence of resorption was 19%, 40%, 67%, 74%, and 73% with injection on days 9, 11, 13, 15, and 17, respectively. In A/Jax females mated with CBA males, the incidence was 0%, 14%, 15%, 41%, and 25%, respectively. The incidence of resorption was much less in CBA mice. In CBA females mated with CBA males, the incidence of resorption was 8%, 3%, 7%, 9%, and 13% with injection on days 9, 11, 13, 15, and 17, respectively, and in CBA females mated with A/Jax males, the incidence was 0%, 9%, 7%, 5%, and 8%, respectively.

The following percent of offspring from dams dosed on the following days had vessel anomalies: day 9, CBA females × A/Jax males—2%; day 13, A/Jax females × A/Jax males—6% and A/Jax females × CBA males—6%; day 15, A/Jax females × A/Jax males—58% and A/Jax females × CBA males—41%; day 17, CBA females × CBA males—3%, A/Jax females × CBA males—3%, and CBA females × A/Jax males—8%.

Skeletal malformations were observed primarily in neonates of dams dosed on day 9 of gestation. Rib anomalies were observed in 49%, 53%, 47%, and 24% of the neonates from A/Jax × A/Jax, CBA × CBA, A/Jax × CBA, and CBA × A/Jax animals that were dosed on day 9 of gestation, respectively, and vertebral anomalies were observed in 35%, 12%, 23%, and 2% of these neonates, respectively. Three percent of the neonates from A/Jax × A/Jax animals dosed on day 11 had rib anomalies and 3% from CBA × CBA animals dosed on day 13 had rib as well as vertebral anomalies. No other neonates had skeletal anomalies. Encephaly and/or gastroschisis were observed in six neonates from CBA × A/Jax animals after dosing on days 9 to 15 of gestation, and exencephaly was observed in one neonate from a CBA × CBA animal. The authors noted that cleft lip was occasionally observed (Larsson and Eriksson 1966).

Eriksson and Larsson (1968) gave a group of 10 gravid A/Jax mice a single IM injection of 100 mg/ml Sodium Salicylate at a dose of 0.1 ml/20 g body weight on day 17 of gestation. Five of the animals delivered on day 17 of gestation, two delivered on day 18, and three delivered on day 19. In the untreated control group three, six, and one gravid animal delivered on days 18,

19, and 20 of gestation, respectively, and in the saline-treated control group, three, three, and four gravid animals delivered on days 18, 19, and 20 of gestation, respectively.

Eriksson (1969) gave groups of 10 to 36 gravid A/Jax and CBA mice a single IM dose of 10 mg/20 g body weight Sodium Salicylate in 0.1 ml of distilled water on day 16, 17, or 18 of gestation; the animals dosed on day 16 or 18 were killed 8 or 24 h after dosing and the animals dosed on day 17 were killed 2, 4, 8, 12, or 24 h after dosing. Of the animals dosed on day 17, one CBA mouse that was to be killed after 8 h and two A/Jax and three CBA mice that were to be killed after 24 h delivered. Of the animals dosed on day 18, all seven A/Jax and all five CBA mice scheduled to be killed after 24 h delivered.

A higher percentage of fetal mortality was observed with A/Jax mice. In this strain, fetal mortality on day 16 of gestation was 46% and 43% after 8 and 24 h, respectively, and on day 17 was 0%, 0%, 19%, 54%, and 39% after 2, 4, 8, 12, and 24 h, respectively. In the CBA groups, fetal mortality on day 16 of gestation was 3% and 7% after 8 and 24 h, respectively, and on day 17 was 0%, 5%, 24%, and 13% after 6, 8, 12, and 12 h, respectively. No fetal mortality was observed 8 h after dosing on day 18.

In A/Jax mice, the incidence of superficial hemorrhage along the spine on day 16 of gestation was 42% and 56% in viable fetuses 8 and 24 h after dosing, respectively, and on day 17 was 3%, 35%, 21%, 52%, and 20% in viable fetuses 2, 4, 8, 12, and 24 h after dosing, respectively. In the CBA groups, the incidence of superficial hemorrhage on day 16 of gestation was 49% and 21% in viable fetuses after 8 and 24 h, respectively, and on day 17 was 33%, 56%, 36%, and 7% in viable fetuses after 6, 8, 12, and 12 h, respectively. No superficial hemorrhages were observed in viable fetuses 8 h after dosing on day 18. Superficial hemorrhage was observed in all dead animals that were examined. In A/Jax mice, the incidence of hepatic hemorrhage on day 16 of gestation was 19% and 26% in viable fetuses 8 and 24 h after dosing, respectively, on day 17 was 3%, 3%, 16%, 30%, and 20% in viable fetuses 2, 4, 8, 12, and 24 h after dosing, respectively, and on day 18 was 2% in viable fetuses after 8 h. In the CBA groups, the incidence of hepatic hemorrhage on day 16 of gestation was 11% and 0% in viable fetuses after 8 and 24 h, respectively; on day 17 was 3%, 6%, 0%, and 0% in viable fetuses after 6, 8, 12, and 12 h, respectively; and on day 18 was 0% in viable fetuses after 8 h. No hepatic hemorrhages were observed in viable fetuses 8 h after dosing on day 18. All dead fetuses except three had hepatic hemorrhage (Eriksson 1969).

Five CFE rats were given a single SC dose of 500 mg/kg Sodium Salicylate in 0.9% saline on day 8 of gestation and two rats were given daily SC doses of 100 mg/kg Sodium Salicylate on days 7 to 11 of gestation; the animals were killed on day 15 or 19 (Lansdown 1970). Controls were given vehicle only. A single dose of 500 mg/kg resulted in 40% maternal toxicity. With this dose, the incidence of resorptions and dead fetuses was 3% and the incidence of malformations was 6%. None of the animals dosed over 5 days died. With daily dosing, the incidence of resorptions and dead fetuses was 40% and the incidence of malformations was 10%. Aberrations in skeletal preosseous cartilage, particularly the cartilage matrix, were observed. The authors concluded that their findings suggested an inhibition of mucopolysaccharide synthesis during skeletal development.

Eriksson (1970) gave groups of 10 gravid A/Jax mice a single IM injection of 3, 10, or 15 mg Sodium Salicylate/20 g body weight in 0.1 ml distilled water on day 17 of gestation and the animals were killed on day 18 of gestation. Four animals of the 15-mg group died within 24 h of dosing, and four animals of this group delivered prior to being killed. Fetal mortality was 4%, 70%, and 100% in the 3, 10, and 15 mg groups, respectively. In the 10-mg group, the incidence of superficial, hepatic, and gastric hemorrhage in living fetuses was 39%, 13%, and 22%, respectively. No hemorrhages were observed in the 3-mg or control groups.

This author also dosed groups of 10 to 20 gravid A/Jax mice with 10 mg Sodium Salicylate/20 g body weight as a single IM injection on day 15, 16, or 17 of gestation (groups 1, 2, and 3, respectively) or as multiple IM injections on days 15, 16, and 17 of gestation (group 4), while another group was dosed with 3 mg/20 g on days 15 and 16 and 10 mg/20 g on day 17 of gestation (group 5). (Ten of the 20 animals of group 3 were from the study described above.) One animal of group 2, two of group 3, and one of group 4 delivered before being killed. The incidences of fetal mortality, hemorrhages in viable fetuses, and vessel anomalies in viable fetuses are summarized in Table 10 (Eriksson 1970).

Five CFE rats were given a single SC dose of 500 mg/kg Sodium Salicylate in 0.9% saline on day 8 of gestation and two rats were given daily SC doses of 100 mg/kg Sodium Salicylate on days 7 to 11 of gestation; the animals were killed on day 15 or 19 (Lansdown 1970). Controls were given vehicle only. A single dose of 500 mg/kg resulted in 40% maternal toxicity. With this dose, the incidence of resorptions and dead fetuses was 3% and the incidence of malformations was 6%. None of the animals dosed over 5 days died. With daily dosing, the incidence of resorptions and dead fetuses was 40% and the incidence of malformations was 10%. Aberrations in skeletal preosseous cartilage, particularly the cartilage matrix, were observed. The authors stated that the findings seem to suggest that mucopolysaccharide synthesis had been inhibited during skeletal development.

Gravid Sprague-Dawley rats were given an SC injection of 50 or 100 mg/kg Sodium Salicylate 18 h prior to being killed on day 22 of gestation and gravid Havana rabbits were dosed subcutaneously with 50 mg/kg Sodium Salicylate 18 h prior to being killed on day 30 of gestation (Sharpe, Larsson, and Thalme 1975). Neonatal rats were whole-body frozen immediately or at 15, 30, or 60 min following delivery, and neonatal rabbits were frozen immediately upon delivery. In the rats, contraction of the intrauterine ductus was significant in neonates from both dose groups at 0, 15, and 30 min and from the 100-mg/kg dose group at 60 min as compared to controls. In test neonatal rabbits, ductal

TABLE 10

Incidence of fetal mortality, hemorrhage, and vessel anomalies in mice treated with Sodium Salicylate (Eriksson 1970)

IM dosing	No. of Litters	Fetal mortality (%)	Superficial hemorrhage (%)	Hepatic hemorrhage (%)	Gastric hemorrhage (%)	Vessel anomalies (%)
10 mg/20 g on gestation day 15	10	39	2	0	0	27
10 mg/20 g on gestation day 16	10	55	42	0	0	0
10 mg/20 g on gestation day 17	20	61	27	12	31	0
10 mg/20 g on gestation days 15, 16, and 17	20	47	4	5	9	33
3 mg/20 g on gestation days 15 and 16; and 10 mg/20 g on gestation day 17	20	21	3	12	36	0

contraction was observed and ductal diameter was one-fifth that of controls.

Groups of 19 to 20 gravid Lakeview outbred (Lak:LVC) golden hamsters were given a single SC injection of Sodium Salicylate on day 8 of gestation, and the animals were killed on day 12 of gestation (Geber 1977). The minimal effective teratogenic dose was 89 mg/kg Sodium Salicylate, which induced 2.8% congenital malformations. Doses of 37 and 45 mg/kg did not produce any congenital malformations.

Groups of six to eight gravid ferrets were given a single SC injection of 125, 250, or 400 mg/kg Sodium Salicylate on day 13 or 18 of gestation, and all animals were killed on day 35 of gestation (Gulamhusein et al. 1980). Control animals were dosed subcutaneously with 0.9% saline. No maternal toxicity was observed. Mean fetal weight was significantly decreased in all test groups compared to controls. The resorption rates were 6%, 33%, 31%, and 91% in the control, 125-, 250-, and 400-mg/kg dose groups dosed on day 13. Resorption rates were 6%, 43%, 37%, and 66% in the control, 125-, 250-, and 400-mg/kg group respectively on day 18.

The incidence of external and internal abnormalities in surviving fetuses was 2%, 11%, 0%, and 86% in the control, 125-, 250-, and 400-mg/kg dose groups respectively dosed on day 13. The incidence of external and internal abnormalities in surviving fetuses of animals dosed on day 18 was 2%, 7%, 33%, and 96% in the control, 125-, 250-, and 400-mg/kg dose groups respectively.

The results in ferrets were compared to those in Wistar rats following a single SC dose of 400 mg/kg Sodium Salicylate given on day 8.5 or 11.5 of gestation; groups of 5 to 10 gravid animals were used. The rats were killed on day 20.5 of gestation. Mean fetal body weights were significantly reduced in both test groups. The resorption rates were 23% and 9% and the incidence of external and internal abnormalities in surviving fetuses was 19% and 11% with dosing on days 8.5 and 11.5, respectively. Sodium Salicylate was more embryotoxic in ferrets than in rats (Gulamhusein et al. 1980).

Gravid golden hamsters were given a single IP dose of 1100 mg/kg Sodium Salicylate on day 8 and killed on day 15 of gestation (Beyer and Geber 1984). Control animals were dosed with saline. A trend toward increased mean lateral ventricle size was observed in test animals as compared to controls.

Gabrielsson et al. (1985) gave groups of gravid Sprague-Dawley rats IV injections of Sodium Salicylate either as single injections or as a constant infusion. Groups of three to nine animals were given single injections of 15, 50, 100, 200, or 500 mg/kg Sodium Salicylate on day 6 of gestation, and blood samples were taken at various intervals from 1 min to 30 h after dosing. Groups of 11 animals were given a single daily IV dose of 75 or 150 mg/kg Sodium Salicylate on days 6 to 13 of gestation, and five animals were given single daily IV doses of 150 mg/kg on days 13 to 19 of gestation. Fourteen or 12 animals were dosed via constant infusion with 1 or 2 mg/h (corresponding to 75 or 150 mg/kg/day) Sodium Salicylate, respectively, on days 6 to 13 of gestation, and blood samples were taken on the days of dosing. Eleven animals were constantly infused with 150 mg/kg/day Sodium Salicylate on days 13 to 19 of gestation. A control group of 10 animals was infused with saline on days 6 to 13 of gestation. The animals were killed on day 19 of gestation.

In the animals given a single IV dose of Sodium Salicylate on day 6 of gestation, fetal body weights were decreased in the 50 to 500-mg/kg test groups when compared to the low-dose group. Compared to the controls, a significant decrease in fetal weight was observed in animals given a continuous infusion of 150 mg/kg and in animals given daily doses of 150 mg/kg on days 6 to 13 of gestation.

The incidence of resorbed and dead fetuses was 0%, 4%, 11.6%, 15%, and 47.6% in the 15-, 50-, 100-, 200-, and 500-mg/kg dose groups, respectively. The incidence was 42% and 81% in the animals dosed daily with 75 and 150 mg/kg/day, respectively,

on days 6 to 13 of gestation and 0% in the animals dosed with 150 mg/kg/day on days 13 to 19 of gestation.

The incidence of resorbed and dead fetuses in animals given continuous infusions of Sodium Salicylate was 4% and 73% in the animals dosed with 75 and 150 mg/kg/day, respectively, on days 6 to 13 of gestation and 6% in the animals dosed with 150 mg/kg/day on days 13 to 19 of gestation. The incidence in controls was 3% (Gabrielsson et al. 1985).

Lukas et al. (1987) gave groups of gravid New Zealand white rabbits a continuous IV infusion of Sodium Salicylate on days 22 to 29 of gestation. The animals were placed in the infusion harnesses on day 19 for purposes of acclimation. Four animals were infused with 60 mg/ml Sodium Salicylate in sterile water at a rate of 1.2 ml/h, with a target maternal plasma salicylate concentration of 10 to 15 mg/dl, and three animals were infused with 80 to 120 mg/dl at the same rate, with a target maternal plasma salicylate concentration of >20 mg/dl. A control group of three animals were infused with normal saline at the rate of 1.2 ml/h. Daily maternal blood samples were drawn to monitor maternal plasma concentrations. The animals were killed on day 29 of gestation.

Maternal weight decreased in the test and control groups. Average litter size was not affected by Sodium Salicylate administration. The only fetal mortality was observed in the high-dose group, in which there were three fetal deaths; this was not significant. The fetal/maternal Sodium Salicylate concentration ratios were 1.02 and 0.86 in the low- and high-dose groups, respectively. Mean fetal weights, fetal crown-rump length, the ratio of fetal weight per centimeter crown-rump length, placental weight, and absolute and relative liver weights were significantly decreased in both test groups as compared to controls. The relative placental weight was significantly increased in the high-dose group. Absolute brain weight was significantly decreased in the high-dose group and the relative brain weight was significantly increased in both test groups compared to controls (Lukas et al. 1987).

The effect of a single IP injection of Sodium Salicylate on fetal joint development was examined using gravid BALB/c mice (Erdoğan, Kadioğlu, and Peker 1996). A dose of 500 mg/kg was administered on day 10 of gestation. Dosing with Sodium Salicylate resulted in lost articulatio cubiti joint spaces and surfaces, fusion between the humerus-radius and ulna, disappearance of some carpometacarpal joint spaces, absence of the fifth phalanx, overgrowth in tibia condyles, and occasional fusions in tarsometatarsal joints and between metatarsal bones with an absence of phalanges.

Table 11 summarizes the in vivo reproductive and developmental toxicity studies described in this section.

Risk Assessment

Corby (1978) examined the possible teratogenic effects of aspirin by reviewing retrospective studies of aspirin consumption during pregnancy. This author concluded that, although direct conclusive evidence of adverse effects in humans is lacking, a potential hazard does exist and thus, the indiscriminate use of aspirin during pregnancy should be contraindicated.

The Procter and Gamble Company (1999a) developed a risk assessment addressing the safety of facial cosmetic products containing ≤2% Salicylic Acid using oral studies on ASA (aspirin) as well as the conclusions of the Teratogen Information Service (TERIS), a computerized database designed to assist physicians or other healthcare professionals in assessing the risks of possible teratogenic exposures in pregnant women. The assessment stated that exposure to cosmetic products intended for use in the face/neck area (daily use) is expected to be in the range of 1.1 g/day, with 95th percentile users applying 1.4 g/day. For a 58-kg female, the average product use would then be approximately 19 mg product/kg/day, with 95th percentile users applying 24 mg product/kg/day. If the product contained 2% Salicylic Acid, this would correspond to topical applied doses of 0.38 and 0.48 mg Salicylic Acid/kg/day, respectively. The assessment stated that oral ingestion of "baby aspirin" (containing 81 mg ASA/62 mg Salicylic Acid) would yield an exposure of 1.05 mg/kg for a 58-kg female. Therefore, systemic salicylate exposure from a facial cosmetic product containing 2% Salicylic Acid is expected to be in range of ~20% of that following ingestion of a single baby aspirin, which the authors asserted was a salicylate dose widely recognized as carrying no maternal or fetal risk. Additionally, the risk assessment concluded that availability of Salicylic Acid from cosmetic products is low and concomitant use of such products with other topical Salicylic Acid containing products would not substantially increase the risk of developmental or reproductive toxicity.

Modulation of Salicylate-Induced Reproductive Effects

Oral Studies

Salicylic Acid

Cekanova et al. (1974) dosed gravid NMRI mice orally with 500 or 1000 mg/kg Salicylic Acid, 500 mg/kg Salicylic Acid plus 500 mg pyridyl-3-methanol, or 1000 or 2000 mg/kg of an ester of Salicylic Acid plus pyridyl-3-methanol (the combination was referred to as S-2063) in 1% carboxylmethyl cellulose at a volume of 0.2 ml/20 g. Groups of 11 to 14 animals were dosed on day 9 and groups of 5 to 13 animals were dosed on day 17 of gestation. Controls were dosed with vehicle. The animals were weighed on day 0 and days 9 to 18, and killed on day 18 of gestation.

Four of the animals dosed with 1000 mg/kg Salicylic Acid and three of those dosed with 2000 mg/kg S-2063 mixture on day 9 of gestation, and three of the animals dosed with 1000 mg/kg Salicylic Acid and three dosed with 2000 mg/kg S-2063 mixture on day 17 of gestation died after dosing. One female of the 500 mg/kg Salicylic Acid and one of the 1000 mg/kg S-2063 mixture dose groups delivered prematurely.

In the animals dosed on day 9, the incidence of resorbed fetuses prior to day 17 was 16.0%, 19.5%, 19.5%, 12.6%, and 25.3% for the 500- and 1000-mg/kg Salicylic Acid groups, the Salicylic Acid and pyridyl-3-methanol group, and the 1000- and

TABLE 11
Reproductive and developmental toxicity studies

Animals	Dose	Methods	Results	Reference
Dermal exposure to:				
Methyl Salicylate 12 rats/group	1, 3, or 6 g/kg of a petroleum-based grease using 3% Methyl Salicylate	Dermal applications were made on gestation days (GDs) 6–15; positive controls were dosed dermally with 2 and 1 g/kg Methyl Salicylate on GDs 6–9 and 10–15; dose was changed due to maternal toxicity (tox.)	No maternal toxicity and no changes in reproductive parameters or malformations were seen; positive controls had 100% incidence of total resorptions	Infurna et al. 1990
LVG hamsters	350 or 525 mg/100 g	Methyl Salicylate was applied to the back of each animal at 7 days 9 h, and the skin was washed 2 h after dosing; fetuses were recovered on d 9 of gestation	Neural tube defects were seen in 6% and 53% of the low- and high-dose litters, respectively	Overman and White 1983
Oral exposure to:				
Salicylic Acid 20 Wistar rats/group	0.06%, 0.1%, 0.2%, or 0.4% in feed	Animals were fed test diets on GDs 8–14; 15 animals/group were killed on GD 20; 5 animals/group delivered	Maternal mortality was 0%; neonatal mortality was 71% in the 0.4% group; significant reproductive effects were seen in the 0.4% group; skeletal anomalies were seen in the 0.2% group; only one dam gave birth to live neonates in the 0.4% group and skeletal anomalies were seen in 0.2% neonates	Tanaka et al. 1973a
20 Wistar rats/group	75, 150, or 300 mg/kg	Animals were dosed orally once daily on GDs 8–14; 15 animals/group were killed on GD 20; 5 animals/group delivered	3 dams of the 300-mg/kg group died; fetal mortality was 26% and 100% in the 150- and 300-mg/kg groups; significant reproductive effects were seen in 150-mg/kg fetuses and neonates	Tanaka et al. 1973b
10 Sprague-Dawley (SD) rats/group	10 mg/kg	Animals were dosed twice daily on GD 20 and 21	The mean gestation period was increased	Waltman et al. 1973
Sodium Salicylate NZW rabbits	100 mg/kg	Animals were dosed on GDs 4–7 and killed on GD 8 or 28	The preimplantation ratio and average litter size were not affected; teratogenic effects were not induced	Fabro et al. 1984

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TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Animals	Dose	Methods	Results	Reference
21 albino rats/group	200 mg/kg (2 groups)	Animals were dosed on GDs 6–15 and killed on GD 20	Significant increase in resorptions and decrease in viable fetuses seen in 1 group; in external and internal abnormalities significanutly increased in 2nd group; and skeletal anomalies in both groups	Keplinger et al. 1974
17–19 SD rats/group	30, 90, or 180 mg/kg	Animals dosed on GDs 6–15 and killed on GD 21	Teratogenicity was 30% in the 180-mg/kg group, and marked embryotoxicity occurred; maternal toxicity was low; growth decreased in the 90- and 180-mg/kg groups (dose dependent)	Fritz and Giese 1990
CD-1 mice SD rats	Mice: 1500 mg/kg Rats: 300 mg/kg	Animals were dosed on GD 7, 8, 9, 10, or 11; mice were killed on GD 18 and rats on GD 21	Mice: fetal mortality increased with dosing on day 10; skeletal anomalies increased w/dosing on days 8 and 9 Rats: skeletal anomalies increased with dosing on day 8 and 10	Beyer and Chernoff 1986
19 or 37 CD-1 mice	2000 and 2600 mg/kg	Animals were dosed on GD 8 and killed on GD 18	2000 mg/kg: 11% maternal mortality, 71% viable litters, 14% fetal mortality; 7% of fetuses with malformations; 2600 mg/kg: 24% maternal mortality, 79% viable litters, 7% fetal mortality; 3% of fetuses with malformations	Kavlock et al. 1985
2 CFE rats/group	500 or 100 mg/kg	Animals were given a single dose of 500 mg/kg on GD 8 and 100 mg/kg on GDs 7–11 and killed on GD 15 or 19	500 mg/kg: 50% maternal toxicity; 53% resorptions and dead fetuses, 13% malformations; 100 mg/kg: 15% incidence of resorptions and dead fetuses	Lansdown et al. 1970
22 CD-1 mice	800 mg/kg	Animals were dosed on GDs 8–12 and allowed to deliver	Average neonatal weight was decreased on postnatal days 1 and 3	Chernoff and Kavlock 1982, 1983
30 ICR/SIM mice	1600 mg/kg	Animals were dosed on GDs 8–12 and allowed to deliver	Seven dams died; neonate survival and average number of viable neonates/litter on days 1 and 3 was significantly decreased and number of dead neonates/ litter on day 1 was significantly increased	Seidenberg et al. 1986
25 A/Jax mice	66.6 mg/ml	Animals were dosed on GD 17; 5 dams were killed 4 h and the remaining 20 were killed 24 h after dosing	One dam delivered between 5–24 h; fetal mortality was 47% and the incidence of superficial, hepatic, and gastric hemorrhage was 6%, 1%, and 2% in the animals killed at 24 h; fetal hepatic glycogen was significantly decreased	Eriksson 1971

12–15 albino rats/group	25, 75, or 150 mg/kg	Animals were dosed on GDs 15–20 and allowed to deliver; neonates were killed on day 42	Parturition was delayed in one and two dams of the 25- and 150-mg/kg groups; in the 150-mg/kg group, neonatal mortality increased in a dose-dependent manner	Fritz and Suter 1985
	4.2, 12.5, or 25 mg/kg	Animals were dosed on GDs 20–21 and allowed to deliver; neonates were killed on day 42	In the 12.5- and 25-mg/kg groups, neonatal mortality increased in a dose-dependent manner	
10 SD rats/group	10 mg/kg	Animals were dosed twice daily on GDs 20 and 21 and allowed to deliver	The duration of and bleeding at parturition was increased; 13/121 neonates were born dead	Waltman et al. 1973
SD and Long-Evans rats	125 or 175 mg/kg	Animals were dosed on GDs 8–10 and allowed to deliver; locomotor activity was tested using clean and homecage bedding	No malformations were seen; alterations in activity were seen (male neonates had more salicylate-related changes than females)	Buelke-Sam et al. 1984
Methyl Salicylate				
LVG hamsters	175 mg/100 g	Animals were dosed at 7 days 9 h of gestation and killed on GD 9	72% of 35 litters had neural defects; Salicylate reached the fetus	Overman and White 1983
24–27 SD rats/group	4000 or 6000 ppm	Animals were fed test diet with calcium carbonate for 60 days prior to mating through weaning at day 20 or 21; procedure was then repeated	No abnormalities noted in offspring; neonate survival at weaning was greater in the test than the control groups	FDA 1966
F_0: 25 mice/sex/group F_{1b}: 30 males/30 females/group	0.25% or 0.5% in feed	Animals were dosed for 30 days prior to mating; F_0 animals were mated twice F_{1a} animals maintained through weaninge F_{1b} animals mated twice	(Results are only from females in each generation that mated twice.) No gross abnormalities were observed with any litter; all surviving neonates appeared normal; no reproductive abnormalities were seen	Abbott and Harrisson, no date
F_0: 25 Wistar rats/sex/group F_{1b}: 30/sex//group	0.25 or 0.5% in feed	Same protocol as above, with the exception that the animals were dosed for 60 days prior to mating	No gross abnormalities were observed with any litter; all surviving neonates appeared normal; mating performance and reproduction and viability indices were decreased. and number of deaths between birth and day 5 were increased in the 0.5% group; litter size was decreased in both test groups	Abbott and Harrisson, no date
F_0: 10 Osborne-Mendel rats/sex/group	500, 1500, 3000, or 5000 ppm in feed	F_0 animals mated after 100 days of dosing; F_{1a} animals were killed at weaning; 20 littermated F_{1b} animals were mated; procedure was repeated until generation 3	No gross abnormalities were observed; various reproductive effects were seen, especially in the 2nd generation	Collins et al. 1971

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TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Animals	Dose	Methods	Results	Reference
Male and female CD-1 mice	25, 50, or 100 mg/kg	Reproductive assessment by continuous breeding; control and high-dose F_1 offspring reproductive and fertility performance was evaluated due to lack of effect in F_0 mice	Reproductive and fertility parameters were generally not affected; also no significant effect on mating behavior, fertility rate, or reproductive performance was seen	Research Triangle Institute 1984; Morrissey et al. 1989; Lamb et al. 1997a
20 CD-1 mice/ sex/group	100, 250, or 500 mg/kg	Reproductive assessment by continuous breeding; crossover mating trial was performed to determine affected sex	Significant decrease seen in the mean number of litters, average number of pups/litter, proportion of live pups, and mean live pup weights in the high-dose group; fertility was poor in all groups, so the affected sex was not determined	Environmental Health Research Testing, Inc. 1984; Morrissey et al. 1989; Lamb et al. 1997b
CD rats	0.05 or 0.1 ml	Animals were dosed on GD 10 and 11 and either killed on GD 21 or allowed to deliver	The 0.1-ml group had decrreased body weight gain, fewer and smaller neonates, and more resorptions and malformed neonates; fetal kidney weight was decreased (GD 21) but was not different from control on postnatal day 6	Woo and Hoar 1972
Parenteral exposures to:				
Salicylic Acid				
17 SD rats	380 mg/kg	Animals were given a divided dose SC on GD 9, and then injected with mineral isotopes; the animals were killed in GD 20	Marked maternal weight loss; mean fetal weight was significantly decreased; the resorption rate was 46.6%, and 5.3% of viable fetuses were malformed; urinary mineral excretion was not affected	Koshakji and Schulert 1973
3–4 SD rats/group	300 or 380 mg/kg	Animals were given a divided dose SC on GD 16, and then injected with mineral isotopes; the animals were killed after 6 or 24 h	The high dose caused hematuria (3 cases) a high rate of fetal mortality and superficial hemorrhage; maternal-fetal uptake of minerals was not affected	Koshakji and Schulert 1973
Sodium Salicylate				
19–20 Lak: LVC golden hamsters/ group	37, 45, or 89 mg/kg	Animals were dosed SC on GD 8 and killed on GD 12	The minimal effective teratogenic dose was 89 mg/kg (produced 2.8% congenital malformations)	Geber 1977

5 CFE rats	500 mg/kg on GD 8 100 mg/kg daily on GDs 7–11	SC dose	The single dose caused 40% maternal toxicity and a 3% and 6% incidence of resorptions and dead fetuses and malformations, respectively; daily dosing resulted in 40% and 10% incidence of resorptions and dead fetuses and malformations, respectively	Lansdown et al. 1970
43 rats	60–180 mg	Animals were dosed SC on GD 9, 10, or 11 and killed on GD 21	Six dams given >120 mg died (no other information as a function of dose given); 24 animals resorbed their fetuses; external abnormalities in 15/100 fetuses; 11 of the 85 fetuses that appeared normal had skeletal anomalies	Warkany and Takacs 1959
9–42 SD rats/group	200–500 mg/kg	Animals were dosed SC on GD 10 and killed on GD 20	Significant numbers of fetal anomalies in the 400- and 500-mg/kg groups	Goldman and Yakovac 1963
6–8 ferrets/group	125, 250, or 400 mg/kg	Animals were dosed SC on GD 13 or 18 and killed on GD 35	Mean fetal weight was significantly decreased in all groups; resorption rates were 33%, 31%, and 91% and 43%, 37%, and 66% for the 125%, 250%, and 400-mg/kg groups dosed on GD 13 and 18, respectively; incidence of abnormalities was 11- and 86% for the 125- and 400-mg/kg group dosed on GDs 13 and 7, 33, and 96% for the 125-, 250-, and 400-mg/kg group dosed on GD 18	Gulamhusein et al. 1980
5–10 Wistar rats/group	400 mg/kg	Animals were dosed SC on GD 8.5 or 11.5 and killed on GD 20.5	Mean fetal weight was significantly decreased in both groups; resorption rates were 23% and 9%, and the incidence of abnormalities was 19% and 11% with dosing on GDs 8 and 11.5, respectively	Gulamhusein et al. 1980
SD rats	50 or 100 mg/kg	Animals were dosed SC 18 h prior to being killed on GD 22; fetuses were frozen immediately, or 15, 30, or 60 min after delivery	Contraction of the intrauterine ductus was significant in both groups at 0, 15, and 30 min and the high-dose group at 60 min	Sharpe et al. 1975
Havana rabbits	50 mg/kg	Animals were dosed SC 18 h prior to be killed on GD 30; fetuses were frozen immediately	Ductal contraction was observed and ductal diameter was 1/5 control values	

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TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Animals	Dose	Methods	Results	Reference
1–5 rats/group	0.20–0.75 g/kg	Animals were given a single SC dose the last wk of pregnancy and killed after 2 days	Maternal mortality was 2/5 and 3/3 in the 0.5- and 0.75-mg/kg groups; all fetuses of surviving animals lived	Jackson 1948
2–4 rabbits/group	0.5 or 1.0 g/kg	Same as above	Maternal mortality was 1/4 and 2/2 in the 0.5 and 1.0 groups; 19/23 fetuses of surviving animals lived	
A/Jax mice	10 mg	Animals dosed IM on one of GDs 7–13 and killed on GD 18	A high incidence of external anomalies was seen with dosing on GD 12 or 13 and of deformities of ribs and vertebrae was seen in all groups	Larsson et al. 1963
A/Jax mice	10 mg	Animals were dosed IM on GD 9 or 12 and killed on GD 18	Incidence of resorption: 18.1% and 43.3%, GDs 9 and 12; vessel anomalies: 11.9%, GD 12; rib anomalies: 52.2%, GD9 vertebral anomalies: 33.3%, GD12	Larsson and Boström 1965
CBA mice			Incidence of resorption: 5.9% and 4.4%, GDs 9 and 12; vessel anomalies: 0%, GD 12; rib anomalies: 16.7%, GD 9; vertebral anomalies: 4.0%, GD 12	
5–8 A/Jax and CBA mice/ group	10 mg/20 g	Animals, which were mated within and across strains, were dosed IM on GD 9, 11, 13, 15, or 17	A/Jax females × A/Jax males: resorption: 19%–73%; vessel anomalies: 6%–58%; rib anomalies (GD 9): 49%; vertebral anomalies (GD 9): 35% A/Jax females × CBA males: incidence of resorption: 0%–41%; vessel anomalies: 3%–41%; rib anomalies (GD9): 47%; vertebral anomalies (GD9): 23% CBA females × CBA males: incidence of resorption: 3%–13%; vessel anomalies: 3%; rib anomalies (GD 9): 53%; vertebral anomalies (GD 9): 12% CBA females × A/Jax males: incidence of resorption: 0%–9%; vessel anomalies: 2%–8%; rib anomalies (GD 9): 24%; vertebral anomalies (GD 9): 2%	Larsson and Eriksson 1966
10 A/Jax mice	10 mg/20 g	Animals were dosed IM on GDs 17	5, 2, and 3 animals delivered on GDs 17, 18, and 19	Eriksson and Larsson 1968

10–36 A/Jax and CBA mice/group	10 mg/20 g	Animals were dosed IM on GD 16 or 18 and killed after 8 or 24 h or dosed on GD 17 and killed after 2, 4, 8, 12, or 24 h	Many animals delivered prematurely; fetal mortality was greater with A/Jax mice; superficial and hepatic hemorrhage was seen in both strains	Eriksson 1969
10 A/Jax mice/group	3, 10, or 15 mg/20 g	Animals were dosed IM on GD 17 and killed on GD 18	In the 15-mg group, 4 animals died within 24 h and 4 delivered prematurely; fetal mortality was 4%, 70%, and 100% in the 3%, 10%, and 15-mg groups; incidence of superficial, hepatic, and gastric hemorrhage was 39%, 13%, and 22% in the 10 mg group	Eriksson 1970
10–20 A/Jax mice/group	10 or 3 mg/20 g	Animals were dosed IM with 10 mg on GD 15, 16, or 17 or on GDs 15–17 or with 3 mg on GD 15 and 16 and 10 mg on GD 17	Some animals delivered prematurely; fetal mortality was 21%–61%, superficial, hepatic, and gastric hemorrhage was 2%–42, 0%–12%, and 0%–36%, and vessel anomaly was 0%–33%	Eriksson 1970
3–14 SD rats/group	15–500 mg/kg (see methods)	Animals were dosed IV as follows: single dose of 15, 50, 100, 200, or 500 mg/kg on GD 6; daily doses of 75 or 150 mg/kg on GDs 6–13; daily dose of 150 mg/kg on GDs 13–19; constant infusion of 75 or 150 mg/kg/day on GDs 6–13; and constant infusion of 150 mg/kg/day on GDs 13–19; the animals were killed on GD 19	Fetal body weights were decreased in the groups given a single dose of 50–500 mg/kg, and daily doses of 150 mg/kg or continuous infusion of 150 mg/kg on GDs 6–13 Incidence of resorbed and dead fetuses for different dose regimes was: Single dose: 15 mg—0%; 50 mg—4%; 100 mg—11.6%; 200 mg—15%; 500 mg—47.6% Daily dose GDs 6–13: 75 mg—42%; 150 mg—81% Daily dose GDs 13–19: 150 mg—0% Infusion GDs 6–13: 75 mg—4%; 150 mg—73% Infusion GDs 13–19: 150 mg—6%	Gabrielsson et al. 1985
3–4 New Zealand white rabbits/ group	60 mg/ml or 80–120 mg/dl	Animals were given cont. infusions on GDs 22–29 and killed on GD 29	Mean fetal weight fetal crown-rump length, ratio of fetal weight/cm crown-rump length, placental weight and absolute and relative liver weights were significantly decreased in both groups	Lukas et al. 1987

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TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Animals	Dose	Methods	Results	Reference
Golden hamsters	1100 mg/kg	Animals were dosed IP on GD 8 and killed on GD 15	A trend toward increased mean lateral ventricle size was observed	Beyer and Geber 1984
5 CFE rats/group	100 or 500 mg/kg	Animals were dosed IP daily with 100 mg/kg on GDs 7–11 or once with 500 mg/kg on GD 8; the animals were killed on GD 15 or 19	The incidence of resorptions and dead fetuses and of malformations was 36% and 8% with the multiple dose and 81% and 16% with the single dose	Lansdown et al. 1970
balb/c mice	500 mg/kg	Animals were dosed IP on GD 10	Effects on fetal joint development were seen	Erdoğan et al. 1996
Methyl Salicylate				
116 Rats	0.1–0.5 cc	Animals were dosed SC on GD 9, 10, or 11 and killed on GD 21	26/69 dams died; 47 resorbed their fetuses; external abnormalities in 45/298 fetuses; 75 of the 253 fetuses that appeared normal had skeletal anomalies (no information given as a function of dose)	Warkany and Takacs 1959
5 CD rats	200 or 400 mg/kg	Animals were dosed IP on GD 8-9 and killed on GD 20	Embryotoxicity was seen at 400 mg/kg; fetal mortality was 2% and 50% in the 200- and 400-mg/kg groups; fetal body weight index was significantly decreased in the 400-mg/kg group; some developmental anomalies were seen in both groups, and dose-related decreases in organ weights were observed	Kavlock et al. 1982
5–16 SD rats	250–450 mg/kg	Animals were dosed IP with 250–450, 200–300, 300–375, or 200–300 mg/kg on GD 11, 10–11, 11–12, or 11–13 and killed on GD 20	Maternal toxicity was observed; fetal weight was significantly decreased (dose dependent); malformations were observed in fetuses of groups dosed with ≥350 mg/kg on GD 11 and ≥300 mg/kg on >1 day; incidence of resorptions was significant in the 400 mg/kg gp dosed on GD 11; kidney development was affected	Daston et al. 1988
SD rats	200, 250, or 300 mg/kg	Animals were dosed IP on GDs 10–13	A high incidence of maternal mortality was seen in the 300-mg/kg group; neonatal mortality was increased in the 250- and 300-mg/kg groups on days 1–2; no external abnormalities were seen in surviving neonates; some effect on urine-concentrating ability was seen in young neonates	Daston et al. 1988

2000-mg/kg S-2063 mixture groups, respectively; the incidence of resorbed fetuses after day 17 was <1%. In the animals dosed on day 17, the incidence of resorbed fetuses prior to day 17 was 11.5%, 17.2%, 9.3%, 11.0%, and 16.7% for the 500- and 1000-mg/kg Salicylic Acid groups, the S-2063 mixture (again, this mixture is Salicylic Acid and pyridyl-3-methanol) group, and the 1000 and 2000 mg/kg S-2063 groups, respectively. The respective incidences of resorbed fetuses after day 17 were 4.1%, 48.3%, 2.7%, 0.7%, and 8.3%; the resorptions occurred in one, two, or three litters. In the animals dosed on day 9, the incidence of malformations was 4.5%, 26.7%, 8.9%, 3.2%, and 23.8% for the 500- and 1000-mg/kg Salicylic Acid groups, the Salicylic Acid and pyridyl-3-methanol group, and the 1000- and 2000-mg/kg groups, respectively (Cekanova et al. 1974).

Methyl Salicylate

In a study described previously, Collins, Hansen, and Keeler (1971) also examined the effect of the addition of calcium carbonate to Methyl Salicylate–supplemented diet. Groups of F_{2b} rats were given 1500 ppm calcium carbonate (600 ppm available as calcium) in addition to 500, 1500, 3000, or 5000 ppm Methyl Salicylate. The animals were mated, and the first and second litters were examined. The addition of calcium carbonate did not markedly alter the effects obtained with Methyl Salicylate only.

Sodium Salicylate

The effect of dietary zinc and genetic strain on salicylate-induced teratogenesis was determined in the rat (Hackman and Hurley 1984). Groups of 3 to 8 gravid Sprague-Dawley and 4 to 10 Wistar rats were fed a zinc-deficient diet containing 0.4 μg zinc/g diet (designated as 0 μg/g), purified diets in which the zinc concentration was adjusted to 4.5, 9, 100, or 1000 μg zinc/g diet, or stock diet (which contained 40 μg zinc/g diet). On day 9 of gestation, the animals were dosed orally with 250, 500, or 750 mg/kg Sodium Salicylate or 0.9% sodium chloride. All animals were killed on day 21 of gestation.

The number of total viable fetuses, and the pooled proportions of resorptions (Res/T) and resorptions + malformations per total sites (Res + Mal/T) and of malformations per total viable fetuses (Mal/Viable) are summarized in Table 12a for the Sprague-Dawley strain and in Table 12b for the Wistar strain. Data for Wistar rats on stock diet were not reported. Wistar rats appeared more sensitive than Sprague-Dawley rats to Sodium Salicylate–induced teratogenesis. The frequency of resorption, malformed fetuses, and total abnormal sites generally decreased with increased zinc concentrations (Hackman and Hurley 1984).

Bergman et al. (1990) fed groups of female Sprague-Dawley rats basal diet containing 0.15 ppm sodium selenite or a diet containing 4.5 ppm sodium selenite for 8 weeks. After 8 weeks,

TABLE 12a

Effect of Zinc on Sodium Salicylate–induced teratogenesis in Sprague-Dawley rats (Hackman and Hurley 1984)

Zinc (μg/g)	Salicylate (mg/kg)	No. of litters	Total viable fetuses	Res/T (%)	(Res + Mal)/T (%)	Mal/viable (%)
Zn deficient (0.4)	0	6	41	30	84	78
Zn deficient (0.4)	250	5	32	37	100	100
Zn deficient (0.4)	500	7	43	41	93	88
Zn deficient (0.4)	750	6	2	96	98	50
4.5	0	7	57	16	19	3
4.5	250	6	43	23	38	14
4.5	500	7	49	31	52	3
4.5	750	6	26	51	66	3
9	0	7	63	10	10	0
9	250	4	34	8	8	0
9	500	7	42	37	43	9
9	750	4	10	73	73	0
100	0	7	64	8	8	0
100	250	5	47	0	2	0
100	500	6	38	35	45	15
100	750	5	41	49	52	4
1000	0	8	70	12	13	1
1000	250	5	51	3	0	0
1000	500	7	52	27	31	5
1000	750	6	24	63	73	29
Stock (40)	0	4	43	0	0	0
Stock (40)	250	3	28	12	12	0
Stock (40)	500	5	49	9	12	4
Stock (40)	750	6	38	39	44	7

TABLE 12b
Effect of Zinc on Sodium Salicylate–induced teratogenesis in Wistar rats (Hackman and Hurley 1984)

Zinc (μg/g)	Salicylate (mg/kg)	No. of litters	Total viable fetuses	Res/T (%)	(Res + Mal)/T (%)	Mal/viable (%)
Zn deficient (0.4)	0	7	41	51	77	54
Zn deficient (0.4)	250	4	34	17	82	79
Zn deficient (0.4)	500	8	11	87	100	100
Zn deficient (0.4)	750	4	0	100	100	0
4.5	0	6	56	13	36	26
4.5	250	5	44	12	48	40
4.5	500	6	40	34	57	35
4.5	750	4	0	100	100	0
9	0	9	92	4	6	2
9	250	5	42	27	34	9
9	500	8	64	20	29	10
9	750	7	17	77	85	35
100	0	9	82	10	11	1
100	250	5	54	8	15	7
100	500	10	41	58	65	17
100	750	5	16	68	76	25
1000	0	10	97	10	10	0
1000	250	5	41	10	17	7
1000	500	10	58	46	50	6
1000	750	7	17	76	77	5

the animals were mated and maintained on their respective diets. Groups of 10 to 18 gravid animals, which were fed a basal or selenite-supplemented diet, were dosed orally with either 250 mg/kg Sodium Salicylate in distilled water or physiological saline once daily on days 6 to 13 of gestation. All animals were killed on day 19 of gestation.

Selenite did not have a reproductive or teratogenic effect in animals given physiological saline. The number of surviving fetuses (42.6% of implants and 4.7 fetuses per litter were resorbed or dead) and fetal weight (0.99 g) was decreased in animals fed the basal diet and dosed with Sodium Salicylate; malformations were observed in 50.4% of the fetuses, a total of 83 malformations were observed in 57 of 113 fetuses.

In animals fed the selenite-supplemented diet and dosed with Sodium Salicylate, an increase in fetal survival was observed compared to the test group fed a basal diet (34.4% of implants and 4.0 fetuses per litter were resorbed or dead), but the incidence of fetal malformations was significantly increased compared to test animals given the basal diet; 66.0% of the fetuses were malformed, and a total of 152 malformations were observed in 95 of 144 fetuses. Selenite supplementation did not affect oral Sodium Salicylate embryotoxicity (Bergman et al. 1990).

The interaction between Sodium Salicylate and murine cytomegalovirus (MCMV) was examined using gravid CD-1 mice (Francis et al. 1990). Groups of 6 to 15 gravid animals were dosed intraperitoneally with 1×10^4 or 5×10^4 plaque-forming units MCMV on day 8 of gestation and orally with 500 or 750 mg/kg Sodium Salicylate on days 9 and 10 of gestation. Controls were given vehicle, MCMV, or Sodium Salicylate only. The animals were killed on day 18 of gestation. No synergistic effects of MCMV and Sodium Salicylate were observed. Sodium Salicylate alone was not fetotoxic.

Parenteral Studies

Sodium Salicylate

A group of 20 gravid A/Jax mice was given IP injections of 1.5 mg pentobarbital/20 g body weight on days 15 and 16 of gestation and an IM injection of 10 mg Sodium Salicylate/20 g body weight on day 17 of gestation, while a control group was given pentobarbital only (Eriksson 1970). Fetal mortality was 31% and 5% in the pentobarbital/Sodium Salicylate and pentobarbital only groups, respectively. Compared to results of a previously described study, pentobarbital pretreatment decreased the damaging effects of Sodium Salicylate (reported 61% mortality). In viable fetuses from animals given pentobarbital and salicylate, the incidence of superficial, hepatic, and gastric hemorrhage was 26%, 9%, and 37%, respectively; in the salicylate-only group, the respective incidences were 1%, 0%, and 1%. The effect of salicylate and pentobarbital on maternal hepatic microsomal hydroxylating enzymes was examined. Salicylate did not affect these enzymes.

Groups of 5 to 14 gravid CBA mice were dosed intramuscularly with 500 mg/kg Sodium Salicylate or 500 mg/kg Sodium Salicylate and 2.5 or 25 mg/kg $PGF_{2\alpha}$ at various times on day 9 of gestation, and the animals were killed on day 16 (Marsk 1980). Nine of 14 animals dosed at 10 AM with Sodium Salicylate

and 25 mg/kg $PGF_{2\alpha}$ died after dosing. In groups dosed with Sodium Salicylate only, the resorption rates were 9.7% or 11.8% with dosing at 10 AM or 2 PM, respectively, and the respective incidences of fetuses with rib malformations were 53.6% or 37.3%. In the group dosed with Sodium Salicylate at 10 AM and 2.5 mg/kg $PGF_{2\alpha}$ at 12 PM, the incidences of resorptions and fetuses with rib malformations were 4.4% and 62.8%, respectively. In the groups dosed with Sodium Salicylate at 10 AM and 25 mg/kg $PGF_{2\alpha}$ at 10 AM, 12 PM, or 2 PM, the incidences of resorption were 74.5%, 24.5%, or 4.4%, respectively, and the respective incidences of fetuses with rib malformations were 100%, 92.5%, or 87.8%.

Bergman et al. (1990) fed groups of female Sprague-Dawley rats basal diet (containing 0.15 ppm sodium selenite) or a diet containing 3.0 ppm sodium selenite for 8 weeks. After 8 weeks, the animals were mated and groups of 16 to 19 gravid animals, fed either a basal or selenite-supplemented diet, were dosed intravenously using an osmotic minipump to maintain a stable Salicylic Acid blood concentration. The animals were given a daily dose on days 6 to 13 of gestation with 150 mg/kg Sodium Salicylate at an infusion rate of 10 μl/h; controls were dosed intravenously with physiological saline. All animals were killed on day 19 of gestation.

Selenite did not have a reproductive or teratogenic effect on animals given physiological saline. The number of surviving fetuses (36.4% of implants and 3.6 fetuses per litter were resorbed or dead) and fetal weight (1.86 g) was decreased in animals fed the basal diet and dosed with Sodium Salicylate; malformations were observed in 5.4% of the fetuses, a total of eight malformations were observed in 7 of 129 fetuses. In animals fed the selenite-supplemented diet and dosed with Sodium Salicylate, an increase in fetal survival was observed compared to the test group fed a basal diet (11.0% of implants and 1.4 fetuses per litter were resorbed or dead). The incidence of fetal malformations was decreased; 1.9% of the fetuses were malformed, and a total of six malformations were observed in 3 of 154 fetuses. A slight but insignificant increase in fetal weight (2.00 g) was observed in selenite supplemented animals (Bergman et al. 1990).

Gravid Sprague-Dawley rats were used in studies examining homeostasis, teratogenic effects, and fetal histopathology (Khera 1991). The animals were dosed subcutaneously with 280 mg/kg/day Sodium Salicylate, and the effects of ammonium chloride or sodium bicarbonate were determined. Dependent on the study, dosing was performed on day 8, days 8 and 9, or days 8 to 10 of gestation. Sodium Salicylate induced mild maternal acidosis, hypokalemia, and hypophosphatemia, with no change in pH. It also induced maternal hemorrhage in extraembryonic cavities, papillary proliferation of the visceral yolk sac endoderm, and failure to form the chorioallantoic labyrinth. Resorptions, hydrocephaly, rib defects, and fetal body weight reduction were observed. Concurrent treatment with ammonium chloride enhanced the teratologic and histologic effects, whereas concurrent treatment with sodium bicarbonate significantly reduced these effects. Neither concurrent treatment affected acid-base values.

GENOTOXICITY

In Vitro Genotoxicity Studies

Salicylic Acid

Salicylic Acid was not mutagenic in a *Salmonella*/microsome test using *S. typhimurium* strains TA100, TA98, TA1535, and TA1537 with metabolic activation (McCann et al. 1975).

A modified Ames test was performed with 1, 10, and 100 μg/plate Salicylic Acid using *S. typhimurium* strains TA1535, TA1537, TA1538, and TA1536 (Commoner 1976). Negative and positive controls were used. The test was performed without metabolic activation and with activation using microsome preparations from seven different tissues from Wistar rats. Salicylic Acid was not mutagenic.

Salicylic Acid was not mutagenic towards *S. typhimurium* TA100 or TA98 with or without metabolic activation (Sugimura et al. 1976; Kawachi et al. 1980a, 1980b), it was not mutagenic towards *E. coli* WP-2 (Sugimura et al. 1976), and it was negative in a *B. subtilis rec assay* without metabolic activation (Kawachi et al. 1980a, 1980b).

Salicylic Acid was used to determine the lethal and mutagenic effects on and the uptake by *Saccharomyces cerevisiae* strain *rad18* cells (Zetterberg 1979). Killing and reversion frequencies were pH and temperature dependent. The undissociated form of Salicylic Acid was taken up more readily.

A chromosome aberration assay was performed using Chinese hamster ovary (CHO) cells with and without metabolic activation to determine the clastogenic potential of Salicylic Acid (Stich et al. 1981). A concentration of 25 mg/ml, half the level which induced mitotic inhibition, was not clastogenic with or without metabolic activation. The addition of Cu^{2+} and Mn^{2+} did not have much effect on the percentage of metaphases with chromosome aberrations induced by 12 mg/ml Salicylic Acid; the percentage with Salicylic Acid only was 1.4% as compared to 1.3% and 0.0% with Cu^{2+} and Mn^{2+}, respectively.

San and Chan (1987) reported that 2.5 to 10.0 mg/ml Salicylic Acid was not mutagenic in an Ames assay using *S. typhimurium* strain TA98. The test was performed with and without metabolic activation. These investigators also studied the effect of 2.5 to 10 mg/ml Salicylic Acid on aflatoxin B_1 (AFB_1)-induced mutagenicity was determined using *S. typhimurium* strain TA98 in the presence of metabolic activation. Salicylic Acid inhibited AFB_1-induced mutagenesis when administered concurrently, but not when Salicylic Acid was added after AFB_1.

In another Ames test, 0.1 mg/disc Salicylic Acid was not mutagenic toward *S. typhimurium* TA98 with or without metabolic activation (Kuboyama and Fujii 1992). Using strain TA100, mutagenic activity was seen with rat, but not mouse, guinea pig, or hamster, with metabolic activation using S9; no mutagenic activity was seen without metabolic activation.

A rec assay was performed using *B. subtilis* strains H17 (Rec^+0 and M45 (Rec^-) (Kuboyama and Fujii 1992). Salicylic Acid was positive; 2 mg had a DNA-damaging tendency.

The effect of Salicylic Acid on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitrosourea (MNU) mutagenicity was evaluated using *Euglena gracilis* (Foltínová and Grones, 1997). Concentrations of 50 to 500 μmol/L Salicylic Acid inhibited MNNG mutagenicity by 24 to 66.2% and of 800 to 1200 μmol/L inhibited MNU mutagenicity by 26 to 36%. A concentration of 185 μmol/L was needed to inhibit MNNG mutagenicity by 50%. Salicylic Acid, 50 to 1200 μmol/L, was not mutagenic to *E. gracilis*.

Butyloctyl Salicylate

The mutagenic potential of Butyloctyl Salicylate in DMSO was determined in a standard plate incorporation assay and a preincubation assay using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain CM891 (WP2uvrA/pKM101) (Huntingdon Life Sciences 1998e). Doses of ≤5000 μg/plate were tested without and with metabolic activation. Negative and positive controls gave expected results. Butyloctyl Salicylate was not mutagenic.

An in vitro mammalian chromosome aberration test was performed using human lymphocytes to determine the mutagenic potential of Butyloctyl Salicylate in DMSO (Huntingdon Life Sciences 1998f). Doses of 20 to 500 μg/plate were tested without metabolic activation and of 500 to 2500 μg/plate were tested with metabolic activation. Negative and positive controls gave expected results. No reproducible increases in the frequency of metaphases with aberrant chromosomes were observed; with a 3-h treatment, 20-h sampling time, a significant increase was observed in one of two cultures treated with 2500 μg/plate with metabolic activation. It was concluded that Butyloctyl Salicylate was not clastogenic.

Ethylhexyl Salicylate

An Ames assay was performed using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 to determine the mutagenic potential of Ethylhexyl (Octyl) Salicylate (Haarmann and Reimer 1991). Concentrations of 3000 to 75,000 μg/plate were tested without metabolic activation and of 100 to 3000 μg/plate with metabolic activation. Ethylhexyl (Octyl) Salicylate was not mutagenic.

Isodecyl Salicylate

The mutagenic potential of Isodecyl Salicylate was determined in an Ames test using *S. typhimurium* strains TA97, TA98, TA100, and TA102 (Vevy Europe 1984). Concentrations of 312, 625, 2500, and 5000 μg/plate were tested in the presence of metabolic activation. Appropriate positive controls and a negative control were used. Isodecyl Salicylate was not mutagenic at the concentrations tested.

Methyl Salicylate

An Ames test was performed using *S. typhimurium* TA92, TA1535, TA100, TA1527, TA94, and TA98 with metabolic activation (Ishidate et al. 1984). Methyl Salicylate, ≤10 mg/plate, was not mutagenic.

The mutagenic potential of 1.0 to 333.3 μg/plate Methyl Salicylate was determined in a *Salmonella*/mammalian microsome assay using strains TA1535, TA1537, TA98, and TA100 with and without metabolic activation (Mortelmans et al. 1986). Positive and negative controls were used. Methyl Salicylate was not mutagenic.

Methyl Salicylate, 0.1 mg/disc, was not mutagenic in an Ames test using *S. typhimurium* TA98 and TA100 without metabolic activation, but it was mutagenic towards TA98 and TA100 in the presence of hamster, but not rat, mouse, or guinea pig, with metabolic activation using S9 (Kuboyama and Fujii 1992). Five mg/disc was negative for DNA damage in a rec assay.

Sodium Salicylate

The mutagenic potential of 1% to 3% Sodium Salicylate was determined using *E. coli* (Demerec, Bertani, and Flint 1951). Sodium Salicylate was not mutagenic.

Sodium Salicylate was negative in a DNA cell-binding assay using Ehrlich ascites cells (Kubinski, Gutzke, and Kubinski 1981).

Sodium Salicylate, 0.1 mg/disc, was not mutagenic in an Ames test using *S. typhimurium* TA98 and TA100 with or without metabolic activation, and 5 mg/disc was negative for DNA damage in a rec assay (Kuboyama and Fujii 1992).

The effect of Sodium Salicylate on MNNG and MNU mutagenicity was evaluated using *E. gracilis* (Foltínová and Grones 1997). Concentrations of 50 to 500 μmol/L Sodium Salicylate inhibited MNNG mutagenicity by 38% to 74% and of 800 to 1200 μmol/L inhibited MNU mutagenicity by 34% to 42%. Concentrations of 85 and 1150 μmol/L were needed to inhibit MNNG and MNU mutagenicity, respectively, by 50%. Sodium Salicylate, 50 to 1200 μmol/L, was not mutagenic to *E. gracilis*.

Tridecyl Salicylate

The mutagenic potential of Tridecyl Salicylate was determined in a *S. typhimurium* reverse mutation assay using *S. typhimurium* strains TA1535, TA1537, TA1538, TA97, and TA98 (Biolab 1997a). Concentrations of 10 to 10,000 μg/plate in DMSO were tested in the presence and absence of metabolic activation. Appropriate positive controls and a negative control were used. Tridecyl Salicylate was not mutagenic at the concentrations tested.

In Vivo Genotoxicity Studies

Salicylic Acid

Three of four male mice were dosed orally with 100 mg/kg Salicylic Acid; the effects on incorporation of tritiated thymidine into testicular DNA were investigated (Seiler 1977). Salicylic

Acid significantly decreased thymidine incorporation compared to controls.

A sister-chromatid exchange (SCE) study was performed using groups of five male Swiss albino mice to determine the clastogenic potential of Salicylic Acid (Giri, Adhikari, and Khan 1996). The animals were injected intraperitoneally with 25, 50, or 100 mg/kg Salicylic Acid in DMSO 1 h after SC implantation of a BrdU tablet or dosed orally with 350 mg/kg Salicylic Acid in 2% gum acacia in distilled water 0.5 h after tablet implantation. Negative controls were dosed with 75 μl DMSO (intraperitoneally) or 0.3 ml gum acacia (orally) and positive controls were dosed with 1.5 mg/kg mitomycin C. Colchicine was injected intraperitoneally 22 h after BrdU-tablet implantation, and bone marrow was removed 2 h later. Salicylic Acid did not induce SCEs.

Giri, Adhikari, and Khan (1996) also performed a chromosome aberration study using male Swiss albino mice. Groups of four animals were dosed intraperitoneally with 50, 100, or 200 mg/kg Salicylic Acid in DMSO and five animals were dosed orally with 350 mg/kg Salicylic Acid in 2% gum acacia in distilled water. Groups of four and five negative-control animals were dosed intraperitoneally with 75 μl DMSO or orally with 0.3 ml 2% gum acacia in distilled water, respectively; five positive-control animals were dosed with 25 mg/kg cyclophosphamide. The animals were injected with 2 mg/kg colchicine 22 h after dosing, and killed 2 h later. In both the IP and oral studies, no significant increase in chromosomal aberrations was seen with any dose of Salicylic Acid. A significant increase in mitotic index was observed with the 50 mg/kg IP dose and the single oral dose.

Ethylhexyl Salicylate

The mutagenic potential of Ethylhexyl (Octyl) Salicylate was determined in a micronucleus test performed according to OECD Test Guideline No. 474 (Haarmann and Reimer 1991). Five male and five female NMRI mice were dosed orally with 2 g/kg Ethylhexyl (Octyl) Salicylate. No increase in micronucleated polychromatic erythrocytes was observed 24, 48,or 72 h after dosing.

Sodium Salicylate

A SCE assay was performed using male Swiss albino mice following the procedure described previously (Giri, Adhikari, and Khan 1996). Sodium Salicylate was given intraperitoneally at doses of 25, 50, or 100 mg/kg and orally as a single 350 mg/kg dose. Sodium Salicylate did not induce SCEs.

A chromosomal aberration study was also performed using male Swiss albino mice following the procedure described previously (Giri, Adhikari, and Khan 1996). Sodium Salicylate was given intraperitoneally at doses of 50, 100, or 200 mg/kg and orally as a single 350-mg/kg dose. A significant increase in chromosomal aberrations was seen with the 200 mg/kg IP dose and the 350-mg/kg oral dose.

CARCINOGENICITY

Salicylic Acid

Salicylic Acid was reported by Sugimura et al. (1976) and Kawachi et al. (1980a) not to be a carcinogen, although details were not provided.

The effect of Salicylic Acid on mouse epidermal JB6 cells, a culture model used to study tumor and anti-tumor promotion, was examined (Dong et al. 1997). Salicylic Acid inhibited tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced transformation in a concentration-dependent manner. No significant effect was observed on ^{3}H-TdR incorporation into DNA. Salicylic Acid inhibited TPA-induced tissue inhibitor of metalloproteinase (TIMP-1) mRNA expression, and it inhibited in a dose-dependent manner the anchorage-independent growth of H-*ras* and c-*jun*-transformed JB6 cells. Salicylic Acid did not affect the protein concentrations of mitogen-activated protein kinase Erk1 or Erk2, even with 24 h pretreatment. Salicylic Acid decreased intracellular pH. The researchers stated that the results "suggest that inhibition of tumor promoter induced-neoplastic transformation in JB6 cells may be through inhibition of AP-1 [activator protein-1] transactivation."

Methyl Salicylate

A skin-painting study was performed in which Methyl Salicylate was applied to the back of 39 mice at biweekly intervals for 400 days (Burdette and Strong 1941). No neoplasms were induced.

Groups of 15 male and 15 female A/He mice were dosed intraperitoneally with 100 or 500 mg/kg Methyl Salicylate in tricaprylin three times per week for 8 weeks for a total of 24 doses (Stoner et al. 1973). Two negative-control groups, one untreated and one dosed with vehicle, and two positive-control groups, given 5 or 20 mg/animal urethan, were used. The animals were killed 24 weeks after the initiation of dosing. Two out of 13 males and 1/14 females of the low-dose group that survived until study termination had lung tumors. One out of 12 males and 5/13 females of the high-dose group that survived until study termination had pulmonary tumors. These compare to 10/46 males and 8/48 females and 8/30 males and 10/28 females with tumors in the untreated and vehicle control groups, respectively.

Sodium Salicylate

Elder et al. (1996) reported that Sodium Salicylate had dose-dependent inhibitory effects on adenoma, in vitro transformants of adenoma, and carcinoma cell lines, with IC_{50} values (not defined) of 1.65 to 7.28 mM. The carcinoma and in vitro transformed adenoma cell lines were more sensitive than the adenoma cell lines.

CLINICAL ASSESSMENT OF SAFETY

Irritation Studies on Normal Skin

Salicylic Acid

Harrison Research Laboratories (HRL), Inc. (1993a) determined the irritation potential of a gel containing 2% Salicylic

Acid in a cumulative irritation study completed by 27 subjects, 15 males and 12 females. An occlusive patch containing 0.2 g of the test material was applied to the back of each subject for 48 (Monday and Wednesday) or 72 h (Friday) three times per week for 2 weeks for a total of six applications. Upon patch removal, the test sites were scored (on a scale of 0 to 4) and the new patches were applied.

The six daily scores were summed to yield an aggregate 14-day score, and the 14-day scores for all subjects were summed to yield a grand total score. The grand total score for the gel containing 2% Salicylic Acid was 14.5. The authors concluded that the gel produced minimal cumulative irritation (HRL Inc. 1993a).

TKL Research, Inc. (1998a) determined the irritation potential of a facial cosmetic cream containing 1.5% Salicylic Acid (pH 2.75; Procter and Gamble Company 1999b) in a 21-day cumulative irritation patch test. Twenty-seven subjects completed the study. Distilled water and 0.2% (*w/v*) SLS served as negative and positive controls, respectively. Occlusive patches containing 0.2 g of the test material were applied to the infrascapular area of the back of each subject for 24 (Monday to Thursday) or 72 h (Friday); the test sites were scored upon patch removal and new patches were applied to the same site. This procedure was repeated for 21 days.

A total score was calculated by summing each individual's scores on each of the 21 days. Normalized scores were calculated by summing the scores of all subjects, dividing by the total number of readings for all subjects, and multiplying by 21 (the number of readings) and by 10 (to normalize to 10 subjects). A facial cosmetic cream containing 1.5% Salicylic Acid, with a total score of 415.0 and a normalized score of 147.5, was classified (using the normalized score) as slightly irritating (TKL Research, Inc. 1998a).

TKL Research, Inc. (1998b) performed a 21-day cumulative irritation patch test following the same procedure described above for a facial skin conditioner cream containing 1.5% Salicylic Acid (pH 2.78; Procter and Gamble Company 1999b) and a facial skin conditioner lotion containing 0.02% Salicylic Acid (pH 3.5; Procter and Gamble Company 1999b), with the exception that the cream was applied under occlusive and semiocclusive patches and the lotion was applied under a semiocclusive patch. Twenty-seven subjects completed the study.

Under occlusive patches, the cream containing 1.5% Salicylic Acid had total and normalized irritation scores of 125.0 and 45.7, respectively. Using semiocclusive patches, the cream containing 1.5% Salicylic Acid had total and normalized scores of 45.0 and 16.5, respectively. Under both test conditions, the cream was classified using the normalized scores as producing no significant irritation. The lotion containing 0.02% Salicylic Acid had total and normalized scores of 50.0 and 18.3, respectively, and it also was classified as producing no significant irritation (TKL Research, Inc. 1998b).

A third 21-day cumulative irritation patch test was performed using the same procedure (TKL Research, Inc. 1998c). Twenty-eight subjects completed the study. A facial skin conditioner cream containing 1.5% Salicylic Acid (pH 2.78; Procter and Gamble Company 1999b) was tested using occlusive and semiocclusive patches. Using occlusive patches, the cream had total and normalized scores of 381.0 and 132.0, respectively, and was slightly irritating (classified using the normalized scores). Using semiocclusive patches, it had total and normalized scores of 69.0 and 23.9, respectively, and was classified as producing no significant irritation.

Ethylhexyl Salicylate

A 48-h occlusive patch test was performed using 4% Ethylhexyl (Octyl) Salicylate in petrolatum (Anonymous, 1976). Ethylhexyl (Octyl) Salicylate was not irritating.

Methyl Salicylate

In a 48 h closed-patch test, 8% Methyl Salicylate in petrolatum did not produce irritation (Opdyke 1978).

In a dermal absorption study described earlier in which five subjects applied products containing 12% to 50% Methyl Salicylate (Roberts et al. 1982), each subject reported pain and erythema at the application site of each product.

Erythema determinations were made on four subjects using thermography in a dermal penetration study (Collins et al. 1984). A product containing 1% *w/w* Methyl Salicylate was applied as a metered aerosol. A visible erythematous reaction developed within approximately 10 min of application.

In the introduction to the cytokine study presented earlier, Wilmer et al. (1994) stated that Methyl Salicylate was a primary contact irritant.

In a dermal absorption study described earlier (Morra et al. 1996) in which six male and six female subjects applied an ointment containing 12.5% Methyl Salicylate twice daily for 4 days, all subjects reported burning, stinging, and erythema at the site of application. All but one subject reported pruritus and prolonged erythema for up to 7 days after the termination of dosing.

TEA-Salicylate

One subject included in a dermal absorption study (described previously) reported prolonged pruritus and erythema (Morra et al. 1996). In the study, 12 subjects, 6 males and 6 females, applied two doses of a cream containing 10% TEA-Salicylate with a 12-h interval.

Tridecyl Salicylate

The dermal irritation potential of Tridecyl Salicylate was determined using 30 male and female subjects (number per sex not stated) in an occlusive patch test performed according to the methods of Draize (Biolab 1997b). The patch was applied to the volar forearm of each subject for 48 h, and the test sites were scored 15 min and 24 h after patch removal. No erythema or edema was observed, and the total irritation and mean irritation indices were 0 at both evaluations. Tridecyl Salicylate was a nonirritant.

Irritation Studies on Diseased Skin

Methyl Salicylate

Occlusive patch tests were performed on five herbal topical medicines using 20 subjects with endogenous eczema or contact dermatitis (Lee and Lam 1990). The oils or ointments contained 3.75% to 67% Methyl Salicylate. One oil containing 67% Methyl Salicylate caused irritation in eight of the subjects and an oil containing 40% Methyl Salicylate caused irritation in two of the subjects. The remaining oils and ointment, containing 15%, 38%, and 3.75% Methyl Salicylate, respectively, did not produce any irritant responses.

Effect on Immediate Contact Reactions to Other Agents

Salicylic Acid

Johansson and Lahti (1988) examined the effect of a 5% Salicylic Acid gel on nonimmunologic immediate contact reactions (NIICRs) to 500 mM benzoic acid, 500 mM cinnamic aldehyde, 50 mM methyl nicotinate, and 14.1 M (100%) DMSO using 16 subjects, 8 males and 8 females, 5 of whom were atopic. On day 1, open applications of 10 μl of the irritants were applied to 1 × 1-cm areas on the back of each subject. A 0.5-ml dose of 5% Salicylic Acid was applied to a 10 × 15-cm area at 0, 8, and 24 h. One hour after the last application, the NIICR test was done on the gel area and the reference area. The test was repeated the next day (day 3) on the same areas but not on the previous test sites. The test sites were wiped 20 min and observed 40 min after application. Reactions were assessed visually and with a laser-Doppler flowmetry (LDF) device. Erythema due to benzoic acid and methyl nicotinate was significantly reduced with Salicylic Acid on days 2 and 3 when assessed using LDF. Upon visual observation, reactivity to cinnamic aldehyde was reduced on day 3; Salicylic Acid did not affect edema.

Sensitization

Fisher (1986) reported that a subject can have an allergic contact dermatitis reaction to a product, but not react to any of the individual ingredients. This can be due to a "physical synergism," in which one ingredient can act as a "penetrating agent," or a "chemical synergism," in which individual nonsensitizing ingredients combine to form a contact allergen. Salicylic Acid was used as an example of an ingredient that can promote skin penetration and be involved in physical synergism. However, an ingredient can also "quench" the allergenic capacity of a product.

Studies of sensitization reactions in study subjects with normal skin (predictive studies) and with diseased skin (provocative studies) of the salicylates are presented below.

Predictive Studies

Salicylic Acid

A maximization study was performed using 25 subjects; induction and challenge concentrations of Salicylic Acid were 20% and 10%, respectively (Kligman 1966). None of the subjects were sensitized.

TKL Research, Inc. (1993a, 1993b, 1993c) performed two repeat-insult patch tests (RIPTs) to evaluate the sensitization potential of a moisturizer cream or lotion containing 2% Salicylic Acid. In the first study evaluating the sensitization potential of a cream, 114 subjects, 20 males and 94 females, enrolled in and 99 subjects, 16 males and 83 females, completed the study. None of the subjects discontinued for test article–related reasons. Two-tenths of a gram of the test material was applied to occlusive patches, and the patches, which were air-dried for 15 to 30 min, were applied to the infrascapular region of the back for 24 h. This procedure was repeated every 48 to 72 h after patch application for a total of nine applications. After a 2-week nontreatment period, a challenge patch was applied to a previously untreated site on each subject. The patches were removed at 24 h and the sites evaluated 48 and 72 h after application. The only responses seen, i.e., "?"—doubtful response, barely perceptible erythema, only slightly different from surrounding skin and "+"—definite erythema without edema, were observed during induction. A moisturizer cream containing 2% Salicylic Acid was not a sensitizer.

In the second RIPT, the sensitization potential of both a moisturizing cream and a moisturizing lotion containing 2% Salicylic Acid was determined. Of the 119 subjects, 14 males and 105 females, enrolled in the study, 101 subjects, 12 males and 89 females, completed the study; half of the subjects had "self-professed sensitive skin." None of the subjects discontinued for test article–related reasons. The procedure was the same as described previously. With both products, "?" and "+" were the only reactions observed during induction. Neither the cream nor the lotion was a sensitizer (TKL Research, Inc. 1993a, 1993b, 1993c).

An RIPT was performed to determine the sensitization potential of a gel containing 2% Salicylic Acid (HRL 1993b). The test was completed by 193 subjects, 52 males and 141 females. Occlusive patches containing 0.2 g of the test material were applied for 24 h to the left upper back of each subject three days per week for 3 weeks for a total of nine induction patches. The test sites were scored on a scale of 0 to 4 at 24 (Monday and Wednesday patches) or 48 h (Friday patches) after patch removal. Following a 2-week nontreatment period, the challenge was performed by applying a 24-h occlusive patch to a previously untreated site on the right upper back of each subject. The induction and challenge sites were scored upon removal of the patch, and a patch was again applied to the challenge site. The sites were scored 48, 72, and 96 h after application of the initial challenge patch. During induction, five subjects had scores of ± (faint, minimal reaction) or 1 (erythema), and during challenge, seven subjects had scores of ± or 1. The authors concluded that the gel containing 2% Salicylic Acid was not a sensitizer.

HRL (1997a) performed a second RIPT of a gel containing 2% Salicylic Acid with 198 subjects, 59 males and 139 females, following the same procedure with the exception that

only one challenge patch was applied. During induction, two subjects had ± reactions, and during challenge, five subjects had reactions of ± or 1. The authors again concluded that the gel containing 2% Salicylic Acid was not a sensitizer.

Ethylhexyl Salicylate

A maximization test was performed using 23 subjects to determine the sensitization potential of 4% Ethylhexyl (Octyl) Salicylate in petrolatum (Anonymous 1976). No sensitization reactions were observed.

Methyl Salicylate

In a maximization test using 27 subjects, 8% Methyl Salicylate in petrolatum produced no sensitization reactions (Opdyke 1978).

Provocative Studies

Salicylic Acid

A group of 230 patients, 72 males and 158 females, with venous leg eczema were patch tested with 5% Salicylic Acid in vaseline (Thune 1969). The patches were applied to the back or anterior aspect of the thigh for 24 h and read daily for 4 days. Three patients had positive reactions (defined as erythema and infiltration for >24 h after patch removal).

Wojnar, Hearn, and Starkweather (1980) examined the augmentation of allergic histamine release from human leukocytes by several anti-inflammatory/analgesic agents, including Sodium Salicylate. Leucocyte donors (nine women and seven men) were selected on the basis of release of histamine from their leukocytes with ragweed or housedust extracts. A 25% augmentation of ragweed-induced histamine release was considered significant and was used as a common basis for comparison. The authors report that a concentration of 120 ± 29 μM Sodium Salicylate is needed to produce a 25% augmentation of ragweed-induced histamine release (as compared to 917 ± 104 μM aspirin, for example).

Salicylic Acid, 5% in petrolatum, was part of a standard patch test battery from 1979 to 1983 (Goh and Ng 1986). Of 9701 patients patch tested, 11 (doubtful) positives were observed. Repeat patch tests were performed with 8 of these 11 patients using 0.5%, 1%, 2%, and 5% Salicylic Acid. One patient, who had a history of immediate type hypersensitivity to oral salicylates, had a positive reaction to 1%, 2%, and 5% Salicylic Acid.

Twenty-seven patients, 13 males and 14 females, with a sensitivity to aspirin were challenged orally with Salicylic Acid (Zhu et al. 1997). The challenge was performed with 25–400 mg Salicylic Acid. The challenge was negative for all patients.

Sodium Salicylate

The allergenic potential of Sodium Salicylate was determined in a number of studies using up to 31 patients, 19 males and 12 females, with a history of aspirin intolerance (Patriarca et al. 1976). In a skin test, 31 patients were given an intradermal injection of 0.02 ml of 0.1% Sodium Salicylate; the results were scored 20 min after dosing. In a Prauxnitz and Küstner passive transfer test (PK test), 23 patients were used and 0.1% Sodium Salicylate was the challenge concentration for passively sensitized sites (0.1 ml serum). A passive cutaneous anaphylaxis (PCA) test with 0.05 g Sodium Salicylate was used to determined $IgG_{1,3,4}$ antibodies in all 31 patients; three guinea pigs were used in each case to confirm the reaction. A lymphocyte transformation test (LTT) was performed in vitro using 26 patients, and 2-^{14}C-thymidine was employed. There was one positive reaction to Sodium Salicylate in the skin test, none in the PK test, two in the PCA test (scores not defined), and two in the LTT test.

Phototoxicity/Photosensitization

Salicylic Acid

Ivy Laboratories (1993a) determined the phototoxic potential of a cream containing 2% Salicylic Acid using five male and five female subjects with type I to III skin. Duplicate 2 × 2-cm occlusive patches containing 0.2 g of the cream, which were allowed to air dry for 15 to 30 min, were applied to the lower back of each subject. A third site which was treated in a similar manner with hydrophilic ointment served as a control. Twenty-four hours after application, one of the test patches and the control patch were removed, and the sites were exposed to 20 J/cm^2 of UVA (320 to 400 nm, peak at 350 nm). A 150-W compact xenon arc source with a UV-reflecting dichroic mirror, a 1-mm-thick Schott WG-345 filter, and a 1-mm-thick UG11 filter served as the light source. UV irradiance was measured at the skin. The second test patch was then uncovered and served as an unirradiated treated control.

The sites were graded at the end of each exposure and 24 and 48 h after irradiation. The authors reported that no phototoxicity was observed, and they concluded that the cream containing 2% Salicylic Acid did not possess a detectable phototoxicity potential in humans (Ivy Laboratories 1993a).

The phototoxic potential of a gel containing 2% Salicylic Acid was determined in a test completed by 10 subjects, 1 male and 9 females, with type I, II, or III skin (HRL, Inc. 1993c). Duplicate occlusive patches containing 0.2 g of the test material were applied to the volar forearms of each subject. The patches were removed 24 h after application and the sites were scored on a scale of 0 to 4. One forearm was then irradiated with UVA light for 15 min. The light source consisted of a set of four F40BL fluorescent tubes with a wavelength range of 320 to 400 nm, with >95% of the relative energy at 360 nm; the dose was measured as 0.22 J/cm^2/min (total dose of 3.3 J) at a distance of 15 cm. Immediately, 24 h, and 48 h after irradiation, the test sites on each forearm were scored. No reactions were observed at the irradiated nor non-irradiated sites, and a gel containing 2% Salicylic Acid was not phototoxic.

Ivy Laboratories (1993b) performed a photocontact allergenicity test using 25 subjects, 8 males and 17 females, to

letermine the photosensitization potential of a cream containing 2% Salicylic Acid. Each subject's minimal erythema dose MED) was determined. Occlusive 2 × 2-cm occlusive patches containing 100 mg of the test material (25 mg/cm^2), which was allowed to air dry for 15 to 20 min, were applied to the lower back of each subject for 24 h. The patches were removed, and he sites were exposed to 3 MEDs from a 150-W compact solar arc simulator equipped with a UV-reflecting dichroic mirror, a 1-mm-thick Schott WG-320 filter (290–400 nm), and a 1-mm-hick UG11 filter. Total irradiance at the skin was measured.

Forty-eight hours after irradiation, patches were reapplied to he same sites and the procedure was repeated. Induction consisted of twice weekly exposures for 3 weeks. A challenge was performed 10 days after the last induction exposure by applying for 24 h duplicate occlusive patches containing 25 mg/cm^2 of the test material to previously untested sites on the back. One patch was removed, and the site was irradiated with 4 J/cm^2 UVA. The second site served as a treated unirradiated control. The test sites were examined 48 and 72 h following UVA exposure. No abnormal responses were observed, and the researchers concluded that the cream containing 2% Salicylic Acid did not possess a detectable photocontact-sensitizing potential in human skin (Ivy Laboratories 1993b).

Ivy Laboratories (1993c) performed a second photocontact allergenicity test using 25 subjects, 1 male and 24 females, to again evaluate the photosensitization potential of a cream containing 2% Salicylic Acid. The procedure described above was generally followed. However, in this test, 0.2 mg of the test material was applied to the patch, and the patch was allowed to air dry for 15 to 30 min prior to application. No unexpected responses were observed; mild to moderate erythema, scaling, and tanning, which can be expected following repeated UV exposure, were observed. The researchers concluded that a cream containing 2% Salicylic Acid did not possess a detectable photocontact-sensitizing potential in human skin.

HRL, Inc. (1993d) determined the photoallergic potential of a gel containing 2% Salicylic Acid in a test completed by 28 subjects, 4 males and 24 females, with type I, II, or III skin. During induction, an occlusive patch containing 0.2 g of the test material was applied for 24 h to the radial aspect of the volar forearm (that was to be irradiated) twice per week for three weeks for a total of six applications. A second patch was applied either to the opposite forearm or the left scapular area of the back, as determined by the subject, and this site was not irradiated. Upon patch removal, the test sites were scored on a scale of 0 to 4, and the appropriate sites were irradiated with UVA and UVB. UVA irradiation was for 15 min. UVB irradiation was based on skin type and MED and was either two MEDs or a maximum of 135 s. The test sites were scored immediately after irradiation. Following a 14-day nontreatment period, the ulnar aspect of the irradiated forearm served as the challenge site and a patch was applied for 24 h to a previously untreated site. For challenge of the nonirradiated site, the patches were applied for 24 h as appropriate to either the ulnar aspect of the forearm or the right scapular area of the back. Following patch removal, the sites were scored and the appropriate forearm was subjected to UVA irradiation only. The test sites were scored immediately, 24 h, and 48 h after irradiation.

The UVA light source consisted of a set of four F40BL fluorescent tubes with a wavelength range of 320 to 400 nm and >95% of the relative energy at 360 nm; the dose was measured as approximately 0.22 J/cm^2/min (for a total dose of 3.3 J) at a distance of 15 ± 2 cm. UVB was from the "Solarium 300," with a wavelength range of 260 to 320 nm and >95% of the relative energy at 300 nm; the dose was measured at approximately 1.2 mJ/cm^2/s (skin type I: 105 s = 126 mJ; skin type II: 120 s = 144 mJ; skin type III: 135 s = 162 mJ) at a distance of 22 ± 2 cm.

During induction, 14 subjects had reactions of ± (minimal erythema) or 1 (erythema and/or slight edema within patch margins) at the irradiated test site and one subject had a ± reaction at the nonirradiated test site. Twelve subjects had reactions of ± or 1 at the irradiated control site. At challenge, one subject had a reaction of 1 at the irradiated and nonirradiated test sites. The authors concluded that the gel containing 2% Salicylic Acid did not induce contact dermal photoallergy nor contact dermal sensitization in human subjects (HRL, Inc. 1993d).

HRL, Inc. (1997b) performed another phototoxicity test on a gel containing 2% Salicylic Acid following the same procedure as in HRL, Inc. (1993c), with the exception that the sites were irradiated for 17 min and were scored immediately and 24, 48, and 72 h after irradiation. Ten subjects, two males and eight females, completed the test. The UVA light source consisted of four F40BL fluorescent tubes with approximately 95% of the output in a range of 320 to 400 nm; the dose was measured as approximately 3.1 ± 0.3 mW/cm^2 (total dose of 3.2 ± 0.3 J) at a distance of 15 cm. One subject had a ± (faint, minimal erythema) reaction at both the irradiated and nonirradiated test sites and one had a ± reaction at the nonirradiated site. The authors concluded that the gel containing 2% Salicylic Acid was not phototoxic.

The photoallergic potential of a gel containing 2% Salicylic Acid was determined in a second study completed by 28 subjects, 5 males and 23 females, that generally followed the same procedure (HRL, Inc. 1997c). UVA irradiation was 17 min and UVB irradiation was either 2 MED or a maximum of 120 s. The UVA light source consisted of a set of four F40BL fluorescent tubes with approximately 95% of the output in the wavelength range 320 to 400 nm; the dose was measured as approximately 3.1 ± 0.3 mW/cm^2 (for a total dose of 3.2 ± 0.3 J) at a distance of 15 ± 2 cm. UVB was from the "Solarium 300," with a wavelength range of 260 to 320 nm and >95% of the relative energy at 300 nm; the dose was measured at approximately 1.2 ± 0.1 mW/cm^2 (skin type I: 90 s = 108 mJ; skin type II: 105 s = 126 mJ; skin type III: 120 s = 144 mJ) at a distance of 22 ± 2 cm. The opposite volar forearm only served as the nonirradiated site. During induction, 21 subjects had reactions of ± (minimal erythema) or 1 (erythema within patch margins) at the irradiated test

site and 2 subjects had ± reactions at the nonirradiated test site. Also during induction, seven subjects had reactions of ± or 1 at the irradiated control site. During challenge, two subjects had reactions of ± at the irradiated test site. The authors stated that the test material (containing 2% Salicylic Acid) did not induce contact dermal photoallergy nor contact dermal sensitization in human subjects.

Photoprotective Effects

Salicylic Acid

The photoprotective effect of Salicylic Acid was evaluated (Kristensen and Kristensen 1991). In vitro, a cream containing 2% Salicylic Acid absorbed in the 295 to 323 nm range, with a peak around 303 nm. In vivo, a test was performed using five subjects. Each subject's MED was determined following irradiation with two Philips TL 40 W/12 (UVB) lamps. Application of 0.5 to 10% Salicylic Acid prior to UV exposure dose-dependently inhibited UV-induced erythema. Application after UV-exposure had no effect.

Ethylhexyl Salicylate

In a study to evaluate the photoprotection ability of a formulation containing Ethylhexyl (Octyl) Salicylate, Gange et al. (1986) first photosensitized subjects to UVA irradiation by ingestion of 0.6 mg/kg 8-MOP. After 1.5 h, 2 μl/cm^2 of a formulation containing 5% Ethylhexyl (Octyl) Salicylate, 7% padimate O, and 3% oxybenzone or the vehicle only was applied to a 2 × 10-cm area of the lower part of the back, and an untreated area served as an unprotected control. The test areas were covered with foil, and 2 h after 8-MOP ingestion, the sites were exposed to a series of 9 or 10 increasing doses of 1.0 to 21 J/cm^2 UVA. The light source was a bank of 12 36-inch UVA tubes with peak emission at 366 nm and 98% of the UV in the range of 320 to 400 nm; irradiance at the skin was 4.9 to 5.1 mW/cm^2. The erythemal response at each site was evaluated 48 and 72 h after UV exposure, and the amount of pigmentation at each site was determined 2 weeks after exposure.

No erythema was seen in the unprotected controls exposed to 21 J/cm^2 UVA. The mean phototoxic protection factor (PPF) for the Ethylhexyl (Octyl) Salicylate formulation, calculated as the minimal phototoxic dose (MPD) of protected skin/MPD of unprotected skin, was 2.9 at 48 h (28 subjects) and 2.9 at 72 h (34 subjects). The PPF ranged from 1.4 to 7.5 at 48 h and from 0.7 to 7.5 at 72 h. For the vehicle controls, the mean PPF was 1.1 at 48 h (37 subjects), with a range of 0.5 to 2.0, and 1.1 at 72 h (38 subjects), with a range of 0.7 to 2.0. After 12 to 18 days, the melanogenic protection factor (MPF) for the Ethylhexyl (Octyl) Salicylate formulation, calculated as the minimal melanogenic dose (MMD) of protected skin/MMD of unprotected skin, was 2.7 (28 subjects), with a range of 0.7 to 5.4. For the vehicle controls, the mean MPF was 1.0 (36 subjects), with a range of 0.5 to 2.0 (Gange et al. 1986).

Urticarial Reactions

Salicylic Acid

Eighteen of 21 subjects that had urticarial reactions to ingested aspirin were given Sodium Salicylate (Moore-Robinson and Warin 1967). Urticaria was exacerbated in 13 of the 18 subjects.

Doeglas (1975) performed a provocative test in 20 aspirin-sensitive patients with chronic urticaria. Six of the 20 patients had positive reactions to Sodium Salicylate.

The ability of 5% Salicylic Acid in petrolatum to induce nonimmunological contact urticaria was examined using 110 patients, 67 males and 43 females; 36, 23, 26, and 25 of the patients were atopic, urticarial, nonatopic, and nonallergic, respectively (Lahti 1980). The potential of 5% Salicylic Acid in petrolatum to induce urticaria was also determined using the chamber method with 138 dermatological patients, 63 males and 75 females; 84 of the patients were atopic and 54 were nonatopic (Lahti 1980). With this method, Salicylic Acid was applied to the backs of the patients for 20 min, and the reactions were scored 10 min after removal. No immediate reactions were seen.

Other Skin Effects

Salicylic Acid

The effect of vehicle on the time required for Salicylic Acid to have keratolytic action on normal human skin was investigated (Strakosch 1943). Ointments containing 1% to 15% Salicylic Acid were prepared using the following six vehicles: petrolatum; petrolatum and hydrous wool fat; a base consisting of 6% of a group of esters of cholesterol (primarily oxycholesterol) in a petrolatum base; a base of fatty acid esters of diethanolamine (DEA) with petrolatum; a stearyl alcohol, liquid petrolatum, water, light petrolatum base; and a zinc oxide, talc, petrolatum base. Using groups of four subjects, open applications of the test materials were made to a 5 × 5-cm site on the anterior aspect of the upper thigh or on the abdomen daily three times per day for 24, 48, or 72 h or 7, 10, or 14 days. Keratolytic changes were first seen with the oxycholesterol-petrolatum base, the fatty acid esters of DEA base, and the stearyl alcohol–containing base, next with the petrolatum and hydrous wool fat base, next with the petrolatum base, and then with the zinc oxide base. The changes generally occurred more quickly with greater concentrations of Salicylic Acid.

Creams and ointments containing 2%, 4%, 6%, or 12% Salicylic Acid were applied to the skin of the upper limbs of four subjects per group for 1 week (Marks, Davies, and Cattell 1975). A control group was treated with vehicle only. Skin biopsies were taken. None of the creams caused an increase in mean labeling index or mean epidermal thickness compared to controls, but a progressive increase was seen with the ointments. However, marked changes in the stratum corneum were seen with the creams; in scanning electron micrographs, wide intercellular gaps were found and surfometric analysis was indicative of an irregular surface contour.

Davies and Marks (1976) used 23 subjects to determine the effect of 2%, 4%, 6%, 8%, 10%, or 12% Salicylic Acid in aqueous cream or 2%, 6%, or 10% Salicylic Acid in white soft paraffin on the skin. The test materials were applied to either the inner aspect of one arm or the lateral aspect of one thigh. The appropriate vehicle was applied to a contralateral site as a control. The materials were applied twice daily and rubbed into the skin for 1 min; the sites were not occluded.

After 1 week, the test areas were biopsied. No differences in the samples were seen upon microscopic examination. In treated cryostat sections, differences were found between the treated and control sites; the treated sites had an irregular and much thinner stratum corneum. The mean epidermal thickness was similar for test and control sites. No differences in labeling indices from tissue incubated with tritiated thymidine were seen between the treated and respective control groups. However, a significant difference was found in the labeling index of the cream and paraffin controls; the values were 7.6 and 5.7, respectively. No differences in labeling were seen between test and control specimens of the 2% Salicylic Acid in cream or 10% Salicylic Acid in paraffin groups incubated with tritiated histidine or cytidine.

In scanning electron micrographs of skin surface biopsies, differences were found between treated and control sites, especially at the greater concentrations of Salicylic Acid. The test samples had marked irregularity in the overall arrangement of the horny layer, with many irregular and loose lamellae composed of several squames, and irregularity occurred in scale apposition with large gaps (3 to 10 μm) between individual squames. The researchers postulated that salicylic acid preparations enhance desquamation by encouraging squame separation by causing the dissolution of intercellular cement material (Davies and Marks 1976).

The effect of Salicylic Acid on the stratum corneum was determined by measuring desquamation, thickness, and mitotic activity (Roberts, Marshall, and Marks 1980). First, 6% Salicylic Acid in 70% alcohol was applied to the forearm of five subjects, two males and three females, who were without generalized skin disorders or systemic disease; 70% alcohol was applied to the other arm as a control. 'Forced' desquamation corneocyte counts using a hand-held scrub apparatus were taken from different sites 1, 2, 3, 4, 6, and 8 h after application.

In a second study, the 6% Salicylic Acid in alcohol was applied twice daily for 10 days to the flexor aspect of the forearms of six subjects, one male and five females, who were without skin disorders or systemic disease; again, 70% alcohol was applied to the opposite arm as a control. Prior to the initial application, a 1-cm^2 portion of the test area was stained with a 1% aqueous silver nitrate solution reduced with a photographic developer, and 24 h prior to the initial application, 5% dansyl chloride was applied to both forearms under an occlusive patch. 'Forced' desquamation was performed on days 2, 4, 6, 8, and 10. The gray-black area that resulted from the application of silver nitrate was photographed every 2 h daily until the "abnormal" color faded. The density of the stain on the photographic negative was measured. To determine the turnover time, the areas treated with dansyl chloride were examined daily with a UV lamp that emitted primarily in the UVA region until fluorescence disappeared. A 4 mm punch biopsy was taken from the treated and control site.

In the first study, the 'forced' desquamation cell count increased on the test arm until the 3-h reading (from 96.8 to 140.2 cells/cm^2/10 s $\times$ 10^3), then it decreased, whereas on the control arm the cell count decreased slightly (from 100.6 to 99.4 cells/cm^2/10 s $\times$ 10^3) until the 2-h reading, then it increased slightly. In the second study, there was a marked persistent decrease in desquamation at the test site, while on the control arm, the corneocyte count initially decreased but reached normal values after 4 days. The difference in corneocyte count between the test and control arms was significant on days 6, 8, and 10.

The authors concluded that the results of the silver nitrate densitometry technique indicated that the stain was released more rapidly from the treated site than the control site, but the difference was not significant. No significant difference in the loss of fluorescence due to dansyl chloride was observed between the test and control sites (16 versus 15.2 days, respectively). The biopsies indicated that the Salicylic Acid treated sites had a much thinner stratum corneum (mean 16.1, units not stated; the mean prior to treatment was 23.3) than the control sites (mean 22). No significant difference in the labeling indices of autoradiographs derived from tissues incubated with tritiated thymidine was seen (Roberts, Marshall, and Marks 1980).

The effect of Salicylic Acid on transepidermal water loss (TEWL) was determined (Guillaume et al. 1981). A dose of 5 mg/cm^2 of 5% Salicylic Acid in a w/o emulsion was applied once a day for 7 days under an occlusive patch to a 5 $\times$ 5 cm area of the ventral forearm of six male and three female subjects. Untreated open and occluded control sites were used, as was an occluded site with vehicle only. TEWL was measured 1 h after patch removal. The average TEWL was 1.47 mg/cm^2/h and the range was 0.53 to 3.24 mg/cm^2/h. TEWL was significantly increased by Salicylic Acid compared to both the open and occluded untreated control site and the occluded vehicle site.

In a second study, these authors applied 5% Salicylic Acid in a w/o emulsion in an open manner twice daily for 10 days to the ventral forearm of eight male and four female subjects. TEWL was measured 1 h after the last application. An untreated and vehicle-control site were used. The average TEWL was 0.63 mg/cm^2/h and the range was 0.37 to 1.05 mg/cm^2/h. TEWL was significantly increased by Salicylic Acid compared to both the untreated and vehicle-control sites (Guillaume et al. 1981).

The keratoplastic effect of Salicylic Acid was examined using the cantharidin blister model (Gloor and Beier 1984). Salicylic Acid, 6% in a 70% isopropyl alcohol solution, was applied to the right lower inner arm of seven male subjects twice daily for 10 days. Isopropyl alcohol only was applied to the left arm,

which served as a control. After the last dose on day 10, a 0.1% cantharidin solution in acetone was applied to both arms, the subepidermal blister which formed was removed, and the number of cell layers in the corneal layer was examined. The number of cell layers was significantly less at the test site than the control site, indicating that Salicylic Acid had a keratoplastic effect.

The effect of Salicylic Acid on corneocyte surface area was determined using 10 male subjects with normal skin (Robinson et al. 1994). Salicylic Acid, 5% in an alcoholic gel, was applied to a 12 × 6-cm area of the back of each subject 6 days per week for 4 weeks. The vehicle, 2.5% *w/w* hydroxypropylcellulose, 0.05% *w/w* butylhydroxytoluene, and ethyl alcohol, was also applied, and an untreated site was used as a control site. The subjects were to avoid exposing the test sites to sunlight. Corneocyte samples were taken prior to dosing and on study days 7, 14, 21, and 29, and surface area measurements were performed using image analysis. Dermatitis, characterized by desquamation with minimal or no erythema and by pruritus, was observed as of week 1. No significant differences were observed between treated and control site corneocytes. At all test sites, cyclical changes in mean surface area with respect to baseline were observed.

A tape-stripping technique was used to determine the keratolytic potential of Salicylic Acid (Lodén, Boström, and Kneczke 1995). Fifty milliliter of 0.5% and 2.0% Salicylic Acid in an aqueous vehicle containing 30% ethyl alcohol were applied to the skin of the inner upper arm of 10 subjects using Finn chambers. At 3 and 6 h after application, the chambers were removed and the test sites were tape stripped six times. The transmission of light through the tape was measured with a digital light measuring instrument.

The authors reported that significantly more material was detached from the site treated with 2% Salicylic Acid for 6 h than was detached from vehicle-treated skin, especially seen at the third and fourth tape stripping; however, less material was removed from the Salicylic Acid–treated area at the first tape stripping as compared to the area treated with vehicle only. The researchers also examined the absorption of Salicylic Acid in vitro by tape stripping human breast skin that was exposed to 0.5% and 2.0% Salicylic Acid for 3 and 6 h. Five to 18 $\mu g/cm^2$ were found in the tape strips; greater amounts were found with 2% Salicylic Acid, especially at the 3 h stripping (Lodén, Boström, and Kneczke 1995).

Six subjects were used in a study examining the effect of Salicylic Acid on the skin (Piérard et al. 1997). Salicylic Acid, 5% in a nonionic *o/w* vehicle, was applied to the forearm of each subject twice daily for 4 weeks. Vehicle alone was also applied, and a nontreated control site was used. The dermal effects were determined using immunohistochemistry and computerized image analysis. Changes in epidermal renewal, modifications in cytokeratin and filaggrin patterns, and TNF-α were examined. Both the Salicylic Acid– and vehicle-treated sites were similar to the untreated control site.

Therapeutic Action

Salicylic Acid

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 Salicylic Acid (Van Scott and Yu 1974). Salicylic Acid was dissolved in either water or ethanol and incorporated into a hydrophilic ointment of plain petrolatum. The ointment, containing 10% Salicylic Acid (pH not specified), was applied twice daily to the appropriate test site for 2 weeks. Daily to weekly observations were made. Salicylic Acid provided 1+ improvement, i.e., a slight improvement over that provided by vehicle alone.

A study investigating the effect of Salicylic Acid on treatment of psoriasis was completed with patients with chronic stable plaque-type psoriasis covering greater than 10% of the skin (Kristensen and Kristensen 1991). The patients had skin types II to IV, and emollient to which 2% Salicylic Acid had been added was used. The patients were irradiated three to five times weekly for up to 6 weeks using UV cabins equipped with 16 Philips TL 12 lamps that gave an output of 1.35 mW/cm^2. Maximum irradiation time was 15 min, giving 1.215 J/cm^2. Salicylic Acid decreased clearing in 8 of the 11 patients (73%) treated with the Salicylic Acid emollient. These results were significant compared to patients treated without Salicylic Acid.

Therapeutic Toxicity

A retrospective study involving seven clinicians examined whether hepatomegaly was associated with salicylate therapy in the management of juvenile rheumatoid arthritis (Abbott and Harrisson, no date). A total of 218 cases were reviewed with salicylate dosages of up to 4800 mg/day for 8 to 10 years. No link between salicylate therapy and hepatomegaly was found. These same authors examined possible changes in the density of metaphyses in affected joints due to salicylate administration (Abbott and Harrisson, no date). X-rays from a combined total of 155 cases were reviewed in which various forms of salicylates were administered at doses of 100 to 3240 mg for several months to intermittent dosage for 14 years. No bone lesions were seen.

Salicylic Acid

Signs and symptoms of Salicylic Acid poisoning include nausea, vomiting, tinnitus, dizziness, headache, dullness, confusion, sweating, rapid pulse and breathing, possible skin eruptions (Sax 1979), lethargy, hyperventilation, tachycardia, and fever (Klein-Schwartz 1983). Severe poisoning can result in delirium, hallucinations, convulsions or coma, and respiratory or cardiovascular collapse. Patients allergic to salicylates have had urticarial, anaphylactic, and erythema multiforme reactions (Goldsmith 1979). "Significant" salicylate concentrations can affect platelet function and alter blood coagulation. Blood concentrations of salicylate that are >2.17 mM (300 $\mu g/ml$) are considered toxic (Moore et al. 1995). Birmingham, Greene, and Rhodes (1979) stated that serum salicylate concentrations >20 mg/dl can cause

toxic symptoms. The adverse effects of aspirin, especially gastric irritation and bleeding, are due to Salicylic Acid (Salako, Fadiran, and Thomas 1989).

Toxic reactions to salicylate generally occur more frequently in children because their extracellular fluid volume is small in comparison to the potential areas of application (Taylor and Halprin 1975). With the elderly, care must be taken in prescribing salicylate-containing drugs (and/or other drugs); systemic clearance of salicylates (mainly by hepatic metabolism) may be reduced with age (Durnas and Cusack 1992).

Methyl Salicylate

Methyl Salicylate taken in quantities of ≥1 teaspoon are reported to be "quite toxic" (21 CFR 201.303). (One teaspoon [5 ml] Methyl Salicylate is equivalent to 7000 mg salicylate or 21.7 325 mg aspirin tablets.) The oral LD_{LO} of Methyl Salicylate was 170 mg/kg (Sax 1979). Accidental acute poisoning is not uncommon, especially in children. Kidney irritation, vomiting, and convulsions occur.

The average lethal dose of Methyl Salicylate is 10 ml for children and 30 ml for adults (Environmental Health Research and Testing, Inc. 1984). Common symptoms of toxicity include nausea, vomiting, acidosis, pulmonary edema, pneumonia, convulsions, and possibly death.

Concomitant use of Methyl Salicylate and drug substances can be problematic. Use of topical analgesic preparations containing Methyl Salicylate in conjunction with oral warfarin can result in adverse reactions and bleeding (Chan 1996).

Sodium Salicylate

Sodium Salicylate is a "powerful irritant" (Sax 1979). It can affect the central nervous system.

Case Studies

Numerous case studies documenting the toxic potential of Salicylic Acid, Sodium Salicylate, and Methyl Salicylate after oral ingestion have been reported (Troll and Menten 1945; Ashworth and McKemie 1944; Ryder, Shaver, and Ferris 1945; Craig 1953; Adams, Bigler, and Green 1957; Winek, Collom, and Nelson Voldeng 1973; Pascher 1978; Lester and Davis 1984; Howrie, Moriarty, and Breit 1985; Litovitz and Manoguerra 1992; Koren 1993; Liebelt and Shannon 1993; Chan, Wong, and Chan 1995).

Toxicity has also been described with dermal application of salicylates for management of skin diseases in which 3 to 21% Salicylic Acid was applied (Cawley, Peterson, and Wheeler 1952; von Weiss and Lever 1964; Lindsey 1968; Luderschmidt and Plewig 1975; Davies, Vella Briffa, and Greaves 1979; Raschke et al. 1991; Abdel-Magid and Ahmed 1994; Dwyer, McCloskey, and Kerr 1994; Germann et al. 1996; Chiaretti et al. 1997); in one case study, toxicity was observed as a result of dermal application of Salicylic Acid with concomitant oral administration of a nonsteroidal anti-inflammatory drug (Shupp and Schroeter 1986). Additionally, toxicity was observed with an elderly subject recovering from acute renal failure following dermal application of a Salicylic Acid ointment (Smith and Lyons 1980). Dermal application of a product containing Methyl Salicylate produced toxicity (Bartle et al. 1992), and topical application of Methyl Salicylate (and menthol) followed by the application of heat resulted in skin and muscle necrosis and interstitial nephritis (Heng 1987).

In a patch test of a patient with acute dermatitis who had been using an ointment containing menthol, camphor, and 12% Methyl Salicylate, positive results were seen with 2% Methyl Salicylate in arachis oil and 2% aqueous Sodium Salicylate (Hindson 1977).

A case study was reported in which Methyl Salicylate caused severe urticaria and angioedema (Speer 1979).

In two case studies of reactions to a wart paint containing Salicylic Acid, patch testing showed that Salicylic Acid (tested at 3% in petrolatum) was not the causative agent (Lachapelle and Leroy 1990).

Rudzki and Koslowska (1976) reported positive reactions to 5% Salicylic Acid in yellow soft paraffin in four patients with dermatitis and one with psoriasis; the four patients with dermatitis had used a 2% "salicylic spirit," and the one with psoriasis had used a "5% unguentum salicylicum."

SUMMARY

Salicylic Acid is an aromatic acid used in cosmetic formulations as a denaturant, a hair conditioning agent, and a skin-conditioning agent—miscellaneous. The Calcium, Magnesium, and MEA salts are used as preservatives. Potassium Salicylate is used as a cosmetic biocide and preservative. Sodium Salicylate is used as a denaturant and preservative. The TEA salt of Salicylic Acid is used as a UV light absorber. Several Salicylic Acid esters are used as skin-conditioning agents—miscellaneous (Capryloyl, C12–15 Alkyl, Isocetyl, Isodecyl, and Tridecyl). Butyloctyl Salicylate and Hexyldodecyl Salicylate are used as hair-conditioning agents and skin-conditioning agents—miscellaneous. Ethylhexyl Salicylate (formerly known as Octyl Salicylate) is used as a fragrance ingredient, sunscreen agent, and UV light absorber, and Methyl Salicylate is used as a denaturant and flavoring agent. Myristyl Salicylate has no reported function.

Salicylic Acid and Methyl Salicylate are soluble in organic solvents, but only slightly soluble in water. Ethylhexyl Salicylate is not soluble in water. Calcium, Potassium, and Sodium Salicylate are soluble in water. Potassium Salicylate is reported to be very soluble in water and alcohol. These ingredients have either no odor or only a faint odor, except for Methyl Salicylate, which has the characteristic odor of wintergreen. Consistent with the several medical treatments involving salicylates, test methodologies have been developed for detecting Salicylic Acid in urine and serum. Heavy metal concentration limitations are described for USP grade Magnesium, Sodium, and Methyl Salicylates and for cosmetic grade Methyl Salicylate. Salicylic Acid and Ethylhexyl Salicylate absorb UVB radiation.

Salicylic Acid is used in 107 cosmetic formulations at concentrations ranging from 0.0008% to 3%. Ethylhexyl Salicylate is used in 87 formulations at 0.001% to 8%. Methyl Salicylate is used in 25 formulations at 0.0001% to 0.6%. Sodium Salicylate is used in seven formulations at 0.09% to 2%. TEA-Salicylate is used in five formulations at 0.0001% to 0.75%. Capryloyl Salicylate is used in five formulations at 0.1% to 1%. Isodecyl Salicylate is used in three formulations, but no concentration of use information was reported. Isocetyl Salicylate is not reported to FDA as used, but is reported to CTFA as being used at concentrations ranging from 3% to 5%. Likewise, Butyloctyl Salicylate is not reported to FDA as being used, but is reported to CTFA as being used at 0.5% to 5%. Methyl Salicylate is used in perfumery.

Salicylic Acid, Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate are allowed for use in cosmetics in the European Union as preservatives at a maximum concentration of 5% (acid), except that these ingredients are not to be used in preparations for children under 3 years of age, except for shampoo formulations, which must bear a label warning against use on children under 3 years of age.

In Japan, Salicylic Acid which conforms to the standards of the *Japanese Standards of Cosmetic Ingredients* (JSCI) has precedent for use at a maximum concentration of 0.2% in all categories except eyeliner preparations, in which it is not used. Sodium Salicylate which conforms to the specifications of the JSCI has precedent for use at a maximum concentration (calculated as total Salicylic Acid) of 1% in cleansing preparations and of 0.2% in hair care, treatment, makeup, fragrance, suntan and sunscreen, and nail makeup preparations; it is not used in eyeliner, lip, oral, or bath preparations. Sodium Salicylate is restricted as to the percent as total Salicylic Acid salts allowed in a formulation. Methyl Salicylate, which conforms to the specifications of the JSCI, has precedent for use at a maximum concentration of 0.1% in all Comprehensive Licensing Standards of Cosmetics (CLS) categories except eyeliner preparations, in which it is not used. Ethylhexyl Salicylate, which conforms to the specifications of the *Japanese Cosmetic Ingredient Codex*, has precedent at a maximum concentration of 10% in suntan/sunscreen preparations and of 1% in all other CLS preparations except eyeliner and bath preparations, in which it is not used. Methyl and Ethylhexyl Salicylates are restricted in that the total percentage of UV absorbers in a formulation shall not exceed 10%.

These ingredients have uses in foods and drugs that are regulated by FDA. Salicylic Acid, Magnesium Salicylate, Sodium Salicylate, and Methyl Salicylate have FDA-specified uses as indirect food additives. Salicylic Acid is an approved active ingredient for use in topical OTC acne drug products at concentrations of 0.5% to 2%; in OTC wart remover drug products at concentrations of 12% to 40% in a plaster vehicle, 5% to 17% in a collodion-like vehicle, and 15% in a karaya gum, glycol plaster vehicle with proper labeling directions; in corn and callus remover OTC drug products at concentrations of 12% to 40% in a plaster vehicle and 12% to 17.6% in a collodion-like vehicle with proper labeling directions; and in OTC drugs for the control of dandruff, seborrheic dermatitis, and psoriasis at a concentration of 1.8% to 3%.

Salicylic Acid has been present in OTC topical acne preparations (at concentrations of 2% to 5%), external analgesics and skin protectants used for poison ivy, oak, and sumac, and topical antifungal drug products. Calcium Salicylate has been present in OTC internal analgesic drug products. Sodium Salicylate has been present in OTC dandruff/seborrheic dermatitis/psoriasis and digestive aid drug products. TEA-Salicylate has been present in OTC external analgesic—fever blister and cold sore; insect bite and sting; and poison ivy, oak, and sumac drug products. Methyl Salicylate has been present in OTC smoking deterrent drugs, boil treatment, dandruff/seborrheic dermatitis/psoriasis, fever blister and cold sore treatment, oral health care, and skin protectant–astringent drug products. However, currently FDA has concluded that there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for these specified OTC uses.

Any drug product intended to be taken orally that contains any salicylate ingredient, except effervescent preparations, must bear a statement warning to keep the product out of the reach of children. Any drug containing >5% Methyl Salicylate must bear a label that warns that misdirected use may be dangerous and that the product should be kept out of the reach of children. TEA-Salicylate is allowed for use as an active ingredient in sunscreens at concentrations of <12%, whereas Ethylhexyl Salicylate is allowed at concentrations of <5%.

In veterinary practice, Salicylic Acid is allowed for use in the removal of scar tissue from the teat canal of milk-producing cows; however, a residue tolerance of 0 has been established for milk from dairy animals. In clinical practice, Salicylic Acid has been used in the treatment of ichthyosiform dermatoses. A traditional use of Methyl Salicylate is as a counterirritant.

Salicylic Acid is used in the manufacture of aspirin. Salicylic Acid is also used in the manufacture of salicylates and resins and as a dyestuff intermediate, prevulcanization inhibitor, analytical reagent, and fungicide. Sodium Salicylate is used as a preservative for paste, mucilage, glues, and hides.

Absorption of salicylates from the stomach is normally rapid. Extensive data are available in animals and humans from oral delivery studies. Metabolism by hepatic microsomal enzyme systems conjugates salicylates to glycine, forms glucuronides, or oxidizes them to hydroxybenzoic acids. Salicylates are also absorbed percutaneously. Urinary metabolites resulting from percutaneous delivery are reportedly quantitatively different from those seen with oral delivery, with more glucuronides found and more unmetabolized Salicylic Acid. Data on percutaneous absorption are available from in vitro and in vivo testing of penetration through animal skin. In vitro data are available for pig, mouse, and rat skin. In vivo percutaneous absorption data are available for rabbits, guinea pigs, rats, mice (including hairless

mice), dogs, and monkeys. Data describing penetration through human skin are also available. These animal and human data describe the following percutaneous absorption patterns: rate of penetration is proportional to concentration applied; absorption is dependent on the vehicle (e.g., ethanol > water); absorption varies as a function of pH; and absorption is greater through damaged skin compared to normal skin. Around 10% of applied salicylates can remain in the skin. Parenteral absorption data are also available.

Salicylic Acid is keratolytic. Salicylic Acid is reported to enhance percutaneous penetration of vitamin A, ammoniated mercury, and triamcinolone acetonide, but not methyl nicotinate (which itself rapidly penetrates skin), hydrocortisone, diflucortolone-21-valerate, or cyclosporine.

One study describes the minimal inhibitory concentrations of Salicylic Acid against bacteria, yeasts, and fungi, asserting that its preservative action is restricted to the pH range 2 to 5. Other data show that Salicylic acid inhibits growth of the following cells in culture: HeLa, human prostatic carcinoma, dog distal renal tubular, pig renal proximal tubular, rat kidney, human hepatoma, *B. subtilis*, and *E. coli*. Sodium Salicylate inhibits growth of human fibroblast and rat hepatoma cells in culture at high doses. Inhibition of iNOS is one hypothesis for the cytotoxicity of Sodium Salicylate in several mammalian cell lines. Methyl Salicylate inhibited HeLa and *B. subtilis* cell growth in culture.

Salicylic Acid has anti-inflammatory effects. Sodium Salicylate influences interferon titres in mice; interferes with neutrophil function in vitro; inhibits induction of chemokine mRNA and activation of NF-κB in bone marrow cells; inhibits TNF-induced activation of c-Jun N-terminal kinase and c-*fos* mRNA in human diploid fibroblasts; and enhances tyrosine phosphorylation and increases p38 kinase activity in COS cells. Methyl Salicylate produced an inflammatory response in the ear of female mice, but in vitro exposure of human epidermal keratinocytes to Methyl Salicylate failed to induce IL-8, TNF-α, or GM-CSF.

Salicylic acid produces pharmacologic/physiologic effects as follows: increases the stability of lysosomal membranes in rats and decreases ALT activity in the medium of cultured rat hepatocytes. Sodium Salicylate influenced blood pH in rats, and markedly increased bile flow in rats dosed intraperitoneally, but few other hepatic changes were seen.

Little acute toxicity (LD_{50} in rats; >2 g/kg) via a dermal exposure route is seen for Salicylic Acid, Methyl Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate. These compare with oral acute LD_{50} values for Salicylic Acid in rats ranging from a low of 0.891 g/kg to a high of 1.58 g/kg; for Sodium Salicylate, between 0.9 g/kg and 1.7 g/kg; for Isodecyl Salicylate, no toxicity at levels as high as 4.83 g/kg; for Methyl Salicylate, between 0.887 g/kg and 1.25 g/kg; for Ethylhexyl (Octyl) Salicylate, >2 g/kg; for Tridecyl Salicylate, >1.98 g/kg; and for Butyloctyl Salicylate, >5 g/kg. Values for acute oral toxicity in other species are consistent with these values. Methyl Salicylate given by inhalation is not lethal in mice and rats. The parenteral LD_{50} for Salicylic Acid in mice is 0.52 g/kg and the acute toxicity of Sodium Salicylate Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, and Tridecyl Salicylate via this route of administration are generally in the one gram per kilogram range.

Short-term oral, inhalation, and parenteral exposures to Methyl Salicylate are available. Inconsistent results are seen regarding bone lesions with oral exposures, but reduced growth and feed consumption are consistently seen. No toxicity is seen with inhalation of Methyl Salicylate in a series of 20 exposures of 7 h each at 0.7 g/m^3 and no bone lesions were seen with parenteral exposure. Sodium Salicylate oral exposures are linked with reduced growth and feed consumption, clear kidney damage, and some liver damage; parenteral exposures result in hyperpnea and profuse diuresis in single animal experiments. Salicylic Acid oral delivery produces liver and plasma enzyme changes.

Subchronic dermal, oral, and inhalation studies are available for Methyl Salicylate. Dermal and inhalation exposures are associated with kidney damage. Inhalation exposures also produce pulmonary focal hemorrhages and hyperplasia. Oral exposure results in reduced weight gain and bone lesions that disappear if Methyl Salicylate is coadministered with Calcium Carbonate. No toxicity is seen with oral subchronic exposure to Isodecyl Salicylate or Tridecyl Salicylate. Oral subchronic exposure to Sodium Salicylate is associated with reduced growth and feed consumption, and indication of some bone lesions and isolated muscle weakness.

Chronic exposure data are available for Methyl Salicylate. Adverse effects are seen as a function of the level of exposure in 2-year rat studies, with 2% producing bone lesions and 0.7% not doing so. Liver damage is seen in dogs exposed to 0.15 g/kg/day in one study, kidney and liver weight increases in another study at the same exposure, but no liver or kidney abnormalities in a study at 0.167 g/kg/day.

Dermal irritation studies are available for Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate. Application of 500 mg (in 0.5 ml) of Isodecyl, Tridecyl, and Butyloctyl Salicylate are not irritating. Undiluted application of Ethylhexyl (Octyl) Salicylate produces minimal to mild irritation. Methyl Salicylate at concentrations of greater than 50% is clearly irritating. One study of the effect of vehicle on Methyl Salicylate irritation shows irritation at concentrations as low as 1% with a 70% ethanol vehicle producing the most irritation and polyethylene glycol producing little or no irritation at Methyl Salicylate concentrations up to 6%.

The ocular irritation potential is negative for Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate.

Data are available on the use of a local lymph node assay to determine the sensitization potential of Salicylic Acid and Methyl Salicylate. Although Salicylic Acid at a concentration of 20% in acetone is positive in this assay, a concentration of 20% in acetone/olive oil is not. Methyl Salicylate is negative at

concentrations up to 25%, independent of vehicle. Maximization tests of Methyl Salicylate are negative, as they are for Ethylhexyl (Octyl) Salicylate and Butyloctyl Salicylate. Neither Salicylic Acid nor Tridecyl Salicylate are photosensitizers.

Salicylic Acid, produced when aspirin is rapidly hydrolyzed to Salicylic Acid after absorption from the gut, was reported to be the causative agent in aspirin teratogenesis in animals. Dermal exposures to Methyl Salicylate, oral exposures to Salicylic Acid, Sodium Salicylate, and Methyl Salicylate, and parenteral exposures to Salicylic Acid, Sodium Salicylate, and Methyl Salicylate are all associated with reproductive and developmental toxicity as a function of blood levels reached as a result of exposure.

An exposure assessment of a representative cosmetic product used on a daily basis is available which estimates that the exposure from the cosmetic product would be only 20% of the level seen with ingestion of a "baby" aspirin (81 mg) on a daily basis. This exposure assessment further contends that the reproductive and developmental toxicity from the daily use of a baby aspirin is not significant.

Studies of the genotoxic potential of Salicylic Acid, Sodium Salicylate, Isodecyl Salicylate, Methyl Salicylate, Ethylhexyl (Octyl) Salicylate, Tridecyl Salicylate, and Butyloctyl Salicylate are negative, except that Salicylic Acid is positive in a *B. subtilis* rec assay (negative in seven other bacterial tests and one mammalian test); Methyl Salicylate is positive in *S. typhimurium* strains TA98 and TA100 with metabolic activation (negative in two other Ames tests); and Sodium Salicylate is positive in an in vivo chromosome aberration study in mice (negative SCE in vivo in mice, and in four in vitro test systems).

Methyl Salicylate, in a mouse skin painting study, does not induce neoplasms. Likewise, Methyl Salicylate is negative in a mouse pulmonary tumor system. In vitro predictors of carcinogenesis are also negative for Salicylic Acid and Sodium Salicylate.

Clinical tests for cumulative irritation are available for the following ingredients at the specified concentrations: Salicylic Acid (2%—minimal cumulative irritation; 1.5%—slight or no irritation); TEA-Salicylate (8%—no irritation); Methyl Salicylate (>12%—pain and erythema; 8%—no irritation; 1% aerosol—erythema); Ethylhexyl (Octyl) Salicylate (4%—no irritation); and Tridecyl Salicylate (no irritation). In 20 patients with eczema or contact dermatitis, Methyl Salicylate at 67% is reported to cause irritation in 8 subjects; at 40%, 2 subjects; and at 38%, 15%, and 3.75%—no irritation in any subject.

If Salicylic Acid is applied after the application of agents (benzoic acid, cinnamic aldehyde, methyl nicotinate, and DMSO) known to cause nonimmunologic immediate contact reactions in the skin, the erythema induced by benzoic acid, cinnamic aldehyde, and methyl nicotinate is reduced, but there is no effect on edema.

In normal skin, Salicylic Acid, Methyl Salicylate, and Ethylhexyl (Octyl) Salicylate are not sensitizers. In patients with venous leg eczema, Salicylic Acid augments histidine release in 3/320 challenged with ragweed pollen. Sodium Salicylate injected in the skin of aspirin intolerant individuals affected several parameters as follows: 1/23 had a positive skin test to Sodium Salicylate; 2/31 were positive in the passive cutaneous anaphalaxis test; and 2/26 were positive in the lymphocyte transformation test. Salicylic Acid is not a photosensitizer, nor is it phototoxic. Salicylic Acid and Ethylhexyl (Octyl) Salicylate are low level photoprotective agents.

Salicylic Acid exacerbates urticarial reactions to aspirin; 13 of 18 patients in one study and 6 of 20 in another. At 5% in petrolatum, however, Salicylic Acid does not cause any urticarial reactions in atopic, urticarial, nonatopic, and nonallergic patients.

Salicylic Acid is well-documented to have keratolytic action on normal human skin. It had a small therapeutic effect in patients with various forms of ichthyosiform dermatoses, but decreased clearing in 8 of 11 psoriasis patients when compared to UV therapy alone. Therapeutic toxicities include nausea, vomiting, tinnitus, dizziness, headache, dullness, confusion, sweating, rapid pulse and breathing, skin eruptions, and fever. One estimate is that a blood concentration >300 μg/ml of a salicylate should be considered toxic. Toxic reactions occur more frequently in children. Care must be taken in prescribing salicylate-containing medications because systemic clearance of salicylates may be reduced with age. Severe poisoning can result in delirium, hallucinations, convulsions, coma, and respiratory or cardiovascular collapse. Reversable hearing loss and tinnitus is a reported side effect of salicylates at therapeutic levels.

Methyl Salicylate taken in quantities greater than or equal to 1 teaspoon are reported to be quite toxic (equivalent of the salicylate that could be derived from 20+ adult aspirin tablets). Accidental poisoning is not uncommon, especially in children; symptions of poisoning include kidney irritation, vomiting, and convulsions. The average lethal dose of Methyl Salicylate is 10 ml for children and 30 ml for adults. Use of topical analgesics with Methyl Salicylate in combination with oral warfarin can result in adverse reactions.

Numerous case studies report toxic reactions to oral ingestion of salicylates. Dermal toxicity is also described in the case literature as follows: dermal application of Salicylic Acid with concomitant oral administration of a nonsteroidal anti-inflammatory drug; following dermal application of a Salicylic Acid ointment in an elderly subject recovering from acute renal failure; topical application of Methyl Salicylate (and menthol) followed by the application of heat (skin and muscle necrosis and interstitial nephritis); and severe urticaria and angioedema with Methyl Salicylate exposure.

In two case studies of reactions to a wart paint containing Salicylic Acid, Salicylic Acid (tested at 3% in petrolatum) was not the causative agent. Two percent Methyl Salicylate in arachis oil and 2% aqueous Sodium Salicylate produced positive positive patch test results in a patient with acute dermatitis who had been using an ointment containing menthol, camphor. Twelve

percent Methyl Salicylate and 5% Salicylic Acid in yellow soft paraffin produced positive patch tests in four patients with dermatitis and one with psoriasis, all with some history of exposure to salicylates.

A review of radiographs taken in 155 cases of juvenile arthritis in which various forms of salicylates had been administered at concentrations ranging from 0.1 to 3.24 g for several months did not find any evidence of bone lesions.

DISCUSSION

The CIR Expert Panel considered that the available information is sufficient to evaluate the safety of these ingredients in cosmetic formulations. In reaching its conclusion, the Panel considered three primary issues: (1) increased sun sensitivity (e.g., UV radiation induced skin damage); (2) skin irritation; and (3) reproductive and developmental toxicity.

The Panel expects that these ingredients will have application as exfoliating agents in cosmetic formultations at concentrations of use at the high end of the currently reported use levels, in addition to the other uses that have been specified. In that regard, the Panel expressed concern that repeated use of Salicylic Acid and the various salicylates may effectively increase exposure of the dermis and epidermis to UV radiation. On the other hand, information is available suggesting that these ingredients absorb UV radiation, which would decrease the exposure. Data are not available that suggest what the balance of these two influences would be vis a vis UV radiation–induced skin damage. Drawing on its previous experience in reviewing the safety of alpha hydroxy acids (AHAs), the Panel compared the relatively mild exfoliating action of Salicylic Acid and the various salicylates with that of AHAs, factored in the ultraviolet radiation absorption by salicylates, and estimated that the small increase in sun sensitivity associated with use of AHAs would likely be smaller still with salicylates.

The Panel considered requesting additional safety testing of these ingredients to resolve this question of the existence and/or magnitude of an increase in sun sensitivity, but was convinced that the exfoliant action alone would always raise the possibility that some increase in UV radiation–induced skin damage would be detected, e.g., if more animals had been used, if a more sensitive assay for damage were available, etc. Were there to be evidence of a small increase in sun sensitivity associated with the use of Salicylic Acid and the several salicylates at exfoliant concentrations, or were the available data to be equivocal, the Panel reasoned that the appropriate conclusion would be that these ingredients could be used safely as exfoliants, if expressly formulated to avoid increasing a user's sun sensitivity. Accordingly, the Panel concluded that the prudent course of action would be to advise the cosmetics industry that there can be a risk of increased UV radiation damage with the use of any exfoliant, including Salicylic Acid and the listed salicylates, and that steps need to be taken to formulate cosmetic products with these ingredients as exfoliating agents so as not to increase sun sensitivity, or when increased sun sensitivity would be expected, to include directions for the daily use of sun protection.

The Panel was concerned that the available data were not sufficient to establish a limit on concentration of these ingredients, or to identify the minimum pH of formulations containing these ingredients, such that no skin irritation would occur. Such limits were established with AHAs. Because the available animal and clinical safety test data demonstrate that these ingredients are generally milder than AHAs, the Panel was convinced that it is possible to formulate cosmetic products in a way such that significant irritation would not be likely. Therefore, the Panel concluded that the cosmetics industry should formulate products containing these ingredients so as to be non-irritating.

Reproductive and developmental toxicity associated with exposures to large, therapeutic serum concentrations of Salicylic Acid (as a metabolite of aspirin) have been extensively demonstrated. The Panel considered the possibility that use of Salicylic Acid or the various salicylates could produce serum levels of Salicylic Acid or, with other sources (e.g., aspirin), contribute to serum levels and thereby present a reproductive and developmental toxicity risk. Beginning with the premise that ingestion of a low-dose regimen (81 mg) aspirin by a 58-kg female would result in an exposure of ~1.4 mg/kg/day and that this exposure level is not considered to present any reproductive or developmental toxicity risk, the Panel considered that a representative exposure to a cosmetic product containing Salicylic Acid could result in exposure to ~0.4 to 0.5 mg/kg/day and would not present a risk. Although simultaneous use of several products containing Salicylic Acid could produce exposures greater than would be seen with a baby aspirin, the Panel also did not consider it likely that consumers would simultaneously use multiple cosmetic products containing Salicylic Acid. Thus, the serum levels of Salicylic Acid that would result from dermal application would likely be less than serum levels from ingestion.

CONCLUSION

Based on the available information, the CIR Expert Panel concluded that Salicylic Acid, the salts Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate; the esters Capryloyl Salicylic Acid, C12–15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate; and Tridecyl Salicylate, and the compounds Butyloctyl Salicylate and Hexyldodecyl Salicylate are safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

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2018 FDA VCRP Data

Butyloctyl Salicylate

03C - Eye Shadow	1
07C - Foundations	1
07E - Lipstick	6
07F - Makeup Bases	1
07I - Other Makeup Preparations	1
12A - Cleansing	1
12C - Face and Neck (exc shave)	3
12F - Moisturizing	2
12G - Night	1
12J - Other Skin Care Preps	1
13A - Suntan Gels, Creams, and Liquids	1
Total	**19**

Calcium Salicylate

No FDA VCRP Data

C12-15 Alkyl Salicylate

No FDA VCRP Data

Capryloyl Salicylic Acid

03D - Eye Lotion	5
03G - Other Eye Makeup Preparations	4
11G - Other Shaving Preparation Products	2
12A - Cleansing	9
12C - Face and Neck (exc shave)	23
12D - Body and Hand (exc shave)	3
12F - Moisturizing	24
12G - Night	10
12J - Other Skin Care Preps	19
13C - Other Suntan Preparations	1
Total	**100**

Ethylhexyl Salicylate

02A - Bath Oils, Tablets, and Salts	2
02B - Bubble Baths	6
02C - Bath Capsules	1
02D - Other Bath Preparations	2
03D - Eye Lotion	2
03G - Other Eye Makeup Preparations	1
04A - Cologne and Toilet waters	1219
04B - Perfumes	649
04C - Powders (dusting and talcum, excluding aftershave talc)	2
04E - Other Fragrance Preparation	422
05A - Hair Conditioner	28

05B - Hair Spray (aerosol fixatives)	17
05F - Shampoos (non-coloring)	27
05G - Tonics, Dressings, and Other Hair Grooming Aids	43
05H - Wave Sets	2
05I - Other Hair Preparations	12
06D - Hair Shampoos (coloring)	1
06H - Other Hair Coloring Preparation	4
07B - Face Powders	1
07C - Foundations	9
07E - Lipstick	54
07F - Makeup Bases	7
07I - Other Makeup Preparations	11
08A - Basecoats and Undercoats	2
08B - Cuticle Softeners	2
08E - Nail Polish and Enamel	2
10A - Bath Soaps and Detergents	413
10B - Deodorants (underarm)	6
10E - Other Personal Cleanliness Products	198
11A - Aftershave Lotion	36
11G - Other Shaving Preparation Products	3
12A - Cleansing	25
12C - Face and Neck (exc shave)	47
12D - Body and Hand (exc shave)	50
12E - Foot Powders and Sprays	1
12F - Moisturizing	84
12G - Night	11
12I - Skin Fresheners	1
12J - Other Skin Care Preps	41
13A - Suntan Gels, Creams, and Liquids	23
13B - Indoor Tanning Preparations	4
13C - Other Suntan Preparations	3
Total	**3474**

Hexyldodecyl Salicylate

No FDA VCRP Data

Isocetyl Salicylate

No FDA VCRP Data

Isodecyl Salicylate

03D - Eye Lotion	1
12C - Face and Neck (exc shave)	7
12F - Moisturizing	8
12G - Night	3
Total	**19**

Magnesium Salicylate

03A - Eyebrow Pencil	1
03F - Mascara	8
03G - Other Eye Makeup Preparations	1
Total	**10**

Methyl Salicylate

01A - Baby Shampoos	1
02A - Bath Oils, Tablets, and Salts	1
07I - Other Makeup Preparations	1
09A - Dentifrices	1
09B - Mouthwashes and Breath Fresheners	11
10A - Bath Soaps and Detergents	1
10E - Other Personal Cleanliness Products	3
12C - Face and Neck (exc shave)	2
12D - Body and Hand (exc shave)	5
12E - Foot Powders and Sprays	1
12F - Moisturizing	4
12G - Night	1
12I - Skin Fresheners	1
12J - Other Skin Care Preps	3
Total	**36**

Myristyl Salicylate

No FDA VCRP Data

Potassium Salicylate

No FDA VCRP Data

Salicylic Acid

01A - Baby Shampoos	1
01C - Other Baby Products	1
02D - Other Bath Preparations	3
03A - Eyebrow Pencil	2
03B - Eyeliner	3
03D - Eye Lotion	8
03E - Eye Makeup Remover	4
03F - Mascara	1
03G - Other Eye Makeup Preparations	8
04E - Other Fragrance Preparation	1
05A - Hair Conditioner	12
05B - Hair Spray (aerosol fixatives)	4
05E - Rinses (non-coloring)	3
05F - Shampoos (non-coloring)	197
05G - Tonics, Dressings, and Other Hair Grooming Aids	22
05I - Other Hair Preparations	15
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1

06D - Hair Shampoos (coloring)	3
06F - Hair Lighteners with Color	1
06G - Hair Bleaches	6
06H - Other Hair Coloring Preparation	31
07B - Face Powders	7
07C - Foundations	18
07E - Lipstick	1
07F - Makeup Bases	2
07I - Other Makeup Preparations	10
08B - Cuticle Softeners	1
08C - Nail Creams and Lotions	2
10A - Bath Soaps and Detergents	171
10B - Deodorants (underarm)	6
10E - Other Personal Cleanliness Products	15
11A - Aftershave Lotion	2
11D - Preshave Lotions (all types)	2
11E - Shaving Cream	2
11F - Shaving Soap	1
11G - Other Shaving Preparation Products	3
12A - Cleansing	191
12B - Depilatories	11
12C - Face and Neck (exc shave)	174
12D - Body and Hand (exc shave)	70
12E - Foot Powders and Sprays	4
12F - Moisturizing	106
12G - Night	22
12H - Paste Masks (mud packs)	34
12I - Skin Fresheners	23
12J - Other Skin Care Preps	89
13A - Suntan Gels, Creams, and Liquids	1
13B - Indoor Tanning Preparations	1
13C - Other Suntan Preparations	4
Total	**1300**

Sodium Salicylate

03B - Eyeliner	1
03D - Eye Lotion	1
03G - Other Eye Makeup Preparations	3
05A - Hair Conditioner	2
05F - Shampoos (non-coloring)	2
05I - Other Hair Preparations	5
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1
07C - Foundations	1
07I - Other Makeup Preparations	1
10A - Bath Soaps and Detergents	71
10E - Other Personal Cleanliness Products	11

12A - Cleansing	6
12B - Depilatories	2
12C - Face and Neck (exc shave)	31
12D - Body and Hand (exc shave)	10
12F - Moisturizing	9
12G - Night	1
12J - Other Skin Care Preps	7
Total	**165**

TEA-Salicylate

05A - Hair Conditioner	1
05I - Other Hair Preparations	4
Total	**5**

Tridecyl Salicylate

03D - Eye Lotion	2
07I - Other Makeup Preparations	2
12C - Face and Neck (exc shave)	3
12D - Body and Hand (exc shave)	1
12F - Moisturizing	3
12G - Night	1
12H - Paste Masks (mud packs)	2
Total	**14**

Amyl Salicylate

05A - Hair Conditioner	8
05F - Shampoos (non-coloring)	1
11B - Beard Softeners	1
Total	**10**

Hexyl Salicylate

12C - Face and Neck (exc shave)	1
13B - Indoor Tanning Preparations	1
Total	**2**

Isotridecyl Salicylate

No FDA VCRP Data

Silver Salicylate

No FDA VCRP Data

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: May 21, 2018

SUBJECT: Concentration of Use by FDA Product Category: Salicylates

Concentration of Use by FDA Product Category – Salicylates*

Butyloctyl Salicylate	Isodecyl Salicylate	TEA-Salicylate
Calcium Salicylate	Magnesium Salicylate	Tridecyl Salicylate
C12-15 Alkyl Salicylate	Methyl Salicylate	Amyl Salicylate
Capryloyl Salicylic Acid	Myristyl Salicylate	Hexyl Salicylate
Ethylhexyl Salicylate	Potassium Salicylate	Isotridecyl Salicylate
Hexyldodecyl Salicylate	Salicylic Acid	Silver Salicylate
Isocetyl Salicylate	Sodium Salicylate	

Ingredient	**Product Category**	**Maximum Concentration of Use**
Butyloctyl Salicylate	Eye shadows	3.6%
Butyloctyl Salicylate	Face powders	3.6%
Butyloctyl Salicylate	Foundations	5%
Butyloctyl Salicylate	Lipstick	35.9%
Butyloctyl Salicylate	Face and neck products Not spray	 1-5%
Butyloctyl Salicylate	Body and hand products Not spray	 10%
Butyloctyl Salicylate	Other skin care preparations	5-10%
Butyloctyl Salicylate	Suntan products Not spray Aerosol Pump spray	 6-9.8% 3% 1%
Capryloyl Salicylic Acid	Colognes and toilet waters	0.1%
Capryloyl Salicylic Acid	Shampoos (noncoloring)	0.1%
Capryloyl Salicylic Acid	Face powders	0.3%
Capryloyl Salicylic Acid	Lipstick	0.1%
Capryloyl Salicylic Acid	Bath soaps and detergents	0.3%
Capryloyl Salicylic Acid	Deodorants Not spray Aerosol	 0.3% 0.3%
Capryloyl Salicylic Acid	Shaving cream	0.29%
Capryloyl Salicylic Acid	Face and neck products Not spray	 0.3%
Capryloyl Salicylic Acid	Body and hand products Not spray	 62.9%
Capryloyl Salicylic Acid	Moisturizing products Not spray	 0.5%
Capryloyl Salicylic Acid	Paste masks and mud packs	0.4%
Capryloyl Salicylic Acid	Other skin care preparations	0.3%
Capryloyl Salicylic Acid	Suntan products Not spray	 0.1%
Ethylhexyl Salicylate	Bath oils, tablets and salts	0.2%
Ethylhexyl Salicylate	Eye lotions	0.1%
Ethylhexyl Salicylate	Colognes and toilet waters	0.38%

Ethylhexyl Salicylate	Other fragrance preparations	0.23%
Ethylhexyl Salicylate	Hair conditioners	0.001-0.05%
Ethylhexyl Salicylate	Hair sprays Aerosol Pump spray	 0.00099-0.0075% 0.2%
Ethylhexyl Salicylate	Shampoos (noncoloring)	0.0057-0.012%
Ethylhexyl Salicylate	Tonics, dressings and other hair grooming aids	0.012-0.05%
Ethylhexyl Salicylate	Other hair preparations (noncoloring)	0.03%
Ethylhexyl Salicylate	Hair dyes and colors	0.012%
Ethylhexyl Salicylate	Blushers (all types)	0.085%
Ethylhexyl Salicylate	Foundations	2.1-5%
Ethylhexyl Salicylate	Lipstick	4-4.5%
Ethylhexyl Salicylate	Makeup bases	3%
Ethylhexyl Salicylate	Cuticle softeners	0.15%
Ethylhexyl Salicylate	Nail polish and enamel	0.01%
Ethylhexyl Salicylate	Bath soaps and detergents	0.0012-0.21%
Ethylhexyl Salicylate	Deodorants Not spray	 0.0016%
Ethylhexyl Salicylate	Aftershave lotions	0.3-4%
Ethylhexyl Salicylate	Skin cleansing (cleansing lotions, liquids and pads)	0.15%
Ethylhexyl Salicylate	Face and neck products Not spray	 4-5.1%
Ethylhexyl Salicylate	Body and hand products Not spray	 0.0003-5%
Ethylhexyl Salicylate	Night products Not spray	 0.1%
Ethylhexyl Salicylate	Paste masks and mud packs	0.0075-0.2%
Ethylhexyl Salicylate	Other skin care preparations	5%
Ethylhexyl Salicylate	Suntan products Not spray Aerosol Pump spray	 5% 5% 5%
Ethylhexyl Salicylate	Other suntan preparations	5%
Isodecyl Salicylate	Face and neck products Not spray	 0.1%
Isodecyl Salicylate	Body and hand products Not spray	 2.5%
Magnesium Salicylate	Eyebrow pencils	0.2%
Magnesium Salicylate	Mascaras	0.2%
Methyl Salicylate	Bath oils, tablets and salts	0.0016%
Methyl Salicylate	Eye lotions	0.0000013-0.000026%
Methyl Salicylate	Eye makeup removers	0.000000038%
Methyl Salicylate	Hair conditioners	0.001-0.0011%
Methyl Salicylate	Hair sprays	

	Aerosol Pump spray	0.0000051-0.000054% 0.00001%
Methyl Salicylate	Shampoos (noncoloring)	0.0002-0.001%
Methyl Salicylate	Tonics, dressings and other hair grooming aids	0.0006%
Methyl Salicylate	Other hair preparations (noncoloring)	0.0000051%
Methyl Salicylate	Hair rinses (coloring)	0.00000002%
Methyl Salicylate	Foundations	0.000011%
Methyl Salicylate	Mouth washes and breath fresheners	0.038%
Methyl Salicylate	Bath soaps and detergents	0.0015-0.0059%
Methyl Salicylate	Deodorants Not spray Aerosol	 0.00006-0.03% 0.000018-0.0018%
Methyl Salicylate	Aftershave lotions	0.0005%
Methyl Salicylate	Preshave lotions	0.0000029%
Methyl Salicylate	Shaving cream	0.00000006-0.005%
Methyl Salicylate	Shaving soap	0.0002%
Methyl Salicylate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00027-0.0015%
Methyl Salicylate	Face and neck products Not spray	 0.000015-1%
Methyl Salicylate	Body and hand products Not spray	 0.000065-0.0004%
Methyl Salicylate	Foot powders and sprays	0.5%
Methyl Salicylate	Night products Not spray	 0.000026%
Methyl Salicylate	Paste masks and mud packs	0.000018%
Methyl Salicylate	Other skin care preparations	0.0000008%
Salicylic Acid	Eyeliners	0.2%
Salicylic Acid	Eye shadows	0.00001%
Salicylic Acid	Eye lotions	0.0001-0.15%
Salicylic Acid	Mascaras	0.2%
Salicylic Acid	Other eye makeup preparations	0.00001-0.19%
Salicylic Acid	Hair conditioners	0.1%
Salicylic Acid	Hair sprays Aerosol Pump spray	 0.1% 0.2%
Salicylic Acid	Permanent waves	0.00075%
Salicylic Acid	Rinses (noncoloring)	0.005%
Salicylic Acid	Shampoos (noncoloring)	0.052-4%
Salicylic Acid	Tonics, dressings and other hair grooming aids	0.004-0.2%
Salicylic Acid	Hair dyes and colors	0.015-0.1%
Salicylic Acid	Blushers	0.00001%
Salicylic Acid	Foundations	0.0001-1%
Salicylic Acid	Makeup bases	0.2%

Salicylic Acid	Rouges	0.0001%
Salicylic Acid	Makeup fixatives	0.2%
Salicylic Acid	Bath soaps and detergents	0.064-0.15%
Salicylic Acid	Other personal cleanliness products	0.2%
Salicylic Acid	Aftershave lotions	0.2%
Salicylic Acid	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01-2.1%
Salicylic Acid	Face and neck products Not spray Spray	 0.001-2% 0.1%
Salicylic Acid	Body and hand products Not spray Spray	 0.0005-0.2% 0.1%
Salicylic Acid	Moisturizing products Not spray	 0.00013-0.2%
Salicylic Acid	Paste masks and mud packs	0.01-1%
Salicylic Acid	Skin fresheners	0.5%
Salicylic Acid	Other skin care preparations Peel	0.01-2% 30%
Salicylic Acid	Suntan products Not spray	 0.45%
Sodium Salicylate	Baby shampoos	0.31%
Sodium Salicylate	Hair conditioners	0.2-0.3%
Sodium Salicylate	Hair straighteners	0.04%
Sodium Salicylate	Permanent waves	0.2%
Sodium Salicylate	Shampoos (noncoloring)	0.0008-0.5%
Sodium Salicylate	Foundations	0.01%
Sodium Salicylate	Bath soaps and detergents	0.25-0.37%
Sodium Salicylate	Face and neck products Not spray	 0.0015-0.0074%
Sodium Salicylate	Body and hand products Not spray	 0.1%
Sodium Salicylate	Paste masks and mud packs	0.5%
Amyl Salicylate	Hair conditioners	0.06%
Amyl Salicylate	Hair sprays Aerosol Pump spray	 0.0023% 0.0058%
Amyl Salicylate	Shampoos (noncoloring)	0.085%
Amyl Salicylate	Tonics, dressings and other hair grooming aids	0.12%
Amyl Salicylate	Foundations	0.000025%
Amyl Salicylate	Bath soaps and detergents	0.26%
Amyl Salicylate	Deodorants Not spray	 0.23%
Amyl Salicylate	Aftershave lotions	0.13%
Amyl Salicylate	Preshave lotions	0.025%

Amyl Salicylate	Shaving cream	0.18%
Amyl Salicylate	Shaving soap	0.059%
Amyl Salicylate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.02-0.042%
Amyl Salicylate	Face and neck products Not spray	 0.001%
Amyl Salicylate	Body and hand products Not spray	 0.038%
Amyl Salicylate	Night products Not spray	 0.000025%
Amyl Salicylate	Paste masks and mud packs	0.0003%
Hexyl Salicylate	Eye lotions	0.00074%
Hexyl Salicylate	Hair conditioners	0.14%
Hexyl Salicylate	Hair sprays Aerosol Pump spray	 0.022% 0.013%
Hexyl Salicylate	Shampoos (noncoloring)	0.21%
Hexyl Salicylate	Tonics, dressings and other hair grooming aids	0.11%
Hexyl Salicylate	Hair bleaches	0.5%
Hexyl Salicylate	Foundations	0.016%
Hexyl Salicylate	Bath soaps and detergents	0.52%
Hexyl Salicylate	Deodorants Not spray	 0.097%
Hexyl Salicylate	Aftershave lotions	0.055%
Hexyl Salicylate	Shaving cream	0.11%
Hexyl Salicylate	Shaving soap	0.11%
Hexyl Salicylate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.032-0.062%
Hexyl Salicylate	Face and neck products Not spray	 0.02-0.03%
Hexyl Salicylate	Body and hand products Not spray Spray	 0.08-0.12% 0.023%
Hexyl Salicylate	Night products Not spray	 0.016%
Hexyl Salicylate	Paste masks and mud packs	0.0038%

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

Information collected in 2018

Table prepared May 21, 2018