BUFF BOOK 2

Salicylic Acid

Benzyl Alcohol, Benzoic Acid,
Sodium Benzoate

Polyquaternium-7

Quaternium-22

CIR EXPERT PANEL MEETING
JUNE 28-29, 2010

Cosmetic Ingredient Review
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Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: Salicylic Acid

Date 28 May 2010

Last year, CIR received a request to consider new information on the Salicylic Acid report from David Steinberg at Steinberg & Associates. There hasn't been space in the agenda to consider this until now.

Mr. Steinberg has suggested that the caveat regarding formulation to "avoid skin irritation or, when increased sun sensitivity would be expected, directions for use should include the daily use of sun protection," while correct for short-chain esters and salts, is not correct for long-chain esters.

As a result of the CIR conclusion, he notes that Health Canada has limited use of Butyloctyl Salicylate in sunscreens to 5% and has prohibited use in other cosmetics.

Mr. Steinberg will be available to present his concerns.

If this safety assessment should be reopened based on this concern, then CIR would undertake an updated literature search and use those data, with the extensive data in the original report, to prepare a draft amended safety assessment for Panel review.

The original 2003 safety assessment for salicylic acid and related ingredients is included in the Panel Book.

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Dr. Alan Anderson
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September 4, 2009

Dear Alan,

I would like to formally request the CIR to review their published opinion on the Salicylic Acid group. The conclusion published, IJT 22(Suppl 3):1-108, 2003 states:

"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 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 <u>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</u>."

Although this is a correct conclusion for Salicylic Acid and its salts and short chain esters, it is incorrect for long chain esters. Long chain esters listed include Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate; and Tridecyl Salicylate, and Butyloctyl Salicylate and Hexyldodecyl Salicylate.

Ethylhexyl Salicylate is a Category I UV filter while the others are emollients often found in creams, lotions and also sunscreen drugs. Because of the CIR

conclusion, Health Canada has established a SnAc on Butyloctyl Salicylate which limits its' use in sunscreens to 5% and prohibits its use in other cosmetics I would be glad to review this issue with the Committee so that the conclusion can be changed to more accurately represent the safety of these ingredients.

Best regards,

David C. Steinberg

CC: John Bailey, PhD, PCPC

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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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Monice Zondlo Fiume, former Scientific Analyst/Report Management Coordinator.

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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.

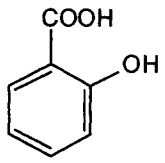
INTRODUCTION

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

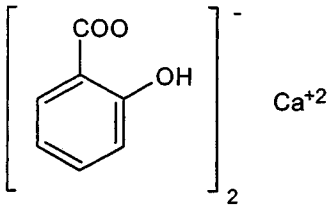
Definition and Structure

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

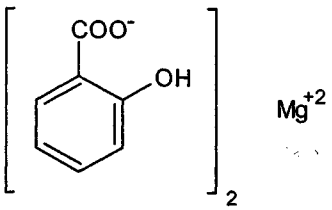


It is also known as 2-Hydroxybenzoic Acid (Pepe, Wenninger, and McEwen 2002; Lide 1993; Lewis 1993a; Budavari 1989); Benzoic Acid, 2-Hydroxy (Pepe, Wenninger, and McEwen 2002; Gennaro 1990); *o*-Hydroxybenzoic Acid (Pepe, Wenninger, and McEwen 2002; Lewis 1993a, 1993b; Gennaro 1990); *o*-Hydroxy Benzoic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

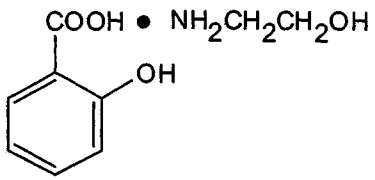


Magnesium Salicylate (CAS no. 18917-89-0) is the magnesium salt of Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



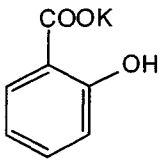
It is also known as Salicylic Acid, Magnesium Salt; Magnesium 2-Hydroxybenzoate (Pepe, Wenninger, and McEwen 2002); 2-Hydroxybenzoic Acid Magnesium Salt (Budavari 1989); Benzoic Acid, 2-Hydroxy-, Magnesium Salt (Pepe, Wenninger, and McEwen 2002); and Magnesium, Bis(2-Hydroxybenzoato-O^1,O^2)- (Gennaro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

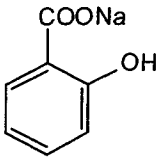


It is also known as Ethanolamine Salicylate; Salicylic Acid, Monoethanolamine Salt; Monoethanolamine 2-Hydroxybenzoate; and Benzoic Acid, 2-Hydroxy-, Monoethanolamine Salt (Pepe, Wenninger, and McEwen 2002).

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):

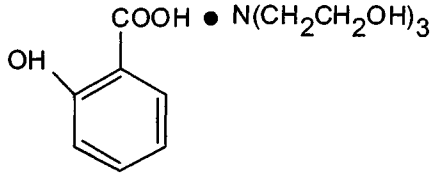


Sodium Salicylate (CAS no. 54-21-7) is the sodium salt of Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



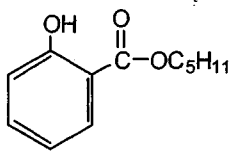
It is also known as Sodium Salicylic Acid; Salicylic Acid, Sodium Salt; Sodium *o*-Hydroxybenzoate; *o*-Hydroxybenzoic Sodium Salt (Lewis 1993a); 2-Hydroxybenzoic Acid, Monosodium Salt (Pepe, Wenninger, and McEwen 2002; Lewis 1993a; Budavari 1989); and Benzoic Acid, 2-Hydroxy-, Monosodium Salt (Pepe,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

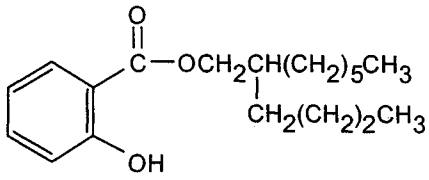


It is also known as Triethanolamine Salicylate; Trolamine Salicylate; 2-Hydroxybenzoic Acid, Compound with 2,2′,2″-Nitrilotris[Ethanol] (1:1); and Benzoic Acid, 2-Hydroxy-, Compound with 2,2′,2″-Nitrilotris[Ethanol] (1:1) (Pepe, Wenninger, and McEwen 2002).

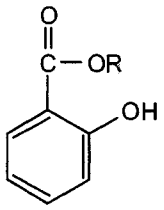
Amyl Salicylate is not addressed in this report because its only current use is as a fragrance ingredient; it is the ester of amyl alcohol and salicylic acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



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):



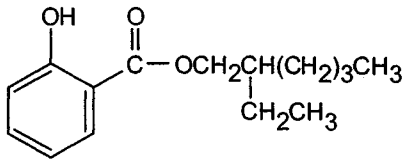
C12–15 Alkyl Salicylate (CAS number not available) is the ester of C12–15 alcohols (q.v.) and Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



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.)

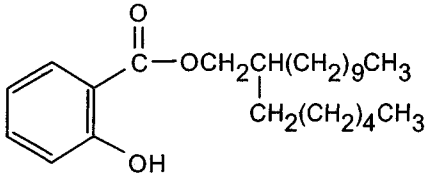
Ethylhexyl Salicylate (CAS no. 118-60-5) is the ester of 2-ethylhexyl alcohol and Salicylic Acid that is also known as Benzoic Acid, 2-Hydroxy-, 2-Ethylhexyl Ester; 2-Ethylhexyl 2-Hydroxybenzoate; Ethyl hexyl salicylate; 2-Ethylhexyl Salicylate; Octyl Salicylate; and Salicylic Acid, 2-Ethylhexyl Ester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



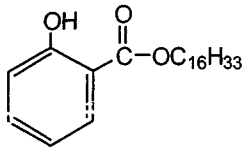
Because this cosmetic ingredient was previously known as Octyl Salicylate (Pepe, Wenninger, and McEwen 2002) and many of the references refer to Octyl Salicylate, this ingredient will be identified as Ethylhexyl (Octyl) Salicylate in the text. Headings will refer to Ethylhexyl Salicylate, the current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

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

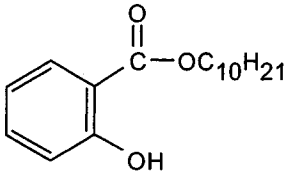
that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



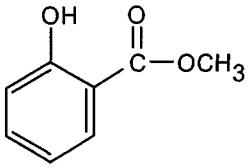
Isocetyl Salicylate (CAS number not available), also known as Salicylic Acid, Isocetyl Ester, is the ester of isocetyl alcohol (q.v.) and Salicylic Acid (q.v.) that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



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):

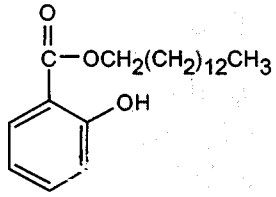


Methyl Salicylate (CAS no. 119-36-8) is the ester of methyl alcohol and Salicylic Acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):

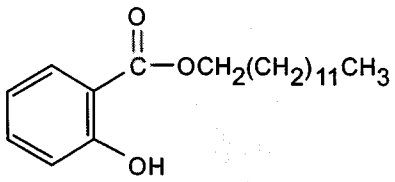


It is also known as Salicylic Acid, Methyl Ester (Lewis 1993a); Methyl 2-Hydroxybenzoate; Benzoic Acid, 2-Hydroxy-, Methyl Ester; (Pepe, Wenninger, and McEwen 2002); 2-Hydroxybenzoic Acid, Methyl Ester (Pepe, Wenninger, and McEwen 2002; Lewis 1993a; Budavari 1989); *o*-Hydroxybenzoic Acid, Methyl Ester;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

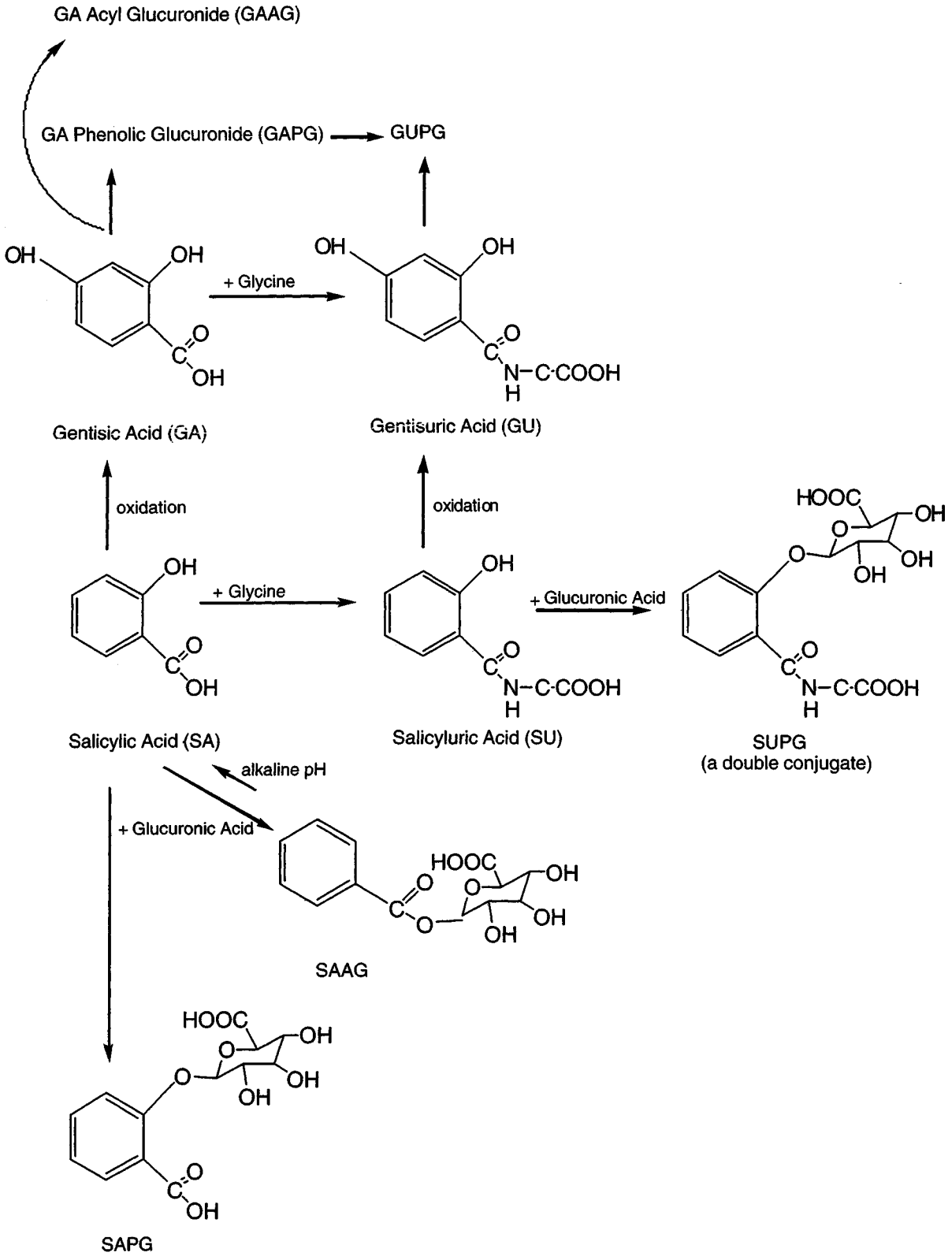


FIGURE 1

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 ± 3.6 and 5.4 ± 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% ± 6% and 56% ± 16% for male and females, respectively, through viable skin and 47% ± 2% and 50% ± 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 α(%) 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

(Continued on next page)

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

(Continued on next page)

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%, ≤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	

(Continued on next page)

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

(Continued on next page)

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 ^{3}H-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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Cosmetic Ingredient Review

Commitment . . . Credibility
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Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: New inhalation toxicity data on Benzyl Alcohol and Benzoic Acid

Date 28 May 2010

In 1998, the CIR Expert Panel completed its final safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate, concluding that they are safe for use in cosmetics up to 5%, but that the data are insufficient to support the safety of these ingredients in which the primary route of exposure is inhalation. Benzyl Alcohol also was safe for use in hair dyes at concentrations up to 10%. The additional data needed were (1) inhalation toxicity data.

The Personal Care Products Council has provided results of a 4-week inhalation toxicity study of aerosolized Benzyl Alcohol and Benzoic Acid in Sprague-Dawley rats. No adverse findings with respect to any parameters were found in either treatment or control groups. The report concluded that the no-observed-effect-level (NOEL) and no-observed-adverse-effect level (NOAEL) were the same at 1,072 mg/m^3 for benzyl alcohol and 12.6 mg/m^3 for benzoic acid.

In addition, a recent comet assay of benzyl alcohol and benzoic acid was published.

Are these data a sufficient basis for the Expert Panel to undertake to amend the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate?

If these data are sufficient and the Panel determines to reopen this safety assessment to amend the conclusion, then there is an opportunity to also consider that the available data support other simple salts of Benzoic Acid (in addition to Sodium Benzoate) such as Calcium Benzoate, Magnesium Benzoate, Potassium Benzoate, and Benzyl Benzoate. The Council has provided use concentration data for these ingredients, and has updated the use concentration data for Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate.

So, there are two questions:

1) Reopen to amend the conclusion? If the answer is no, then no further action is needed.
2) If yes, then should the four additional salts be added as CIR prepares an amended safety assessment?

The original safety assessment, the first 50 or so pages of the new inhalation toxicity study, new use concentration data from PCPC survey (and the PCPC transmittal memo) are in the Panel Book.

Additional Materials available online at http://www.cir-safety.org/jun10.shtml

- Complete new unpublished inhalation toxicity study
- New published comet assay study

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Final Report on the Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate[1]

Benzyl Alcohol is an aromatic alcohol used in a wide variety of cosmetic formulations as a fragrance component, preservative, solvent, and viscosity-decreasing agent. Benzoic Acid is an aromatic acid used in a wide variety of cosmetics as a pH adjuster and preservative. Sodium Benzoate is the sodium salt of Benzoic Acid used as a preservative, also in a wide range of cosmetic product types. Benzyl Alcohol is metabolized to Benzoic Acid, which reacts with glycine and excreted as hippuric acid in the human body. Acceptable daily intakes were established by the World Health Organization at 5 mg/kg for Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. Benzoic Acid and Sodium Benzoate are generally recognized as safe in foods according to the U.S. Food and Drug Administration. No adverse effects of Benzyl Alcohol were seen in chronic exposure animal studies using rats and mice. Effects of Benzoic Acid and Sodium Benzoate in chronic exposure animal studies were limited to reduced feed intake and reduced growth. Some differences between control and Benzyl Alcohol–treated populations were noted in one reproductive toxicity study using mice, but these were limited to lower maternal body weights and decreased mean litter weights. Another study also noted that fetal weight was decreased compared to controls, but a third study showed no differences between control and Benzyl Alcohol–treated groups. Benzoic Acid was associated with an increased number of resorptions and malformations in hamsters, but there were no reproductive or developmental toxicty findings in studies using mice and rats exposed to Sodium Benzoate, and, likewise, Benzoic Acid was negative in two rat studies. Genotoxicity tests for these ingredients were mostly negative, but there were some assays that were positive. Carcinogenicity studies, however, were negative. Clinical data indicated that these ingredients can produce nonimmunologic contact urticaria and nonimmunologic immediate contact reactions, characterized by the appearance of wheals, erythema, and pruritis. In one study, 5% Benzyl Alcohol elicited a reaction, and in another study, 2% Benzoic Acid did likewise. Benzyl Alcohol, however, was not a sensitizer at 10%, nor was Benzoic Acid a sensitizer at 2%. Recognizing that the nonimmunologic reactions are strictly cutaneous, likely involving a cholinergic mechanism, it was concluded that these ingredients could be used safely at concentrations up to 5%, but that manufacturers should consider the nonimmunologic phenomena when using these ingredients in cosmetic formulations designed for infants and children. Additionally, Benzyl Alcohol was considered safe up to 10% for use in hair dyes. The limited body exposure, the duration of use, and the frequency of use were considered in concluding that the nonimmunologic reactions would not be a concern. Because of the wide variety of product types in which these ingredients may be used, it is likely that inhalation may be a route of exposure. The available safety tests are not considered sufficient to support the safety of these ingredients in formulations where inhalation is a route of exposure. Inhalation toxicity data are needed to complete the safety assessment of these ingredients where inhalation can occur.

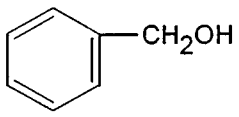
INTRODUCTION

This report is a compilation of data concerning Benzyl Alcohol (CAS No. 100-51-6), Benzoic Acid (CAS No. 65-85-0), and Sodium Benzoate (CAS No. 532-32-1). Reviews of early literature (1920–1977) were prepared for the Food and Drug Administration (FDA) on Benzyl Alcohol and Benzoic Acid (Flavor and Extract Manufacturers' Association 1984) and on Benzoic Acid and Sodium Benzoate (Informatics, Inc. 1972; Federation of American Societies for Experimental Biology [FASEB] 1973). This Cosmetic Ingredient Review (CIR) report includes relevant studies cited in the earlier reviews as well as recent animal and clinical studies.

CHEMISTRY

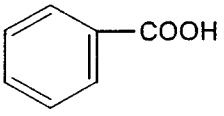
Definition and Structure

Benzyl Alcohol is an aromatic alcohol that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



Synonyms for Benzyl Alcohol include Benzenemethanol (Wenninger, Canterbery, and McEwen 2000), Phenyl-methanol, Phenylcarbinol, Phenylmethyl Alcohol (Food and Agricultural Organization of the United Nations/World Health Organization [FAO/WHO] 1994); Hydroxytoluene, α-Hydroxytoluene, (Lewis 1993).

Benzoic Acid is an aromatic acid that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



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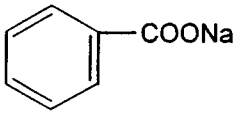
Synonyms for Benzoic Acid include Benzeneformic acid, Benzenemethanoic Acid, Benzoate, Carboxybenzene, Dracylic Acid, Phenylformic Acid, Benzenecarboxylic Acid, Phenylcarboxylic Acid (Budavari 1989; Lewis 1993).

The sodium salt of Benzoic Acid, Sodium Benzoate conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



Synonyms for Sodium Benzoate include sodium salt of benzenecarboxylic acid, sodium salt of phenylcarboxylic acid (FAO/WHO 1994).

Physical and Chemical Properties

Table 1 lists physical properties of Benzyl Alcohol and Benzoic Acid.

Benzyl Alcohol is a combustible liquid. When heated to decomposition it emits acrid smoke and fumes (Lewis 1993).

Benzoic Acid burns rapidly in oxygen and is combustible when exposed to heat or flame. It can react with oxidizing materials. When heated to decomposition it emits acrid smoke and fumes (Lewis 1993).

When Sodium Benzoate is heated to decomposition it emits toxic fumes of Na_2O. It is cautioned that oral doses of 8 to 10 g can cause nausea and vomiting; small doses have little or no effect (Lewis 1993).

Method of Manufacture

Benzyl Alcohol is found naturally in many foods such as apricots, snap beans, cocoa, cranberries, mushrooms, and honey (Flavor and Extract Manufacturers' Association 1984). Benzyl Alcohol is also found in the essential oil of many plants including jasmine, hyacinth, and ylang-ylang (Lewis 1993). Large scale production of Benzyl Alcohol is achieved by the action of sodium or potassium carbonate on benzyl chloride (Budavari 1989).

Benzoic Acid is also found naturally in many foods such as apricots, snap beans, cocoa, cranberries, mushrooms, and honey (Flavor and Extract Manufacturers' Association 1984).

Analytical Methods

The Benzoic Acid content of cosmetic formulations can be determined by high-performance liquid chromatography (Gagliardi et al. 1984).

Impurities

The Cosmetic, Toiletry, and Fragrance Association (CTFA) lists the following specifications for Benzyl Alcohol, 0.2% maximum aldehyde (as benzaldehyde) and 0.005% maximum sulfated ash. Other characteristics such as chlorinated compounds, specific gravity, refractive index, and distilling range must match the standards of the National Formulary (NF) or United States Pharmacopeia (USP) (Nikitakis and McEwen 1990).

CTFA specifications for Benzoic Acid and Sodium Benzoate with regard to congealing range, equivalent weight, water content, and alkalinity follow standards set by the USP and NF (Nikitakis and McEwen 1990).

USE

Cosmetic

Benzyl Alcohol

Benzyl Alcohol is used in cosmetics as a fragrance component, preservative, solvent, and viscosity-decreasing agent (Wenninger, Canterbery, and McEwen 2000). In January 1998, Benzyl Alcohol was reported to be used in 322 cosmetic formulations (FDA 1998). (See Table 2.) Concentration of use data

TABLE 1
Physical properties of Benzyl Alcohol and Benzoic Acid

Property	Benzyl alcohol	Benzoic acid	Reference
Appearance	Liquid	—	Budavari 1989
	—	White crystalline powder	Lewis 1993
Odor/taste	Faint aromatic odor, sharp burning taste	—	Budavari 1989
Molecular weight	108.14 Da	122.12 Da	Lide 1993
Boiling point (°C)	205.3	249	Lide 1993
Melting point (°C)	−15.3	122.13	Lide 1993
Density	1.0419	1.0749, 1.2659	Lide 1993
Solubility	Water; alcohol; ether; acetone; benzene	Alcohol; ether; acetone; chloroform; benzene	Lide 1993
Flash point (°F)	213 (CC)	250 (CC)	Lewis 1993
Refractive index	−0.002	—	Nikitakis and McEwen 1990

TABLE 2
Frequency of use of Benzyl Alcohol (FDA 1998)

Product category	No. of formulations in category	No. containing ingredient
Baby shampoos	21	5
Baby lotions, oils, powders, creams	53	3
Other baby products	29	2
Bath oils, tablets, and salts	124	2
Other bath preparations	159	3
Eyeliner	514	3
Eye shadow	506	4
Eye makeup remover	84	10
Mascara	167	5
Other eye makeup preparations	120	4
Colognes and toilet waters	656	1
Other fragrance preparations	148	2
Hair conditioners	636	7
Hair sprays (aerosol fixatives)	261	4
Rinses (noncoloring)	40	1
Shampoos (noncoloring)	860	7
Tonics, dressings, and other hair grooming aids	549	14
Hair dyes and colors	1572	130
Hair rinses (coloring)	33	18
Hair color sprays (aerosol)	4	1
Other hair-coloring preparations	59	2
Face powders	250	1
Foundations	287	2
Lipstick	790	1
Makeup bases	132	1
Other makeup preparations	135	2
Deodorants (underarm)	250	1
Feminine hygiene deodorants	4	2
Other personal cleanliness products	291	1
Aftershave lotion	216	4
Preshave lotions (all types)	14	1
Shaving cream	139	3
Cleansing	653	10
Face and neck skin care (excluding shaving)	263	12
Body and hand skin care (excluding shaving)	796	16
Foot powders and sprays	35	2
Moisturizing	769	8
Night skin care	188	3
Paste masks (mud packs)	255	4
Skin fresheners	184	4
Other skin care preparations	692	11
Suntan gels, creams, and liquids	136	2
Other suntan preparations	38	3
1998 total for Benzyl Alcohol		**322**

are no longer reported to the FDA (FDA 1992). Data from FDA (1984) indicated that Benzyl Alcohol was used at concentrations ≤25%. Studies cited in the Clinical Assessment of Safety section of this report tested mascara formulations containing 0.65% Benzyl Alcohol (Hill Top Research 1997a, 1997b).

Benzoic Acid and Sodium Benzoate

Benzoic Acid is used as a pH adjustor and preservative and Sodium Benzoate is used as a preservative (Wenninger, Canterbery, and McEwen 2000). In January 1998, Benzoic Acid and Sodium Benzoate were reported to be used in 223 and 156 cosmetic formulations, respectively (FDA 1998) (See Table 3). Data from 1984 indicated that although Benzoic Acid and Sodium Benzoate were used at up to 5% and 25%, respectively, the majority of use of both ingredients was at ≤1% (FDA 1984).

Studies cited in the Clinical Assessment of Safety section of this report tested eye shadow formulations containing 0.1% Benzoic Acid (Biosearch Inc. 1991; TKL Research 1991) and liquid/powder foundation formulations containing 0.2% Benzoic Acid (Biosearch Inc. 1992a, 1992b, 1992c, 1992d; Education and Research Foundation 1992).

International

Benzyl Alcohol

The European Union (EU) has stipulated that when used as a preservative, Benzyl Alcohol is restricted to a maximum concentration of 1% (EU 1995).

Benzyl Alcohol is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS)*. Benzyl Alcohol, which conforms to the specifications of the *Japanese Standards of Cosmetic Ingredients*, has precedent for use without restriction in the following *CLS* categories: soaps, face cleansing products, shampoos, hair rinses, hair coloring preparations, and eye creams, eyeshadows, and mascaras. There has been precedent for use of Benzyl Alcohol at concentrations up to 5% in the following categories: hair care products, creams and milky lotions, shaving creams and lotions, suntan and sun cream, lotion and oil formulations, shaving lotions, cosmetic oils, powders, foundations, perfumes, packs, nail creams, nail enamels, nail makeup removers, cheek color products, eyebrow products, and bath preparations. There has been no precedent regarding its use in eyeliners, lipsticks, lip creams, and dentifrices (Santucci 1999).

According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, Benzyl Alcohol is not prohibited or restricted (beyond the limits in the CLS discussed above) in its use beyond a basic obligation of manufacturers to use all ingredients in a manner which guarantees safety (Japan Ministry of Health and Welfare 2000).

Benzoic Acid and Sodium Benzoate

The EU has stipulated that when used as preservatives, Benzoic Acid, its salts and esters are restricted to a maximum concentration of 0.5% (acid) (EU 1995).

Benzoic Acid and Sodium Benzoate are listed in the *CLS* and must conform to the specifications of the *Japanese Standards of Cosmetic Ingredients*. Precedent to use Benzoic Acid at concentrations up to 0.2% has been established in all *CLS* cosmetic categories except eyeliners. Precedent to use Sodium Benzoate at concentrations up to 1% has been established in all *CLS* categories (Santucci 1999).

According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, use of Benzoic Acid is restricted to 0.2 g per 100 g of any cosmetic formulation (Japan Ministry of Health and Welfare 2000).

Noncosmetic

Benzyl Alcohol

Benzyl Alcohol is approved for use as a food additive (Rothschild 1990). In 1979, the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) established an acceptable daily intake (ADI) level of 0 to 5 mg/kg body weight. This ADI concerned Benzyl Alcohol and benzyl benzoate and "applies to the benzyl/benzoic moiety related to benzoic acid representing total benzoate from all food additive sources" (FAO/WHO 1994).

Benzyl Alcohol can be used as an active ingredient in over-the-counter (OTC) drug preparations (Wenninger, Canterbery, and McEwen 2000). In 1982, the FDA Panel on OTC Dentifrices and Dental Care Products recommended that Benzyl Alcohol not be used in the treatment of dental pain in children younger than 2 years of age (Grad and Grushka 1986).

In human pain studies, Benzyl Alcohol was an effective local anesthetic. Subjects complained of less pain after receiving intramuscular (IM) injections of various medications that contained Benzyl Alcohol (Wightman and Vaughan 1976; Gouyette et al. 1982; Rasmussen, Zachmann, and Nilsson 1989; Frenken, van Lier, and Koene 1994; Jørgensen 1994; Williams and Howe 1994).

Benzoic Acid and Sodium Benzoate

Benzoic Acid and Sodium Benzoate both have the status "generally recognized as safe" (GRAS) (Rothschild 1990). In 1983, the JECFA established an ADI of 0 to 5 mg/kg body weight for Benzoic Acid, its salts, benzyl acetate, alcohol and benzoate (FAO/WHO 1994).

Benzoic Acid (USP) is a component of benzoic and salicylic acid ointments (USP), a topical antifungal agent (Taylor 1988).

Since the late 1970s, Sodium Benzoate has been used in the treatment of hyperammonemia in patients with inborn errors of the urea cycle. The treatment is based on the synthesis and excretion of hippurate as an alternative pathway to eliminate nitrogen versus urea synthesis (Brusilow et al. 1979, 1980).

An extensive review of this therapeutic use of benzoates by Tremblay and Qureshi (1993) noted that laboratory models have yet to corroborate clinical findings. Further, animal studies demonstrated that Sodium Benzoate, at some doses, potentiated

TABLE 3
Frequency of use of Benzoic Acid and Sodium Benzoate (FDA 1998)

Product category	No. of formulations in category	No. containing ingredient
Benzoic Acid		
Other baby products	29	1
Bath oils, tablets, and salts	124	2
Bubble baths	200	26
Other bath preparations	159	32
Eye shadow	506	5
Eye makeup remover	84	3
Mascara	167	1
Other eye makeup preparations	120	1
Other fragrance preparations	148	2
Hair conditioners	636	2
Hair straighteners	63	1
Shampoos (noncoloring)	860	3
Tonics, dressings, and other hair grooming aids	549	5
Other hair preparations	276	2
Blushers (all types)	238	1
Face powders	250	3
Foundations	287	7
Lipstick	790	37
Makeup bases	132	1
Other makeup preparations	135	1
Cuticle softeners	19	1
Other manicuring preparations	61	1
Mouthwashes and breath fresheners	49	12
Other oral hygiene products	6	2
Bath soaps and detergents	385	8
Aftershave lotion	216	4
Shaving cream	139	2
Cleansing	653	12
Face and neck skin care (excluding shaving)	263	2
Body and hand skin care (excluding shaving)	796	8
Moisturizing	769	8
Night skin care	188	1
Paste masks (mud packs)	255	3
Skin fresheners	184	9
Other skin care preparations	692	9
Suntan gels, creams and liquids	136	2
Indoor tanning preparations	62	3
1998 total for Benzoic Acid		**223**
Sodium Benzoate		
Bath oils, tablets and salts	124	1
Other bath preparations	159	1
Eyeliner	514	3
Eye shadow	506	4
Eye makeup remover	84	5
Other eye makeup preparations	120	2
Hair conditioners	636	5
Hair sprays (aerosol fixatives)	261	24

(Continued on next page)

TABLE 3
Frequency of use of Benzoic Acid and Sodium Benzoate (FDA 1998) *(Continued)*

Product category	No. of formulations in category	No. containing ingredient
Hair straighteners	63	1
Rinses (noncoloring)	40	3
Shampoos (noncoloring)	860	20
Tonics, dressings, and other hair grooming aids	549	5
Wave sets	55	1
Hair bleaches	113	2
Face powders	250	2
Other makeup preparations	135	1
Dentifrices	38	6
Mouthwashes and breath fresheners	49	1
Other oral hygiene products	6	2
Bath soaps and detergents	385	1
Deodorants (underarm)	250	1
Other personal cleanliness products	291	4
Aftershave lotion	216	15
Shaving cream	139	7
Other shaving preparation products	60	1
Cleansing	653	5
Face and neck skin care (excluding shaving)	263	4
Body and hand skin care (excluding shaving)	796	8
Moisturizing	769	6
Night skin care	188	3
Paste masks (mud packs)	255	2
Skin fresheners	184	1
Other skin care preparations	692	9
1998 total for Sodium Benzoate		**156**

ammonia toxicity in mice (O'Connor et al. 1982, 1989) and rats (Maswoswe et al. 1986).

Conditions for benzoate therapy typically include reduced nitrogen intake and a priming intravenous (IV) dose of 250 mg/kg administered over 0.5 to 2 hours, followed by an additional 250 to 500 mg/kg/day administered with meals (Tremblay and Qureshi 1993).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

The available human absorption, distribution, metabolism, and excretion data were sufficiently extensive that animal data were not included. Therefore, the following section cites clinical studies only.

Benzyl Alcohol

When metabolized, Benzyl Alcohol is converted to Benzoic Acid by simple oxidation (Flavor and Extract Manufacturers' Association 1984). The relevant data, therefore, relate to Benzoic Acid and Sodium Benzoate.

Benzoic Acid and Sodium Benzoate

Even after administration of high doses of Sodium Benzoate, the hourly excretion of hippuric acid increases to a maximum and then remains constant until all but a small portion is eliminated (Quick 1931). The rate of hippuric acid formation in humans after oral administration of 5 g Benzoic Acid increased with the concomitant administration of glycine.

Bridges et al. (1970) reported on the metabolism of Benzoic Acid in humans and various animal species. The FASEB (1973) and GRAS reports (Informatics 1972) reported that Benzoic Acid and Sodium Benzoate are rapidly absorbed from the gastrointestinal tract of mammals and conjugated with glycine in the liver. The resulting hippuric acid is excreted in the urine rapidly (75% to 100% of the dose is excreted within 6 hours; the remaining dose is excreted within 2 to 3 days). The availability of glycine was the rate-limiting factor in the formation of hippuric acid. When insufficient glycine was available benzoyl glucuronide was formed.

Feldman and Maibach (1970) reported that 42.6% ± 16.5% of a dermally applied [^{14}C]-Benzoic Acid dose (4 μg/cm^2; in acetone) was excreted in the urine within 24 hours. When applied

in petrolatum, 60.5% of the dose was absorbed (Bronaugh and Franz 1986).

By quantifying 24-hour urine excretion, Rougier et al. (1986) demonstrated that dermal application of 1000 nmol [^{14}C]-Benzoic Acid (10^{-3} μCi/nmol) produced the following penetration scale: forehead > abdomen > thigh > chest > arm > back. The 4-day penetration through the forehead (27.65 ± 3.61 nmol/cm^2) was three times greater than absorption through the back (8.55 ± 1.32 nmol/cm^2). Benzoic Acid had been applied to two sites of each body area; one site was tape stripped to determine the amount of test material in the stratum corneum. The quantified values from the urine were comparable to predicted values estimated from the tape stripping.

In a study investigating the effects of aging on dermal absorption, Roskos, Maibach, and Guy (1989) applied [^{14}C]-Benzoic Acid (in acetone) to the forearm of two groups of panelists, "young" (22 to 40 years) and "old" (>65 years). A 24-hour protective patch was placed on the skin and the site was washed after patch removal. A second protective patch was then applied and remained in place until day 7. Analysis of 7-day urine excretion indicated that 36.2% ± 4.6% of the applied dose was absorbed by the young panelists, whereas 19.5% ± 1.6% was absorbed by the old panelists. The difference was statistically significant ($p < .01$).

Kubota and Ishizaki (1991) demonstrated that biotransformation of Benzoic Acid to hippuric acid follows saturable or Michaelis-Menten kinetics in humans following ingestion of Sodium Benzoate.

No statistical difference ($p > .05$) was found by Lotte et al. (1993) in the percutaneous absorption of Benzoic Acid by Asian, Black, and Caucasian panelists. [^{14}C]-Benzoic Acid (1 μmol/cm^2) was applied to two sites of the upper arm and the sites were washed after 30 minutes of contact. (The two applications occurred on contralateral arms and were made 48 hours apart.) Urine was collected for 24 hours and one site was tape stripped to measure Benzoic Acid in the stratum corneum. Amounts absorbed were 1.43% ± 0.27% by Asian skin, 1.07% ± 0.17% by black skin, and 1.2% ± 0.19% by Caucasian skin.

Gregus et al. (1993, 1996) reported that both lipoic acid (1993) and valproic acid (1996) reduced the clearance of Benzoic Acid in rats that had been "loaded" with glycine. Both acids reduced the availability of hepatic coenzyme A that is needed for the adenosine triphosphate (ATP)-dependent conjugation with glycine.

Cellular Effects

Benzyl Alcohol

Benzyl Alcohol is a membrane "fluidizer" that affects lipid bilayer structure (Ebihara et al. 1979). It has been demonstrated to act on membranes of erythrocytes (Burgen et al. 1970; Bassé et al. 1992) and hepatocytes (Gordon et al. 1980).

Studies reported Benzyl Alcohol to increase activity of membrane-bound Ca^{2+}-dependent enzymes such as adenylate cyclase (Voorheis and Martin 1982; Martin, McConkey, and Stokes 1985; Needham and Houslay 1988) and thiol proteinase (Ahkong et al. 1980). Conversely, Benzyl Alcohol inhibited activities of various glycosyltransferases of the rat liver Golgi membrane (Mitranic, Boggs, and Moscarello 1982). The activities of erythrocyte-bound *p*-nitrophenylphosphatase and acetylcholinesterase were increased at some concentrations of Benzyl Alcohol and inhibited by others (Tanaka 1984). The effect on cell membranes was considered the mechanism by which Benzyl Alcohol inhibited lymphocyte-mediated cytolysis in vitro (Kemp and Berke 1973a, 1973b).

Benzyl Alcohol induced time-, dose-, and temperature-dependent hemolysis of erythrocytes (Ohmiya and Nakai 1978).

Benzoic Acid and Sodium Benzoate

Sodium Benzoate inhibited activity of D-amino acid oxidase (Brada and Bulba 1980; London and Gabel 1988).

In an in vitro study, Sodium Benzoate at doses ≥500 μg/ml suppressed the activities of marker enzymes in the mitochondria and cytosol of rat liver hepatocytes. Suppression of DNA synthesis was noted at 100 μg/ml (Oyanagi et al. 1987).

Radical Scavenging Activity

Benzoic Acid and Sodium Benzoate

Benzoic Acid and Sodium Benzoate are recognized as hydroxyl radical scavengers and researchers have reported that benzoates inhibited mechanisms that generated free radicals. In in vitro studies, benzoates reduced the cytotoxicity of drugs/chemicals such as hydroxyurea in L5178Y cells (Przybyszewski and Malec 1982), 6-hydroxydopamine in mouse pancreatic islets (Grankvist, Sehlin, and Taeljedal 1986), doxorubicin in a Dox-sensitive human ovarian cancer cell line (Cervantes et al. 1988), and inhibited argemone oil–induced enzymatic and nonenzymatic hepatic lipid peroxidation in rat cells (Upreti, Das, and Khanna 1991). In other in vitro studies, benzoates inhibited some chemical-induced DNA lesions (Kaneko et al. 1984; Sugioka et al. 1984; Daniel, Mao, and Saffiotti 1983; Mahmood et al. 1993). In in vivo studies using rats, Sodium Benzoate had a protective effect against gentamicin-induced renal failure (Walker and Shah 1988), and demonstrated a dose-dependent reduction in ethanol-induced gastric lesions (Evangelista and Meli 1985). However, Rotstein and Slaga (1988) reported that the scavenging activity of Sodium Benzoate did not significantly inhibit tumor progression when tested in a murine skin multistage carcinogenesis model.

Benzoic Acid and Sodium Benzoate also inhibited immune responses that relied on reactive oxygen intermediates such as natural killer cells (Suthanthiran et al. 1984), some neutrophil activity (Cheung, Archibald, and Robinson 1984; Thomas, Smith, and Pang 1991; Kumar, Anand, and Ganguly 1993), and phagocytes (Weitzman and Stossel 1982; Weitberg et al. 1985). However, Kraut, Segal, and Sagone (1982) reported that granulocyte aggregation in response to cell membrane injury was not affected by oxygen radical scavengers.

Glycine Competition

Benzoic Acid and Sodium Benzoate

The metabolism of the benzoates depletes glycine concentrations and can therefore alter the glycine-dependent metabolism of other compounds. Amsel and Levy (1969) and Levy, Amsel, and Elliott (1969) demonstrated that Benzoic Acid or Sodium Benzoate successfully competed with aspirin for glycine, resulting in increased concentration and persistence of salicylic acid in the body. Almost total inhibition of salicyluric acid formation in humans was achieved using either 2.7 g Benzoic Acid or 3.2 g Sodium Benzoate.

The GRAS report (Informatics Inc. 1972) cited studies in which ingestion of Sodium Benzoate reduced the glycine-dependent formation of creatine, glutamine, urea, and uric acid and increased the effects of procaine, lidocaine, cocaine, tetracaine, and dibucaine. Under conditions of severely restricted fluid and salt intake, benzoates increased and prolonged the concentration of serum penicillin.

Enzyme Inhibition

Benzyl Alcohol

Messiha (1991) reported that short-term intake of 2% Benzyl Alcohol in the drinking water resulted in an inhibition of hepatic alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase isoenzyme activities in female rats. The effects were not noted in male rats.

Compared to control rats, Benzyl Alcohol noncompetitively inhibited activity of hepatic alcohol dehydrogenase (L-ADH) of rats maintained for a short term on 5% ethanol (Messiha, Pasi, and Morniroli 1992).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Benzyl Alcohol

The literature review by the Flavor and Extract Manufacturers' Association (1984) cited the following oral LD_{50} values for Benzyl Alcohol: mouse 1580 mg/kg; rat 1230 to 3200 mg/kg (four studies), and rabbit 1040 mg/kg.

Benzoic Acid and Sodium Benzoate

The RTECS (Registry of Toxic Effects of Chemical Substances) cited that the human low lethal oral dose of Benzoic Acid was 500 mg/kg (RTECS 1995).

The oral LD_{50} of Benzoic Acid in mice was 1996 mg/kg (Flavor and Extract Manufacturers' Association, 1984). In rats, the oral LD_{50} for Benzoic Acid was 2000 to 2500 mg/kg, for Sodium Benzoate it was 2100 to 4070 mg/kg. The LD_{50} of Sodium Benzoate in rabbits and dogs was 2000 mg/kg. The oral LD_{100s} for Benzoic Acid for rabbits, cats, and dogs were 1520 to 2000, 2000, and 2000 mg/kg, respectively (FASEB 1973).

Short-Term Oral Toxicity

Benzyl Alcohol

In a gavage study by the National Toxicology Program (NTP 1989), technical grade Benzyl Alcohol (99% pure) in corn oil at doses of 125, 250, 500, 1000, or 2000 mg/kg was administered to groups of 10 F344/N rats and $B6C3F_1$ mice (5 of each sex). Animals were dosed 5 days a week for 16 days (total of 12 doses). Feed and water were provided *ad libitum*. On days 8 and 9, both rats and mice of the 125-mg/kg group received doses that were 10-fold too high.

All rats that received 2000 mg/kg and two of five males and three of five females that received 1000 mg/kg Benzyl Alcohol died before the end of the study. Rats of the two highest dose groups had blood around the nose and mouth, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts. Final body weight of male rats of the 1000-mg/kg group was 18% less than that of vehicle controls. Lethargy was observed in rats of the two highest dose groups; rough coats were noted in males of the 500- and 1000-mg/kg groups and in females of the 250- and 500-mg/kg groups. No compound-related histopathologic changes were noted.

All mice that received 2000 mg/kg and one of five males and two of five females that received 1000 mg/kg Benzyl Alcohol died before the end of the study. Lethargy and rough coats were noted in males that received $\geq$500 mg/kg and in females that received $\geq$1000 mg/kg. Blood in the urinary bladder was noted at necropsy in mice of the two highest dose groups. No compound-related histopathologic changes were noted (NTP 1989).

Reviewing this study, the United States Environmental Protection Agency (EPA) determined that the lowest-observable-adverse-effect level (LOAEL) was $\leq$500 mg/kg for male rats, and $\leq$250 mg/kg for female rats. EPA determined that the no-observable-adverse-effect level (NOAEL) was $\leq$250 mg/kg for male mice and $\leq$500 mg/kg for female mice (EPA 1989).

Benzoic Acid and Sodium Benzoate

Fujitani (1993) fed groups of 10 F344/N rats and $B6C3F_1$ mice (5 of each sex) 1.81%, 2.09%, or 2.40% (rats) or 2.08%, 2.50%, or 3.00% (mice) Sodium Benzoate for 10 days. The doses were selected based on earlier reports that repeated dosing with 2.5% Sodium Benzoate was lethal to rats.

One male rat of the high-dose group had signs of "hypersensitivity" and died on day 8. Rats of the mid- and high-dose groups had significantly reduced mean body weight as compared to nontreated controls. Relative liver and kidney weights, as well as serum concentrations of albumin and total protein, were significantly increased in male rats of the mid- and high-dose group and in female rats of the high-dose group. Serum γ-glutamyltranspeptidase activity was significantly increased in males and significantly decreased in females of the high-dose group. Serum cholesterol was significantly decreased in males of the high-dose group and in all dosed females as compared to controls. Changes in other parameters such as serum phospholipid

and uric acid concentrations were sometimes significant but were non–dose-dependent. Enlarged hepatocytes with glassy cytoplasm were noted at microscopic examination of tissues from males of the high-dose group.

All mice of the high-dose group had signs of "hypersensitivity"; 3 of 10 had convulsions and 2 of the 3 (both females) died before the end of the study. Mean body weights of mice of the treated groups were not significantly different from untreated controls. A dose-dependent increase in absolute and relative liver weight was noted; the increase was significant in mice of the high-dose group. Female mice of the high-dose group also had greater relative kidney weights. Serum cholesterol and phospholipid concentrations in male mice of the high-dose group, serum cholinesterase activities in male mice of the mid- and high-dose groups, and serum γ-glutamyltranspeptidase activities of female mice of the mid-dose group were significantly greater than those of the control group. No significant changes were noted in serum concentrations of triglyceride, uric acid, and urea nitrogen, and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) or in the AST/ALT ratio. Enlargement, vacuolation and necrosis of hepatocytes were noted in male mice of the 3.0% group (Fujitani 1993).

The GRAS report (Informatics, Inc. 1972) and the literature review by the Flavor and Extract Manufacturers' Association (1984) cited several short-term oral studies of Benzoic Acid and Sodium Benzoate toxicity (Table 4). Supporting the above findings of Fujitani, Kreis et al. (1967) reported significantly decreased weight gain in rats dosed with 1.1% Benzoic Acid for 35 days, and toxicity following five days of dosing with 3%. Studies in which approximately 2% Sodium Benzoate was administered for 4 to 8 weeks recorded: no adverse effects (Kramer and Tarjan 1962), lesions (Smyth and Carpenter 1948), or significant weight reduction (noted in male rats only) (Fanelli and Halliday 1963). Severe reduction of growth rate was recorded at slightly larger doses of Sodium Benzoate (White 1941). Sodium Benzoate was toxic at 5% (Kieckebusch and Lang, 1960; Fanelli and Halliday 1963).

Subchronic Oral Toxicity

Benzyl Alcohol

Groups of 20 F344/N rats and B6C3F$_1$ mice (10 of each sex) received 50, 100, 200, 400, or 800 mg/kg Benzyl Alcohol, 5 days a weeks for 13 weeks (NTP 1989). Experimental conditions were the same as in the 16-day study. The death of five rats was attributed to rupture caused by the gavage procedure. Gavage-related deaths were considered to result from the trauma of the gavage procedure combined with the neurotoxic/anesthetic effect of the compound. Aside from these, four male rats and one female of the 800-mg/kg group, as well as one female of the 400-mg/kg group and one male of the 200-mg/kg group died on study. The 800-mg/kg group had signs of neurotoxicity, including staggering, labored breathing, and lethargy after dosing. Blood around the nose and mouth was noted in 5 of 10 males of this group after week 8. Compared to vehicle controls, final mean body weights were 7% and 5% smaller, respectively, in male and female rats of the highest dose group. At histopathologic evaluation, lesions observed in rats of the highest dose group included necrosis of the dentate gyrus of the hippocampus in 7 of 7 males and 9 of 9 females; skeletal muscle necrosis in 5 of 10 males, thymic congestion, hemorrhage, and atrophy in 8 of 10 males, and nephrosis in 6 of 9 males. Renal lesions were similar to those noted in age-related spontaneous renal disease.

Nine of 10 deaths (mice) were attributed to the gavage procedure. Final mean body weights of females of the 400- and 800-mg/kg groups were 5% and 8% lower, respectively, than the vehicle control. Staggering was noted during the first and second weeks of dosing in mice of the high-dose group. No compound-related histopathologic alterations were observed. A Sendai virus infection was suspected (NTP 1989).

Reviewing the 91-day study, the EPA extrapolated the high dose for rats and mice into human doses of 84 and 39 mg/kg/day, respectively (for a 70-kg person). The EPA determined the NOAEL was 143 mg/kg for female rats, "which were the more sensitive sex." Using this level, and applying an uncertainty factor of 100 (10 for interspecies extrapolation multiplied by 10 to protect unusually sensitive individuals), resulted in a human reference dose (RfD) for subchronic oral exposure of 1.43 mg/kg/day, which was rounded to 1 mg/kg/day. The subchronic or partial lifetime RfD was described as "an estimate of an exposure level which would not be expected to cause adverse effects when exposure occurs during a limited time interval, i.e., for an interval which does not constitute a significant portion of the lifespan" (EPA 1989).

Benzoic Acid and Sodium Benzoate

Subchronic oral studies on the benzoates which were cited in the GRAS report (Informatics Inc. 1972) and in the literature review by the Flavor and Extract Manufacturers' Association (1984) are summarized in Table 4.

Reviewing the studies, the GRAS report (Informatics Inc. 1972) concluded that " . . . at a level of approximately 1%, the benzoates are at maximum nontoxic level; higher than this, they result in decreased food intake, depressed growth, and toxic effects on test animals."

Despite feed consumption comparable to controls, significant reduction in weight gain was noted in mice treated for 3 months with 80 mg/kg/day Benzoic Acid (Shtenberg and Ignatév 1970), and in rats treated for 90 days with 8% Sodium Benzoate (Deuel et al. 1954).

Chronic Oral Toxicity

Benzyl Alcohol

Groups of 100 F344/N rats (50 each sex) were dosed with 200 or 400 mg/kg Benzyl Alcohol in corn oil, 5 days per week for 103 weeks. Groups of 100 B6C3F$_1$ mice were dosed with 100 or 200 mg/kg Benzyl Alcohol following the same schedule. During week 80, mice were mistakenly dosed for four days

TABLE 4
Multiple-dose oral toxicity studies on Benzoic Acid and Sodium Benzoate

Protocol	Results/comments	Reference
Benzoic Acid		
40 Sprague-Dawley rats (20 each sex) received feed containing either 0.5% or 2% for 1 year. Some other groups also received sorbic acid	No effect noted at 0.5%; slight reduction of growth rate noted at 2%. No additive toxicity noted of Benzoic Acid plus sorbic acid	Ohno et al. 1978*
Royal Wistar rats dosed with 3% for 1, 2, 3, or 5 days (~1500 mg/kg/day); basal diet followed for 19 to 30 days	Fourteen of 35 rats dosed for 5 days died; necrosis of parenchymal cells noted in brain in all 5-day treated rats and occasionally in 3-day treated rats	Kreis et al. 1967
Royal Wistar rats (number not stated) dosed with 1.1% for 7, 14, or 35 days (~550 mg/kg/day)	Significantly poor weight gain; no signs of neurotoxicity or pathological changes in the brain	Kreis et al. 1967
100 mice (50 each sex) dosed for 3 months with 80 mg/kg/day (oral intubation)	Weight gain in treated animals was 66% (females) and 71% (females) of gain in controls, values significant; however, feed intake comparable	Shtenberg and Ignatév 1970
50 mice (25 each sex) dosed with 40 mg/kg/day; fed as a paste for 17 months, followed by 5 days of oral intubation	Major finding was a reduced response to physiological stress in treated animals compared to controls	Shtenberg and Ignatév 1970
Mice (number not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 months	Negative effect on body weight and viability; treatment-related carcinogenic effects noted (not specified); increased liver weights, enlarged spleens, ovaries, and lungs	Ignatév 1965
20 rats (10 each sex) dosed with 40 mg/kg/day; fed as a paste for 18 months, followed by 13 days of oral intubation	Developed increased tolerance to lethal doses of Sodium Benzoate; daily feed and water intake significantly less for treated males; limited data reported	Shtenberg and Ignatév 1970
Rats (number not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 months	No apparent effect on body weight or viability; no changes noted in parenchymatous organs; developed increased tolerance to lethal doses of Benzoic Acid	Ignatév 1965
50 Wistar rats (20 female, 30 male), 20 male Wistar rats, and 20 male Osborne-Mendel rats, dosed with 1.5% in feed for 18 months	Decreased feed intake and reduced growth	Marquardt 1960
Four generations of Bayer-Elberfeid rats dosed with 0.5 or 1.0% in feed	No adverse effect noted; increased life span noted in treated rats	Kieckebusch and Lang 1960
Sodium Benzoate		
28 rats dosed with 5% in feed	Nineteen of 28 died within two weeks of dosing; remaining 9 died by end of week 3	Kieckebusch and Lang 1960
12 Sherman rats (6 each sex) dosed with 2% or 5% in feed for 28 days	Slight weight depression (significant in males) noted at 2%; 5% toxic to all rats	Fanelli and Halliday 1963
Groups of 10 Sherman rats (5 each sex) dosed with 16 to 1090 mg/kg/day (four doses) for 30 days	No toxic effects; increased body weight, reduced appetite (compared to control), noted. Lesions of adrenal glands, upper intestine, kidneys, liver, and spleen	Smyth and Carpenter 1948

(Continued on next page)

TABLE 4
Multiple-dose oral toxicity studies on Benzoic Acid and Sodium Benzoate *(continued)*

Protocol	Results/comments	Reference
Rats (numbers not stated) dosed with 1947 to 2195 mg/kg/day for 3 to 6 weeks	Severe reduction of growth rate	White 1941
Wistar rats dosed with 1.5% in feed for 6 or 8 weeks (after week 4, carotene was added to diet)	No significant effect noted. Vitamin A content in liver and kidneys comparable to control	Kramer and Tarjan 1962
Groups of 10 Sherman rats (5 each sex) dosed with 1%, 2%, 4%, and 8% in feed for 90 days	No adverse effects at ≤4%. At 8% reduced growth rate (feed consumption comparable to control), significantly increased liver and kidney weight with lesions noted	Deuel et al. 1954
White rats (number not stated) dosed with 1.5%, 2.0%, 2.5%, 3.0% in feed for unknown duration	No effects noted in rats of ≤2.5% groups; distinct growth reduction noted in rats of 3.0% group though feed intake was comparable to control. One third of rats of this group died	Griffith 1929

*Study completed since the GRAS report (see text), but included for completeness.

with 375 (low-dose group) and 750 mg/kg (high-dose group) of α-methylbenzyl alcohol. No adverse effects were apparent (NTP 1989).

Mean body weights were comparable among dosed and vehicle control rats throughout the study. A number of accidental deaths was due to gavage procedures in female rats of both dose groups (17 deaths, low-dose; 13 deaths, high-dose) and in males of the 400 mg/kg group (14 deaths). Survival of female rats of the low- and high-dose groups was significantly lower than that of vehicle controls after weeks 71 and 50, respectively. At the end of the study, 17 female rats survived from each of the dose groups, compared to 35 female vehicle-controls; 27 low-dose males and 24 high-dose males survived, compared to 28 male vehicle controls. Clinical signs characteristic of sialodacryoadenitis (cervical swelling, pink eyes, red exudate around eyes) were observed in dosed and vehicle-control rats. The diagnosis was confirmed by serum analysis. Epithelial hyperplasia of the nonglandular stomach was noted in four high-dose males. A squamous cell papilloma was noted in 1 of 19 low-dose and 1 of 50 high-dose males. (It was not stated why only 19 low-dose male rats were examined.) No other compound-related clinical signs were observed.

Mean body weight was comparable among dosed and vehicle control mice throughout the study. Survival of female vehicle controls was significantly lower than that of the high-dose group after week 74 (female: vehicle control, 26/50; low dose, 32/50; high dose, 36/50). Corpora amylacea (foci of mineralization in the thalamus) was observed at an increased incidence in high-dose mice (male: vehicle control, 15/49; low dose, 21/48; high dose, 22/50; female: 14/50; 15/48; 25/50), but was noted to be a common and spontaneously occurring lesion (NTP 1989).

Reviewing the 2-year study, the EPA extrapolated the rat high dose to a human dose of 52 mg/kg/day (for a 70-kg person). The EPA determined that the LOAEL was 286 mg/kg for male rats. (Data from male rats were selected because mortality in female rats was not definitively associated with Benzyl Alcohol treatment.) Using this level, and applying an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 to protect unusually sensitive individuals, and 10 to extrapolate a LOAEL to a NOAEL) resulted in a human RfD for chronic oral exposure of 0.286 mg/kg/day, which was rounded up to 0.3 mg/kg/day (EPA 1989).

Benzoic Acid and Sodium Benzoate

Table 4 summarizes chronic oral studies of the benzoates that were included in the GRAS report (Informatics Inc. 1972) and in the literature review by the Flavor and Extract Manufacturers' Association (1984). Decreased feed intake and reduced growth were noted in rats fed 1.5% Benzoic Acid for up to 18 months (Marquardt 1960). No adverse effects were noted in most rat studies that used ≤1% Benzoic Acid (Ohno et al. 1978; Ignatév 1965; Kieckebusch and Lang 1960). One 18-month study reported significantly decreased feed and water intake in male rats fed 40 mg/kg/day (Shtenberg and Ignatév 1970). A dose-dependent response to doses of Benzoic Acid well below 1% was noted in mice (Ignatév 1965; Shtenberg and Ignatév 1970).

Acute Inhalation Toxicity

Benzyl Alcohol

Three groups of six Sherman rats were exposed for 4 hours to a 2000-ppm concentration of Benzyl Alcohol vapor in normal atmosphere. Nine rats died within 14 days of exposure. The investigators considered the compound to be a moderate hazard (Carpenter, Smyth, and Pozzani 1949).

Smyth, Carpenter, and Weil (1951) reported that rats could inhale air saturated with Benzyl Alcohol vapor for a maximum of 2 hours. Similar to the results reported by Carpenter, Smyth, and Pozzani (1949), inhalation at a concentration of 1000 ppm for 8 hours caused death of three of six animals within 14 days of exposure.

Acute Parenteral Toxicity

Benzyl Alcohol

In a study to determine the toxicity of various vehicles, Montaguti, Melloni, and Cavalletti (1994) administered undiluted Benzyl Alcohol intravenously (via the tail vein) to groups of 10 mice (5 of each sex). Three different mice strains were used with the following dose ranges. CD_2F_1 mice received 0.05 to 0.2 ml/kg, $B_6D_2F_1$ mice received 0.05 to 0.4 ml/kg, and C57BL/6N mice received 0.025 to 0.1 ml/kg. All mice weighed between 14 and 18 g. The highest dose given did not exceed the LD_{50}. Body weight was determined prior to the start of dosing, and 1 week thereafter. Animals were observed for 14 days and postmortem examinations were performed on day 15. Blood samples were withdrawn from the abdominal aorta and analyzed for hemolysis and precipitation potential.

Convulsions, dyspnea, and reduced mobility were noted at the first 24-hour observation in mice treated with all but the lowest dose of Benzyl Alcohol. Decreased body weight gain or slight decrease in body weight was noted in $B_6D_2F_1$ and C57BL/6N mice treated with all but the lowest dose. Postmortem alteration included hyperemia and edema in most animals that had died during the observation period (number not reported). Occasional hemorrhagic foci were observed in the spleen of C57BL/6N mice from all dose groups that had survived Benzyl Alcohol treatment. The blood from Benzyl Alcohol–treated mice had a potential for hemolysis and precipitation. Undiluted Benzyl Alcohol was ranked the most toxic of the five vehicles tested, which included dimethyl sulfoxide, polyethylene glycol 400, dimethylformamide, and absolute ethanol (Montaguti, Melloni, and Cavalletti 1994).

The literature review by the Flavor and Extract Manufacturers' Association (1984) cites several earlier animal studies in which Benzyl Alcohol was administered as either single or multiple doses via the intraperitoneal, intravenous, and subcutaneous routes.

Neurotoxicity

Benzoic Acid and Sodium Benzoate

In response to concerns about the role of food additives in cases of childhood hyperactivity, Crane and Lachance (1985) performed a neurobiological study of Sodium Benzoate using rats. Groups of eight Wistar dams received feed containing 0.1%, 0.5%, or 1.0% Sodium Benzoate beginning on gestation day (GD) 5 and continuing throughout pregnancy and lactation. The control group was untreated. At birth, the number of pups in each litter was equalized to eight. Locomotor activity of the pups was measured on various days. One pup from each litter was killed on days 9, 15, and 21 and the brain removed and examined. On day 22, pups were weaned onto the same diet as their respective dam. On day 24, one male pup from each litter was caged individually and monitored for spontaneous locomotor activity. Rats were killed on day 45 and brain concentrations of norepinephrine, dopamine, and serotonin were measured. No significant difference was noted in feed intake and body weight gain of dams and pups of the treated groups compared to controls. No consistent differences in motor activity and monoamine concentrations were noted.

Dermal Irritation

Benzyl Alcohol

In a primary irritation study 10% Benzyl Alcohol in squalane was applied (0.3 ml) in a 24-hour occlusive patch to the back of eight male albino rabbits. The sites had been clipped free of hair and were abraded in four rabbits. Sites were evaluated according to the Draize scoring system at the time of patch removal and 72 hours later. No irritation was observed; there was a score of zero on a scale of 0 to 8 (Shiseido Research Center 1972).

In a cumulative irritation study, three male albino guinea pigs received a daily open application of 10% Benzyl Alcohol in squalane (0.3 ml) on the back for 3 successive days. Sites were evaluated for erythema and edema 24 hours after each application and scored on a scale of 0 to 4. Benzyl Alcohol in squalane received a cumulative score of 0.4, falling in the ≤ 2.0 range of "none to weak irritant" (Shiseido Research Center 1972).

A polyvinyl chloride (PVC) cup containing 10% *w/v* Benzyl Alcohol was fastened (using surgical tape) to the dorsal side of three male nude mice for 24 hours of contact. The mice (MF 1 h) were 4 weeks old and weighed 10 to 22 g. Following exposure, mice were immediately killed and specimens of the exposed areas and of an adjacent untreated area were taken for microscopic examination. The skin sections were fixed in formalin, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin and scored using the Ingram & Grasso system. A typical section from Benzyl Alcohol–treated areas had severe compact hyperkeratosis, acanthosis, spongiosis, intracellular edema, and some areas of ulceration of the epidermis. The collagen bundles in the dermis appeared slightly fragmented and slight cell infiltration of the area was noted. The final score for Benzyl Alcohol was 22, the modal score for at least three animals. Scores greater than 21 were considered "unacceptably severe damage." The investigators acknowledged that male nude mice were not an ideal model for human skin; however, the study was done to establish the relative dermal tolerance of various penetration enhancers (Lashmar, Hadgraft, and Thomas 1989).

Benzoic Acid and Sodium Benzoate

RTECS (1995) cited that the human low toxic dermal dose of Benzoic Acid was 6 mg/kg.

Phototoxicity

In Vitro

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

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral Studies

Benzyl Alcohol

In a study by Inveresk Research International Ltd. (1983), a group of 50 specific pathogen–free (SPF) CD-1 mice received 750 mg/kg/day Benzyl Alcohol (in distilled water) by gavage on GDs 7 to 14. (Earlier toxicity studies had determined the maximum tolerated dose was between 645 and 1300 mg/kg/day, and the 750-mg/kg/day dose was selected for the reproduction study.) The concurrent vehicle control had 50 mice. Mice were individually caged and feed and water were available *ad libitum*. Clinical observations were made daily. Maternal body weights were recorded prior to dosing, on day 18, and on postnatal day 3; the weight on day 7 determined the dose volume administered over the entire treatment period. Mice were allowed to deliver their litters and nurse the pups for 3 days.

There were 18 compound-related deaths during the dosing period, and one on GD 15. Mice that died were discarded without necropsy. No procedure-related deaths (i.e., gavage error) were recorded. Body tremors, hunching, subdued behavior, prostration, ataxia, swelling, and/or cyanosis of the abdomen and piloerection were noted in mice that died during the study as well as those that produced litters.

No significant differences in reproductive and gestation indices, or in mean gestation length were noted between treated and control mice. A significantly lower day 18 mean body weight and a marginally reduced maternal weight on postpartum day 3 were noted in dosed dams. Decreased mean litter mean pup weight was noted on postpartum days 1 ($p < .01$) and 3 ($p < .001$). On postpartum days 1 to 3, a decreased mean litter weight change ($p < .05$) and decreased mean litter mean pup weight ($p < .001$) were noted. No significant differences were noted between treated and control pups in group litter viability. The investigators considered Benzyl Alcohol a suspect reproductive hazard and recommended further investigation (Inveresk Research International Ltd. 1983). Citing that study, the EPA (1989) noted that the extrapolated human dose (for a 70-kg person) was 58 mg/kg/day.

In screening a new developmental toxicity assay, 50 pregnant CD-1 mice were gavaged on GDs 6 to 13 with Benzyl Alcohol at a rate of 750 mg/kg/day. The dose selected was the LD_{10} value determined in preliminary dose-finding studies. Mice were allowed to deliver. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were measured. Nineteen (38%) of the dams of the Benzyl Alcohol group died prior to delivery; the corresponding vehicle control group (which received water) had no maternal death. (Mice that died were not necropsied.) Maternal weight was significantly less changed in the Benzyl Alcohol group (6.2 ± 3.6 g) as compared to controls (7.9 ± 2.3 g). Viability in the Benzyl Alcohol group was 21 of 22 litters (controls had 29/29 viability) with an average of 10.0 liveborns per litter. Birth weight (1.6 g/pup) and 3-day weight gain (0.5 g/pup) for pups of the Benzyl Alcohol treatment group were significantly less ($p < .05$) than the corresponding values in controls (1.7 and 0.7 g/pup, respectively). The reduced birth weight was classified as "some evidence of developmental toxicity." The researchers noted the 10% false-negative rate for toluene, a "presumptive teratogen" (Hardin et al. 1987).

A group of 50 pregnant SPF CD-1 albino mice was dosed with 550 mg Benzyl Alcohol/kg/day on GDs 6 to 15 by gavage. The Benzyl Alcohol was dissolved in corn oil; a vehicle-control group was maintained. Maternal status (survival, body weight changes), gestation index (length of gestation), reproductive index, postnatal survival, average litter weight, and average pup weight were comparable between treated and control animals (Environmental Health Research & Testing, Inc. 1986).

Benzoic Acid and Sodium Benzoate

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

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

No evidence of teratogenicity was noted in rats administered 510 mg/kg of Sodium Benzoate by gavage on GDs 9 to 11 (Kimmel, Wilson, and Schumacher 1971).

Sodium Benzoate at doses of 1.75, 8, 38, and 175 mg/kg was administered by oral intubation to groups of at least 20 pregnant

TABLE 5
Benzyl Alcohol genotoxicity studies

Assay	Concentration/method	Results/comments	Reference
Bacterial Cells			
Ames: *S. typhimurium* TA 100	100, 250, 500, 1000 μg/plate	Negative	Ball Foxall-Van Aken, and Jensen 1984
Ames: *S. typhimurium* TA 98, TA 100	Not stated	Negative	Rogan et al. 1986
Ames: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537	100; 333; 1000; 3333; 5000; 6666 μg/plate ± S9	Negative	NTP 1989
Mammalian Cells			
L5178Y tk+/tk− forward mutation in mouse lymphoma cell line	[cells incubated with agent for 4 h, then plated to determine thymidine resistance]		McGregor et al. 1988
	(−) S9:		
	156.25; 312.5; 625; 1250; 2500; 5000 μg/ml	Negative ≤2500; lethal at 5000 μg/ml	
	2500; 3000; 3500; 4000; 4500; 5000 μg/ml	POSITIVE at 4500; lethal at 5000 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	3200; 3400; 3600; 3800 μg/ml	Negative	
	(+) S9:		
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
L5178Y tk+/tk− forward mutation in mouse lymphoma cell line	(−) S9:		NTP 1989
	156.25; 312.5; 625; 1250; 2500; 5000 μg/ml	POSITIVE at 5000 μg/ml	
	2500; 3000; 3500; 4000; 4500; 5000 μg/ml	POSITIVE at 4500; lethal at 5000 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	(+) S9:		
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
Chromosome aberration in Chinese hamster ovary cells (ABS in CHO)	(−) S9: [cells incubated with agent for 8–10 h]		NTP 1989
	160; 500; 1600; 5000 μg/ml	Negative	
	2000; 3000; 4000; 5000 μg/ml	POSITIVE	
	250; 500; 1600; 3000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	Negative	
	(+) S9: [cells incubated with agent for 2 h]		
	50; 160; 500; 1600; 5000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	WEAK POSITIVE	
	1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE	
	1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE	

ABS in CHO	(−) S9:		Anderson et al. 1990
	160; 500; 1600; 5000 μg/ml	Negative	
	2000; 3000; 4000; 5000 μg/ml	Negative	
	250; 500; 1600 μg/ml	Negative	
	500; 1600; 3000 μg/ml	Negative	
	(+) S9:		
	50; 160; 500; 1600; 5000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
	1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
	1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
Sister Chromatid Exchange (SCE) in CHO	(−) S9: [cells incubated with agent for 2 h, Brd-U added, then incubated for another 24 h]		NTP 1989
	16; 50; 160; 500; 1600 μg/ml	Negative ≤500; lethal at 1600 μg/ml	
	500; 750; 1000; 1250; 1500 μg/ml	WEAK POSITIVE; lethal at 1500 μg/ml	
	(+) S9: [cells incubated with agent for 2 h, Brd-U added after cells washed]		
	16; 50; 160; 500; 1600; 5000 μg/ml	Negative ≤1600; lethal at 5000 μg/ml	
	500; 1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE; lethal at 5000 μg/ml	
SCE in CHO	(−) S9:		Anderson et al. 1990
	16; 50; 160; 500 μg/ml	Negative	
	500; 750; 1000; 1250 μg/ml	WEAK POSITIVE at 1250 μg/ml	
	(+) S9:		
	16; 50; 160; 500; 1600 μg/ml	Questionable results	
	500; 1600; 3000; 4000 μg/ml	WEAK POSITIVE at 4000 μg/ml	
In vivo: Fruit Fly			
Sex-linked recessive lethal in *Drosophila melanogaster*	Following dosing either orally with 5000 ppm or injected with 8000 ppm, males were mated with untreated females, progeny observed as indication of mutagenic activity	Negative	Foureman et al. 1994
In vivo: Mammalian			
Replicative DNA synthesis (RDS)	Male rats were killed at various times post dosing (300 or 600 mg/kg by either single oral gavage or SC injection) and hepatocytes obtained from perfused liver samples were incubated with [^{3}H]thymidine. RDS measured as percentage of [^{3}H]thymidine-incorporating cells relative to 2000 hepatocytes	Negative; at various times cell viability for Benzyl Alcohol–treated cells was significantly reduced	Uno et al. 1994

albino CD-1 outbred mice and Wistar albino rats on GDs 6 to 15. Groups of 21 to 22 pregnant hamsters were dosed with 3, 14, 65 or 300 mg Sodium Benzoate/kg on GDs 6 to 10. Groups of 10 Dutch-belted rabbits were artificially inseminated and then dosed by oral intubation with 2.5, 12, 54 or 250 mg Sodium Benzoate/kg on GDs 6 to 18. Dams were individually caged and feed and water were available ad libitum. Positive-control groups for mice, rats, and hamsters received aspirin. A positive-control group of rabbits received 6-aminonicotinamide. Sham groups for each animal type served as negative controls. Caesareans were performed on mice, rats, hamsters, and rabbits on days 17, 20, 14, and 29, respectively. Neither adverse effects on maternal or fetal survival nor a significant increase in fetal abnormalities in either soft or skeletal tissues was noted in any of the animals (Food and Drug Research Labs Inc. 1972).

Parenteral Studies

Benzyl Alcohol

In a study which assayed the teratogenic activity of ethinyloestradiol sulfonate in Wistar rats, a vehicle control group that was treated with Benzyl Alcohol/peanut oil was maintained. On GDs 10, 13, 6 to 10, or 10 to 14, rats (number not stated) received intraperitoneal (IP) injections of either the test material or an unspecified amount of vehicle. Fetuses were removed on day 21 and examined. No teratogenic effect was noted (Chemnitius, Oettel, and Lemke 1979).

Benzoic Acid and Sodium Benzoate

Sprague-Dawley rats were injected intraperitoneally with 100, 315, or 1000 mg/kg Sodium Benzoate on GDs 9 to 11 or 12 to 14. Reduced fetal body weight, increased in utero deaths (by 12%), and gross anomalies were noted at the highest dose (Minor and Becker 1971).

GENOTOXICITY

Benzyl Alcohol

Benzyl Alcohol was negative in the Ames test with and without metabolic activation (Ball, Foxall-Van Aken, Jensen 1984; Rogan et al. 1986; NTP 1989), sex-linked recessive lethal (flies) (Foureman et al. 1994), and replicative DNA synthesis (male rats) (Uno et al. 1994) assays. McGregor et al. (1988) considered results of a mouse lymphoma forward mutation assay in the absence of S9 activation to be "questionable," whereas NTP (1989) reported a positive response at concentrations associated with toxicity. Both studies were negative with S9 activation. Benzyl Alcohol, with S9 activation, was positive in the chromosome aberration test in Chinese hamster ovary (CHO) cells (NTP 1989; Anderson et al. 1990). Equivocal results were noted in the sister chromatid exchange (SCE) assay (NTP 1989; Anderson et al. 1990). Genotoxicity studies concerning Benzyl Alcohol are summarized in Table 5.

Benzoic Acid and Sodium Benzoate

Benzoic Acid was negative in the Ames (Fujita and Sasaki 1986; Zeiger et al. 1988) and SCE assays (Oikawa et al. 1980).

Sodium Benzoate was negative in the host-mediated (Litton Bionetics 1974), Ames (Prival, Simmon, and Mortelmans 1991), dominant lethal (rats) (Litton Bionetics 1974), and cytogenetics (both in vitro and in rats) (Litton Bionetics 1974) assays. When tested in the CHO cell line, Sodium Benzoate was positive in the chromosomal aberrations assay (Ishidate and Odashima 1977) and, at a high dose (2 mM), in the SCE assay (Abe and Sasaki 1977).

Njagi and Gopalan (1980) reported that incubation of adenosine, guanosine, uridine, or calf thymus DNA with Sodium Benzoate for up to 12 hours resulted in small shifts in the UV spectra. No shifts in the absorption peaks of the nucleoside cytidine were noted following incubation with Sodium Benzoate. The investigators noted that "DNA fragments do not have such shifts, thus the DNA must have remained intact during the course of incubation." Sodium Benzoate was considered not to act at the genetic level.

Genotoxicity studies concerning Benzoic Acid and Sodium Benzoate are summarized in Table 6.

CARCINOGENICITY

Oral Studies

Benzyl Alcohol

The 2-year gavage study performed by the NTP (detailed in the Oral Toxicity—Chronic section of this report) also tested for Benzyl Alcohol-induced carcinogenicity in rats and mice. Dose-related negative trends were noted in the incidences of anterior pituitary gland neoplasms in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Epithelial hyperplasia of the nonglandular stomach was noted in 4 of 50 high-dose male rats; it was not found in controls or low-dose male rats. An increased incidence of adenomas of the adrenal cortex noted in high-dose male mice (0/48; 0/44; 3/48) was within historical range and not considered compound-related (NTP 1989). The NTP investigators considered the study negative for Benzyl Alcohol–induced carcinogenicity. However, reviewing the study, the EPA (1989) considered the 3 of 48 incidence of adrenal cortex adenoma to be "equivocal evidence of carcinogenic activity rather than negative."

Benzoic Acid and Sodium Benzoate

For 18 to 24 months, groups of Fischer 344 rats (50 males and 52 females per group) received feed containing 2% or 1% Sodium Benzoate. The doses corresponded to the maximum tolerated dose (MTD) and $^1/_2$MTD as determined in 6-week toxicity studies. A control group of 25 male and 43 female rats received untreated feed. Average daily Sodium Benzoate intake was 280 and 202 mg, respectively, for male and female rats of the 2% group, and 141 and 102 mg, respectively, for male and female

TABLE 6

Benzoic Acid and Sodium Benzoate genotoxicity studies

Assay	Concentration/method	Results/comments	Reference
Bacterial Cells			
Host-Mediated	Mice orally dosed (either single dose or five doses each 24 h apart) with 50, 500, 5000 mg/kg Sodium Benzoate, then inoculated with *Salmonella* TA-1530, G-46, and *Saccharomyces* D3; 3 h later animals were killed and the bacteria were removed (by peritoneal wash) and plated	Negative (slight increases in mutation frequencies noted; non-dose dependent)	Litton Bionetics 1974
Ames: *S. typhimurium* TA 97A, TA 102	33–10000 μg Benzoic Acid/plate ± S9	Negative	Fujita and Sasaki 1986
Ames: *S. typhimurium* TA 97, TA 98, TA 100, TA 1535, TA 1537	Benzoic Acid at 100–6666 μg/plate or 100–10,000 μg/plate ± S9 (either rat or hamster liver)	Negative	Zeiger et al. 1988
Ames: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538; *E. coli* WP2	0.033–10 mg Sodium Benzoate per plate ± S9	Negative	Prival, Simmon, and Mortelmans 1991
Mammalian Cells			
SCE in CHO	1, 3, 10 mM Benzoic Acid	Negative	Oikawa et al. 1980
SCE in CHO	1, 2, 5, 10 mM Sodium Benzoate	POSITIVE at ≥2 mM (considered a high dose)	Abe and Sasaki 1977
ABS in CHO	Maximum effective dose: 2.00 mg/ml (138.8×10^{-4} M) Sodium Benzoate	POSITIVE: aberrations noted in 38%	Ishidate and Odashima 1977
Cytogenetics (human embryonic lung cells)	2, 20, 200 mg/kg Sodium Benzoate	Negative (checked for aberrations in anaphase chromosomes)	Litton Bionetics 1974
In vivo: Mammalian			
Dominant lethal (rats)	Following dosing by oral intubation (50, 500, 5000 mg/kg Sodium Benzoate either single dose or five doses each 24 h apart), male rats were mated with two females per week for 8 weeks. Corpora lutea, early and late fetal deaths, and total implantations monitored	Negative	Litton Bionetics 1974
Cytogenetic (rats)	Rats dosed by gastric intubation (50, 500, 5000 mg/kg Sodium Benzoate either single dose or five doses each 24 h apart, killed at various times after dosing (were given colcemid to arrest cells in metaphase)	Negative (checked for aberrations in bone marrow metaphase chromosomes)	Litton Bionetics 1974

rats of the 1% group. No clinical signs of toxicity, differences in average body weight or mortality rates were noted in treated rats when compared with controls. Neoplasms that were present in treated rats were similar in type and number to those in controls. No evidence of Sodium Benzoate–related carcinogenicity was observed (Sodemoto and Enomoto 1980).

In a life time drinking water study, 100 Albino Swiss mice (50 of each sex) were supplied with water containing 2% Sodium Benzoate. A control group of 200 mice was supplied with untreated water. Average daily intake of Sodium Benzoate was 124.0 and 119.2 mg for males and females, respectively. Sodium Benzoate treatment did not affect survival. No carcinogenic effect attributable to treatment was noted at necropsy (Toth 1984).

In Vitro Studies

Benzoic Acid and Sodium Benzoate

In an in vitro study hippurate and its parent compound Sodium Benzoate had antitumor effects on cells derived from Skalsky lymphoma, Németh-Kellner lymphoma (LYNK), L-asparaginase–sensitive 6C3HED Gardner lymphoma (GS), and LP-2 plasmacytoma. In a follow-up in vivo study, mice received subcutaneous implantations of GS or LYNK cells followed by twice daily IP injections of hippurate. A "high level of significance" in inhibition of tumor growth was reported. The in vivo study was not done using Sodium Benzoate (Spustová and Oravec 1989).

Dermal Studies

Jacobs et al. (1984) performed a skin-painting study using groups of 120 Eppley Swiss mice (60 each sex). A nonoxidative hair dye containing 2.0% Benzyl Alcohol and 0.016% Benzoic Acid was painted onto the skin at a dose of 0.05 ml/application, three times weekly for 20 months. Sites were shaved of hair 24 hours before each application and a new bottle of dye was used each week. Two groups of control animals were shaved but not treated. Nine months into the study, 10 mice/sex/group were killed. Body weights and survival differed little between treatment and control groups. Varying degrees of chronic dermal inflammation were noted in all groups, including the controls. A significant ($p < .01$) increase in malignant lymphomas was noted in treated females (23/60). However, the researchers noted that one concurrent control group had a very low incidence (7/60 or 12%) for that tumor type. The rate was 22% for the other control group and had averaged 33% for three control groups in previous studies. Thus, the findings were not considered treatment related. The incidence of pulmonary adenomas and hepatic hemangiomas, which are common to this mouse strain, were similar between treated and control groups. No unusual neoplasms were observed.

CLINICAL ASSESSMENT OF SAFETY

Clinical Experience

Benzyl Alcohol

In 1981 and 1982 several neonatal deaths were ascribed to Benzyl Alcohol present as a preservative in isotonic saline (9 mg/ml) that had been used to flush catheters (Brown et al. 1982; Gershanik et al. 1982). The syndrome consisted of metabolic acidosis, central neural depression, respiratory distress progressing to gasping respiration, hypotension, renal failure, and sometimes seizures and intracranial hemorrhages. In alerting pediatricians of the findings, the FDA (1982) reported an estimated daily intake of 99 to 404 mg/kg, which was 20 to 90 times the 4.5-mg/kg dose considered safe for healthy adults (Kimura et al. 1971). Although the infants involved had "serious underlying disease," biochemical evidence of Benzyl Alcohol toxicity was found. Blood and urine specimens contained high concentrations of Benzyl Alcohol, Benzoic Acid, and hippuric acid. The FDA stated that no cases of toxicity were observed in older infants, children, or adults.

In May 1985, FDA published a notice of intent that it was considering prohibiting use of all antimicrobial preservatives in single-dose containers of parenteral drug products for human use, and requiring multiple-dose parenteral drugs that contain any antimicrobial preservative to bear a warning that caution should be used in the administration to newborn infants. This intent was withdrawn in 1989 with the explanation that manufacturers of bacteriostatic water for injection and bacteriostatic sodium chloride injection had voluntarily agreed in 1982 that these two classes of products would contain a warning label against their use in newborns. Further, a 1983 revision of the US Pharmacopeia monograph required that these two classes of products bear the warning "not for use in newborns." The withdrawal by the FDA noted that the increased awareness brought about by these steps, in conjunction with the lack of additional reports of toxicity, prompted the decision that further regulation was not necessary.

Studies that compared infants born before and after the discontinuation of Benzyl Alcohol–containing solutions have generally supported the above measures (Menon et al. 1984; Benda, Hiller, and Reynolds 1986; Hiller et al. 1986; LeBel et al. 1988; Jardine and Rogers 1989; Cronin, Brown, and Ahdab-Barmada 1991).

Reports are available contraindicating the use of neuromuscular blocking agents containing Benzyl Alcohol (Craig and Habib 1977; Hahn, Feasby, and Gilbert 1983). Use of these agents was not advised in neonates (van der Hal et al. 1987) or in the epidural space (King and Hart 1994).

Reynolds and Smith (1995) reported that nebulizers of bacteriostatic saline containing Benzyl Alcohol as a preservative can cause bronchitis in healthy adults.

Benzoic Acid and Sodium Benzoate

FASEB (1973) reported no adverse effects following ingestion of Benzoic Acid at doses of 100 mg/day (82 doses in 86 days), 500 and 1000 mg/day (for 44 days), and 1000 mg/day (88 doses in 92 days). The number of participants was not reported.

In another study, participants initially ingested Benzoic Acid at 1000 mg/day for 5 days and progressed to 1500 then 2000

and finally 2500 mg/day staying at each protocol for 5 days before increasing the dose. Three of the 12 participants took the entire dose of 35 g in 20 days. This total dose produced marked symptoms of discomfort and malaise, which included nausea, headache, weakness, esophageal burning and irritation, hunger, and indigestion (Wiley and Bigelow 1908).

In an early study (Lucas 1909), 12 men drank apple juice containing 0.1% Sodium Benzoate and had the following signs and symptoms: burning taste, fullness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, constipation, decreased urine flow, increased specific gravity of the urine, and albuminuria. A liter of filtered cider containing 0.2% to 0.3% Sodium Benzoate (2 to 3 g) caused albuminuria within 3 hours of ingestion. However, Lucas himself ingested as much as 6 g/day for 3 successive days without adverse effect.

A single oral dose of 33 g of Sodium Benzoate to a 60-kg individual resulted in "clear signs of poisoning." Signs and symptoms including deep pallor, weak and infrequent pulse, general discomfort, cephalea, and nausea. Similar effects were noted after ingestion of 50 g of Sodium Benzoate over a 5-hour period (Bignami and Boraccia 1924).

Dermal Irritation

Benzyl Alcohol

Benzyl Alcohol (3%) was applied in a polypropylene chamber to the same site on the back of nine healthy female panelists for 4 consecutive days. The duration of exposure was not specified. Sites were visually evaluated on the fifth day. Benzyl Alcohol was an irritant according to the Frosch-Kligman scoring system (Harvell et al. 1994).

Benzoic Acid and Sodium Benzoate

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

Forty-eight female panelists participated in an in-use study that investigated the acnegenic and irritation potential of a liquid/powder foundation containing 0.2% Benzoic Acid. Complying with the test protocol, approximately half of the test population had "mild to moderate" acne. Panelists were instructed to apply the product to the entire face and neck area at least twice a day for 45 days. Acne lesions and irritation were evaluated by a dermatologist on days 0, 3, 7, 10, 28, and 45. Objective and subjective evaluations of irritation were made by a nurse or technician on days 15, 21, and 35. Panelists also maintained daily response logs. The dermatologist noted "no significant changes the lesion counts of the non acne subjects and the acne subjects had a decrease. All objective irritation grades were 0's." Transient grade 1 irritation was noted by the technician; panelists' logs recorded occasional instances of dryness, itching, and flakiness (Education and Research Foundation, Inc. 1992).

Dermal Sensitization

DeGroot (1994) compiled the following recommended patch test concentrations from the published literature: Benzyl Alcohol at 1%, 5%, or 10% in petrolatum, Benzoic Acid at 2%, 5%, or 10% in petrolatum (with an advised test concentration of <5%), and Sodium Benzoate at 2% or 5% in petrolatum (with a note that the 5% concentration may be an irritant).

Benzyl Alcohol

A repeat-insult patch test (RIPT) was conducted using a nonexclusive group of 110 panelists. Two mascara formulations each containing 0.65% Benzyl Alcohol were tested. During a 3-week induction period nine occlusive 24-hour patches (containing ~0.15 g of test material) were applied to the same site on either the upper arm or back. Sites were evaluated 24 hours after patch removal (i.e., prior to application of subsequent patch). Following a 12- to 20-day nontreatment period, a challenge patch was applied to both the original site and a previously unexposed site. Challenge sites were evaluated at 24 and 48 hours after patch removal. No reactions were noted during induction or at challenge to either formulation (Hill Top Research 1997a, 1997b).

Patch testing with Benzyl Alcohol (5% in petrolatum) was part of the American standard series (Adams 1982) and the North American Contact Dermatitis Group (NACDG) perfume screening series (Emmons and Marks 1985).

The Research Institute for Fragrance Materials, Inc. (RIFM) report on Benzyl Alcohol cited an unpublished Kligman Maximization study that tested 10% Benzyl Alcohol in petrolatum using 25 male volunteers (skin types: 10 were Caucasian and 15 were Black). Benzyl Alcohol (and three other test materials) was applied under occlusive patches to the forearm of panelists. A total of five 48-hour exposures occurred during induction and each was preceded by a 24-hour occlusive pretreatment of the sites with 5% aqueous sodium lauryl sulfate (SLS). Following a 10-day nontreatment period, panelists were challenged on the scapular back with a 48 hour patch. Challenge sites were pretreated for 1 hour with 10% SLS. Challenge sites were examined at 48 and 72 hours. No reactions were observed (Kligman 1970; Opdyke 1973).

Adams and Maibach (1985) compiled patch test results from 12 dermatologists over a 6-year period. Patches had been applied to the upper back for 48 hours of contact, and sites were evaluated at 48 and 72 hours. Three cutaneous reactions to 5% Benzyl Alcohol in petrolatum were noted among 713 cosmetic dermatitis patients.

Van Joost, Stolz, and Van der Hoek (1985) reported four positive patch tests to 6.5% Benzyl Alcohol among 242 patients with histories of contact allergy of varying origin. An index of simultaneous reactivity in which the number of reactions to other perfume ingredients was divided by the number of positive

reactions to Benzyl Alcohol had a value of 0.50 (one individual responded to eugenol and another to isoeugenol).

Broeckx et al. (1987) reported results of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction was noted in 48 (0.92% incidence) to Benzyl Alcohol. Reactions were noted in 2 of the 155 patients with cosmetic allergy.

Cross-sensitization to Benzyl Alcohol has been reported in subjects sensitized to Peru balsam (Opdyke 1973).

Benzoic Acid and Sodium Benzoate

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

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

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

Broeckx et al. (1987) reported results of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction to Benzoic Acid was noted in 34 (0.7% incidence). A reaction was noted in 1 of the 155 patients with cosmetic allergy.

Urticarial Reactions

Benzyl Alcohol and especially Benzoic Acid and Sodium Benzoate are among various compounds (such as some food additives) recognized in the published literature to induce nonimmunologic contact reactions in certain populations. Lahti (1980) reported that these agents "produce the reaction without any previous sensitization in most or almost all exposed persons." The hypersensitivity has been indicated by flexural dermatitis, rhinitis, and/or asthma. However, cutaneous changes such as urticaria, angioneurotic edema, and contact urticaria were the more common manifestations (Emmons and Marks 1985; Hannuksela and Haahtela 1987; Fisher 1990; DeGroot 1994). The terms nonimmunologic contact urticaria or nonimmunologic immediate contact reactions were used to describe the occurrence. Using Benzoic Acid, Kligman (1990) demonstrated that immediate reactions to urticariogens were concentration dependent and ranged from wheals induced with the highest test concentration, erythema with a fivefold dilution, and pruritus alone with a 25-fold dilution. (The panelists had been selected because they had developed a raised wheal to 1.0% Benzoic Acid.)

The methodology, test population, and results of various clinical studies demonstrating urticarial reactions are cited in Table 7.

In addition to the study cited in Table 7, Lahti (1980) presented results of various tests using dermatologic patients. Significantly more ($p < .001$) redness and edema were produced by Benzoic Acid (5% in petrolatum) under conditions of the open-test method as compared to the chamber test (both tests were conducted on upper back of 51 atopic and 55 nonatopic patients). Further, reactions were significantly more frequent ($p < .01$) in nonatopic dermatologic patients than in atopic patients when tested using the chamber test; the difference was almost significant in the open test ($p < .05$). However, when summarizing the findings Lahti ultimately concluded that "no significant differences were found in the frequency or strength of the nonimmunologic contact urticaria reactions between atopics and nonatopics." Most reactions appeared within 45 minutes and disappeared within 2 hours, with some persisting for longer than 24 hours (0.1 ml of test material was applied to the volar forearm of 29 atopic and 74 nonatopic physicians and nurses, evaluations were made every 15 minutes for 6 hours). The substance produced more reactions in a water vehicle than when applied in petrolatum; the lowest concentration needed to elicit a wheal and flare reaction was 0.050% Benzoic Acid in water or 0.10% Benzoic Acid in petrolatum (tested on 16 atopic and 16 nonatopic patients). The back, chest, dorsal sides of the forearm and upper arm, and thighs were the most sensitive areas. Neither scratching (11 atopics and 11 nonatopics) nor stripping (7 patients) of the skin prior to Benzoic Acid application increased the severity of the reaction. A diminished skin response was noted after repeated application of Benzoic Acid. In this aspect of the study, 0.1 ml of 5% Benzoic Acid in petrolatum was applied for 40 minutes to the dorsal side of the forearm of 17 nurses and physicians and 1 patient with a venous leg ulcer. The application was repeated on the same site 14 times at 2-hour intervals on the two subsequent days. Sites were evaluated after each exposure. A histamine scratch test was performed on two subjects after the skin had stopped reacting to Benzoic Acid (on the second day). Similar reactivity to histamine was noted between the test site forearm and the control arm. The finding suggested that the decreased reaction to Benzoic Acid resulted from an "emptying of the storage of mediator(s) in the skin rather than . . . fatigue of the dermal vessels and thus a failure to react." Lastly, Lahti

TABLE 7
Clinical patch and oral provocation tests demonstrating urticarial reactions

Test Population; method	No. of reactions (incidence)/comments	Reference
Benzyl Alcohol		
5% Benzyl Alcohol in petrolatum, 15 patients with eczematous dermatitis, 16 with history of cosmetic sensitivity, 19 controls; open testing (45-min exposure) and 48-h patch test	Open testing: contact urticaria noted in 7/15 (47%), 10/16 (63%), and 15/19 (79%) No positive patch tests	Emmons and Marks 1985
Benzoic Acid and Sodium Benzoate		
200 volunteers with no specific skin condition at test site; 125 and 500 mM applied for 20 min to volar forearm using Finn Chamber	To 125 mM: (on scale of 0–8) no. with erythema/no. with edema: score of 0 was 53/175, score of 1 was 44/19, score of 2 was 41/5, score of 3 was 25/1, score of 4 was 26/0, score of 5 was 10/0, score of 6 was 1/0 (none scored higher) To 500 mM: erythema/edema score of 0 was 43/164, score of 1 was 35/30, score of 2 was 41/5, score of 3 was 31/1, score of 4 was 39/0, score of 5 was 9/0, score of 6 was 2/0 (none scored higher)	Basketter and Wilhelm 1996
110 dermatological patients (36 atopics, 23 chronic urticaria, 26 nonatopic dermatitis, 25 non-allergic patients); chamber method with 20-min occlusion to upper back	5% Benzoic Acid in petrolatum, 43 positive reactions (39%)	Lahti 1980
80 housewives (none with known perfume allergy); 20-min occlusive patch to forearm	2% Benzoic Acid in petrolatum, 76 erythematous reactions of varying severity (95%), 15 incidences of edema (19%), 17 incidences of itching, stinging, burning, irritation sensations (21%)	Safford et al. 1990
125 children; patch tested over 7-year period (observed at 20 min for palpable pruritic erythema)	14 positive reactions (11%) to Benzoic Acid	Rademaker and Forsyth 1989
40 patients with urticaria, bronchial asthma, or chronic rhinitis; oral provocation test	Intolerance demonstrated in 2.5% of rhinitis patients and in 11.5% of asthma patients	Wüthrich and Fabro 1981
25 patients with clinical symptoms suggestive of food allergy; oral provocation test to Sodium Benzoate and 4-methylhydroxybenzoate	Positive reaction to benzoates noted in 34.21%	Ibero et al. 1982
132 patients with chronic urticaria and angioneurotic edema (suggested link to food additives); in a double-blind, placebo control study; oral provocation	5 positive reactions (4%) to Sodium Benzoate	Montaño García and Orea 1989
10 subjects with chronic urticaria and angioneurotic edema who had $\geq$1 positive reaction in histamine equivalent skin test; oral provocation test	1 positive reaction (10%) to Benzoic Acid	Malanin and Kalimo 1989
100 mg Sodium Benzoate—46 patients with history of chronic or acute urticaria/angioedema, chronic urticaria, chronic angioedema, or anaphylactoid reactions; DBPC oral challenge	15 positive reactions (32.6%); 12 reactions were in the 37 patients with chronic urticaria/angioedema	Sanchez-Borges and Suarez-Chacon 1992

noted the lack of correlation between dermal and oral exposure studies and concluded that the skin test "could not be used to predict sensitivity to preservatives taken perorally."

Shriner and Maibach (1996) investigated the variation of the nonimmunologic contact urticaria response in different areas of the body within and between two groups of panelists, "young" (10 women aged 23 to 47), and "old" (5 women aged 72 to 90). Panelists were not selected if they had a current or chronic history of dermatitis and/or current antihistamine use. A closed application method was used to apply 2.5% Benzoic Acid to the forehead, nose, nasolabial area, cheek, perioral area, chin, neck, and volar forearm for 20 minutes of exposure. In both age groups, the neck area was the most reactive followed by the perioral and nasolabial areas; the forearm was the least reactive. The younger group consistently demonstrated greater reactivity to Benzoic Acid at each site. The investigators noted that an earlier study had found the cheek to be the most responsive site.

Studying the mechanism behind these reactions, many investigators suggested a nonspecific histamine release (Forsbeck and Skog 1977; Guin et al. 1984; Larsen 1985), whereas Lahti (1987) argued for other (undetermined) mechanisms.

A 29- to 8000-fold increase in plasma concentrations of prostaglandin PGD_2 and a 72- to 370-fold increase in 9α, 11β-PGF_2 concentrations (the stable metabolite of PGD_2) was noted in four healthy panelists following topical application of 10% Benzoic Acid in petrolatum. Benzoic Acid had been applied to the forearm and covered with plastic wrap for 60 minutes of contact; blood had been drawn from the antecubital vein from the treated sites. The changes were not observed in blood drawn from the contralateral arm. The increased PGD_2 biosynthesis was dose-dependent over a concentration range of 0.01% to 15%. No cutaneous erythema was noted at <1%, patchy erythema was noted at 1%, maximal and confluent erythema was noted with ≥5% Benzoic Acid. Pretreatment with oral acetylsalicylic acid resulted in no erythema. The increased PGD_2 synthesis was not accompanied by histamine release. The investigators concluded that PGD_2 mediated the vasodilation associated with topical application of Benzoic Acid (Downard, Roberts, and Morrow 1995).

Lahti, Pylvänen, and Hannuksela (1995) reported that washing of the upper arm skin with a liquid detergent enhanced the immediate reactivity of the skin to Benzoic Acid. Benzoic Acid (10 μl) was applied without occlusive patches to test sites on the upper left and right arms of 12 healthy panelists on days 0, 3, and 6. The upper right arm was treated with the vehicle, a mixture of 2-propyl alcohol and 1,2-propylene glycol. Panelists were instructed to wash their upper left arm with a diluted dishwashing liquid, twice a day for 6 days. Sites were graded visually, and blood flow (measured by a LDF flowmeter), skin color, transepidermal water loss (TEWL), and electrical capacity were measured. Washing alone increased TEWL and decreased electrical capitance. Benzoic Acid produced immediate skin reactions in all panelists. The reactions were stronger on washed skin, suggesting that "even subclinical changes in the skin caused by repeated washing increase the skin response to benzoic acid."

Phototoxicity/Photosensitization

Benzoic Acid and Sodium Benzoate

Clinical studies have demonstrated that ultraviolet (UV) light can produce a dose-dependent inhibition of Benzoic Acid–induced nonimmunologic immediate contact reactions (Larmi, Lahti, and Hannuksela 1988; Larmi 1989a, 1989b).

Biosearch Inc. (1991) tested a matte eye shadow and base formulation each containing 0.1% Benzoic Acid under the conditions of the Draize-Shelanski repeat-insult patch test using 77 panelists. The test materials were applied in 48-hour occlusive patches to one of three sites on the back. Every third patch was applied to the same site. (This protocol allowed for the observation of delayed reactions.) Sites on the back were irradiated for 1 minute with UV light (365 nm, at a distance of 12 inches) following removal of induction patches 1, 4, 7, and 10. At the same time, the materials were applied in 48-hour open patches to the volar aspect of the right forearm. The protocol was followed for a total of 10 applications within a $3\frac{1}{2}$-week period.

Following a 2-week nontreatment period, closed and open challenge patches were applied to previously unexposed sites. Sites on the back were irradiated after removal of the challenge patch. No reactions were noted during induction or at challenge and no reactions were noted in response to irradiation (Biosearch Inc. 1991).

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

A liquid/powder foundation containing 0.2% Benzoic Acid was tested in a photosensitization study using 30 panelists with varying Fitzpatrick Skin Types (degree of pigmentation was stated not to interfere with UV light response or skin reaction evaluation). During induction, six 24-hour occlusive patches were applied to the back within a 3-week period. At the time of patch removal sites were irradiated with 2.0 MEDs of UVB light and 4 Joules/cm^2 of UVA light. Following an 18-day nontreatment period panelists were challenged at two previously unexposed sites. Challenge sites were scored after 24 hours of exposure and one site was then irradiated with 0.5 MED of UVB and 4 Joules/cm^2 of UVA. Another site, not dermally treated, was also irradiated at challenge and served as the UV light

control. Challenge sites were evaluated at 24, 48, and 72 hours postirradiation. Panelists were instructed to avoid natural or artificial sunlight exposure throughout the study. No reactions were observed (Biosearch Inc. 1992d).

Ocular

Benzyl Alcohol

In each of two double-blind studies, 25 patients suffering from early progressive idiopathic cataracts, subcapsular or cortical in site, received one drop of saline containing 0.07% Benzyl Alcohol every 8 hours (Testa et al. 1987). The eyelid was held open for at least 2 minutes. Treatment continued for 22 months. In one study, a control group received placebo, whereas in the other study, the control group received an anticataract medication. Clinical findings were recorded every 30 days for the first 14 months, then patients were followed for up to 18 and 22 months.

A significant ($p < .01$) increase in visual acuity (VA) was observed in patients treated with Benzyl Alcohol after 30 and 60 days as compared to those receiving either placebo or the medication. Compared to those placebo or medication treated, a significant ($p < .01$) decrease in lens opacity was noted in 19 and 17 patients treated with Benzyl Alcohol, respectively.

In the course of the studies, a significant increase in the number of surgeries for cataracts was noted in patients not receiving Benzyl Alcohol. One patient treated with Benzyl Alcohol required surgery after 22 months compared to 38 total who had received either placebo or medication. Benzyl Alcohol was well tolerated except in two patients (4%) where tolerance was fair in one and poor in the other. The investigators encouraged a large scale prospective trial noting that Benzyl Alcohol is already contained (though not at anticataract concentrations) in ophthalmic solutions (Testa et al. 1987).

Benzoic Acid and Sodium Benzoate

An eye shadow plus base containing 0.1% Benzoic Acid was tested in a 28-day in-use study using 52 women. Half of the panelists were contact lens wearers. Panelists were instructed to use the product (one of four eye shadow colors plus base) at least twice a day. Examinations were made at the beginning and end of the study by an ophthalmologist, weekly by a nurse or technician, and the panelists maintained daily logs. Slight conjunctival hyperemia without chemosis was noted in eight women; the condition was nonpersistent in all cases. Another two women presented with slight hyperemia and were also experiencing allergy symptoms; erythema was noted around the eye of one of these two panelists. One panelist had an incidence of red and mildly swollen caruncles at the fifth observation. Four other panelists reported occasional itching; dryness was reported by another two panelists. One panelist reported redness, puffiness, and irritation of the left upper eyelid that prevented her from wearing her contact lenses for 2 days. The investigators concluded that "any reactions observed or sensations perceived were minor, transient and/or sporadic and there were no apparent differences among the four shades of eye shadow. Based on these findings, all of the four products tested were regarded as safe for their intended use" (TKL Research 1991).

SUMMARY

Benzyl Alcohol

Benzyl Alcohol is an aromatic alcohol that is used in cosmetics as a fragrance component, preservative, solvent, and/or viscosity decreasing agent. As of January 1998 it was used in 322 formulations. Data from 1984 indicated use at ≤25%.

Benzyl Alcohol is used as a food additive, in OTC drug preparations, and in clinical settings. It is a membrane fluidizer and a local anesthetic.

Benzyl Alcohol is metabolized to Benzoic Acid, which is then conjugated with glycine and excreted as hippuric acid. EPA reviews of mouse and rat oral-dosing studies conducted by the NTP determined subchronic and chronic oral reference doses for humans of 1 and 0.3 mg/kg/day, respectively. Earlier, the WHO established an ADI of up to 5 mg/kg.

Investigators considered Benzyl Alcohol to be a moderate respiratory hazard and toxic when administered by the parenteral route. It produced severe irritation when applied to the skin of nude mice.

In oral-dose teratogenicity studies using mice, Benzyl Alcohol was negative in one study (550 mg/kg/day), gave questionable results in another (750 mg/kg/day), and was considered a suspect reproductive hazard in the third (750 mg/kg/day [which EPA extrapolated to a human dose of 58 mg/kg/day]).

Mutagenicity studies reported both positive and negative results. It was negative for carcinogenicity when dermally tested on mice at 2.00% in a nonoxidative hair dye. NTP considered it negative for carcinogenicity following 2 years of oral dosing in rats and mice, but EPA considered the results equivocal.

In clinical settings, Benzyl Alcohol can produce nonimmunologic contact urticaria or nonimmunologic immediate contact reactions. It was not a sensitizer when tested in a maximization test at 10% in petrolatum, and demonstrated a low incidence of sensitization in provocation studies. Therapeutic ocular studies indicated it may be beneficial in the management of cataracts.

Benzoic Acid and Sodium Benzoate

Benzoic Acid is an aromatic acid that is used in cosmetics as a pH adjustor and/or preservative. Sodium Benzoate is its sodium salt and is used in cosmetics as a preservative. As of January 1998 they were used in 223 and 156 cosmetic formulations, respectively. Data from 1984 indicated use primarily at ≤1% (with some use at 5% and 25%, respectively).

Both substances are GRAS ingredients. WHO established an ADI of up to 5 mg/kg. Benzoic Acid can be used in ointments and antifungal agents. Sodium Benzoate has been used clinically in the treatment of hyperammonemia. The benzoates are recognized hydroxy radical scavengers.

Benzoic Acid is rapidly absorbed following dermal application. Its metabolism can deplete glycine supplies.

In animal multiple-dose oral toxicity studies decreased feed consumption, depressed growth, and toxic effects were noted at doses of Benzoic Acid or Sodium Benzoate >1%. A neurobiological study was negative.

In oral-dose teratogenicity studies, Benzoic Acid (600 mg/kg) produced significant results in hamsters, but was negative in two rat studies (up to at least 500 mg/kg/day). Sodium Benzoate was negative for teratogenicity in mice and rats (175 mg/kg/day), hamsters (300 mg/kg/day), and rabbits (250 mg/kg/day).

Benzoic Acid was negative in mutagenicity studies. Sodium Benzoate was positive in assays done on the CHO cell line, but negative in other studies. Benzoic Acid was negative for carcinogenicity when dermally tested on mice at 0.016% in a nonoxidative hair dye. Sodium Benzoate was negative for carcinogenicity when administered orally at up to 2% to rats (in feed for up to 2 years) or mice (in a life-time drinking water study).

In clinical studies, toxic symptoms were noted following doses far exceeding the ADI established by the WHO. The benzoates are recognized to produce nonimmunologic contact urticaria or nonimmunologic immediate contact reactions, but it is not clear whether the reactions, are histamine or prostaglandin mediated. Dermal sensitization, phototoxicity, and photosensitization studies were negative.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel was satisfied that results of toxicity, mutagenicity, carcinogenicity, reproductive/developmental, and sensitization studies cited in this report support the safety of these ingredients in cosmetic formulations.

The focus of the Panel's safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate concerned the ability of these ingredients to induce contact urticaria or other contact reactions. The Panel was interested in knowing the threshold and frequency of occurrence of these so-called nonimmunologic reactions. The Panel used the studies cited in Table 7 to establish a pattern. Dermal studies demonstrated that 5% Benzyl Alcohol elicited a reaction in a sizeable portion of the population. One study noted reactions in almost all panelists following brief exposure to 2% Benzoic Acid. The Panel was of the opinion that these urticarial reactions were strictly cutaneous, possibly involving a cholinergic mechanism and not immunoglubolin E (IgE) mediated. Further, predictive clinical sensitization studies using the maximization protocol indicated that 10% Benzyl Alcohol and 2% Benzoic Acid were not sensitizers. In provocative studies, Benzyl Alcohol had a low incidence of sensitization. Utilizing all of the dermal exposure data, the CIR Expert Panel was of the opinion that these ingredients could be used safely at concentrations up to 5%. However, cosmetic manufacturers should consider the nonimmunologic contact urticaria phenomena when using these ingredients in formulation, especially in products designed for use on infants and children.

The Expert Panel received comments suggesting that the available data support the safety of Benzyl Alcohol in hair dyes at concentrations up to 10%. The Panel recognized that hair dye use involves limited body area exposure, has a controlled exposure time per use, and has limited frequency of use (weeks or months between uses). Because of this pattern of use, the Expert Panel concluded that contact urticaria would not be a concern. Therefore, the Panel was of the opinion that Benzyl Alcohol could be used up to 10% in hair dye formulations.

Frequency of use data indicated that these ingredients are used in formulations where inhalation is a route of exposure. The Expert Panel decided that the toxicity data contained in this report were insufficient to assess the inhalation risk of these ingredients. Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data were not sufficient for determination whether the ingredients, under relevant conditions of use, were either safe or unsafe. The Panel released a Notice of Insufficient Data on April 4, 1997, requesting inhalation toxicity data. No comments were received.

CONCLUSION

Based on the available data, the CIR Expert Panel concludes that Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate are safe for use in cosmetic formulations at concentrations up to 5%. The available data are insufficient to support the safety of these ingredients in cosmetic products in which a primary route of exposure is inhalation. Benzyl Alcohol is safe for use in hair dyes at concentrations up to 10%.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 25, 2010

SUBJECT: Inhalation study of Benzyl Alcohol and Benzoic Acid

WIL Research Laboratories, LLC. 2010. A 4-week inhalation toxicity study of aerosolized benzyl alcohol and benzoic acid in Sprague-Dawley rats. WIL-703002.

FINAL REPORT

Volume 1 of 4
(Text, Figures 1-2, Tables 1-36, and Appendix A - Tables A1-A8)

STUDY TITLE

A 4-WEEK INHALATION TOXICITY STUDY OF AEROSOLIZED BENZYL ALCOHOL AND BENZOIC ACID IN SPRAGUE-DAWLEY RATS

STUDY NUMBER

WIL-703002

DATA REQUIREMENT

OECD Guideline, Section 412

STUDY DIRECTOR

Jason M. Roper, PhD

STUDY INITIATION DATE

13 March 2009

STUDY COMPLETION DATE

8 February 2010

PERFORMING LABORATORY

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

SPONSOR

The Personal Care Products Council
1101 17th Street NW
Washington D.C. 20036

COMPLIANCE STATEMENT

This study, designated WIL-703002, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792), 16 October 1989; the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C(97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC; and the protocol as approved by the Sponsor.

The protocol was designed and the study was conducted in general accordance with the following guidelines: Organisation for Economic Cooperation and Development (OECD): Guidelines for Testing Chemicals, Section 412.

Jason M. Roper, PhD Senior Toxicologist, Inhalation Toxicology Study Director	8 FEB 2010 Date
Linda Loretz, PhD, DABT Sponsor Representative	Feb. 2, 2010 Date
Applicant/Submitter	Date

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1. SUMMARY

1.1. OBJECTIVE

The objective of this study was to evaluate the potential toxic effects of aerosolized benzyl alcohol when administered to rats by nose-only inhalation on a 5-day per week basis for a period of 4 weeks (minimum of 20 exposures). In addition to a control group exposed to filtered air and treatment groups exposed to 4 concentrations of benzyl alcohol, 2 additional groups were exposed to benzoic acid.

1.2. STUDY DESIGN

Aerosolized benzyl alcohol was administered via nose-only inhalation for 6 hours per day on a 5-day/week basis for a period of 4 weeks (a minimum of 20 exposures/animal) to 4 groups (Groups 2-5) of Crl:CD(SD) rats. Target exposure concentrations were 30, 100, 300, and 1000 mg/m^3 for Groups 2, 3, 4, and 5, respectively. Aerosolized benzoic acid was administered via nose-only inhalation exposure on a 5-day/week basis for a period of 4 weeks (a minimum of 20 exposures/animal) to 2 groups (Groups 6 and 7) of Crl:CD(SD) rats. Target exposure concentrations were 2.5 and 12.5 mg/m^3 for Groups 6 and 7, respectively. A concurrent control group (Group 1) was exposed to filtered air on a comparable regimen. Each group consisted of 10 animals/sex. All animals were euthanized on the day following the last exposure. The first day and week of exposure were designated as study day 0 and study week 0, respectively.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were conducted prior to exposure, at the approximate mid-point of each exposure (beginning on study day 2; third exposure), 0 to 1 hour following exposure, and once daily on non-exposure days; detailed physical examinations were performed at least weekly. Individual body weights and food consumption were recorded approximately weekly. Ophthalmic examinations were conducted once during the pretest period (study week -2) and near the end of the exposure period (study week 3). Blood samples for clinical pathology evaluations (hematology, coagulation, and serum chemistry) were collected from all animals at the scheduled necropsy (study week 3). Complete

necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all surviving animals in the control (Group 1) and high dose benzyl alcohol and benzoic acid groups (Groups 5 and 7); gross lesions were examined from all animals in all groups.

1.3. RESULTS

With the exception of the low-concentration benzyl alcohol group (Group 2), which had a mean exposure atmosphere concentration of 136% of the target concentration, all exposure atmosphere mean concentrations were within 96.7% to 107.2% of the respective target concentrations. There were no test substance-related mortalities, clinical or ophthalmic observations, or effects on body weights, food consumption, clinical pathology parameters, and organ weights. There were no test substance-related macroscopic or microscopic findings.

1.4. CONCLUSIONS

Based on the results of this study, 6-hour nose-only inhalation exposure to benzyl alcohol at mean exposure concentrations of 41, 102, 290 and 1,072 mg/m^3 or exposure to benzoic acid at target exposure concentrations of 2.5 and 12.6 mg/m^3 for 4 weeks (minimum of 20 exposures) was well-tolerated with no effects at any exposure level. The no-observed-effect-level (NOEL) and no-observed-adverse-effect level (NOAEL) were considered to be 1,072 mg/m^3 for benzyl alcohol and 12.6 mg/m^3 for benzoic acid.

2. INTRODUCTION

The objective of this study was to evaluate the potential toxic effects of aerosolized benzyl alcohol when administered to rats by nose-only inhalation on a 5-day per week basis for a period of 4 weeks (minimum of 20 exposures). In addition to a control group exposed to filtered air and treatment groups exposed to 4 concentrations of benzyl alcohol, 2 additional groups were exposed to benzoic acid.

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A 4-Week Inhalation Toxicity Study of Aerosolized Benzyl Alcohol and Benzoic Acid in Sprague-Dawley Rats." Due to software spacing constraints, the study title appears as "4-Week Inhalation Study of Benzyl Alcohol & Benzoic Acid in Rats" on the report tables. The first day and week of exposure were designated as study day 0 and study week 0, respectively.

The animals were euthanized and necropsied on the day following their last exposure (study day 26, 27, or 28). Although study day 28 was technically the first day of study week 4, all necropsy days are referred to as study week 3 throughout the report text and tables.

The following computer protocols were used for data collection during the study:

Computer Protocol	Type of Data Collected
WIL-703002M	Main study data (males)
WIL-703002F	Main study data (females)
WIL-703002P	Pretest data (males)
WIL-703002Q	Pretest data (females)

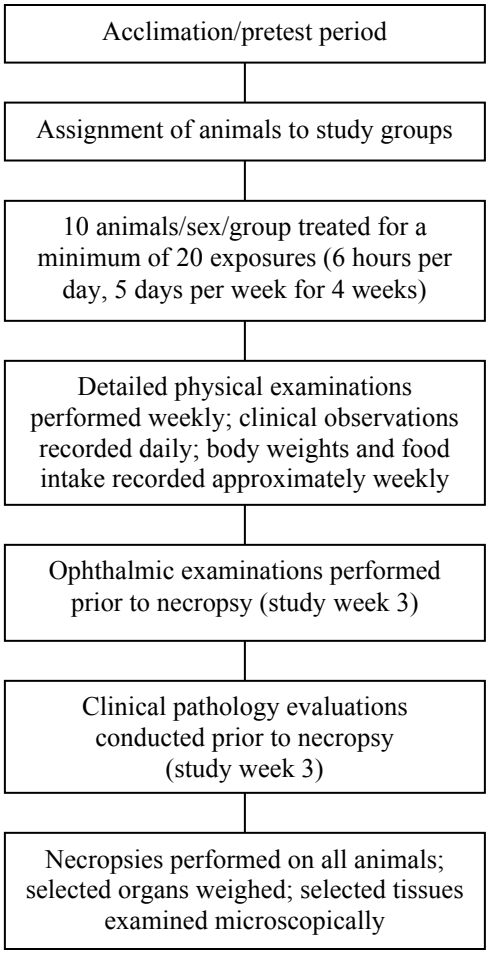
2.2. KEY STUDY DATES

Date(s)	Event(s)
31 March 2009	Experimental starting date (animal receipt)
13 April 2009	Assignment to study groups
22 April 2009	Experimental start date (initiation of dose administration; study day 0)
18, 19, 20 May 2009	Scheduled necropsy (study week 3)
9 July 2009	Experimental termination (completion) date (last histopathological examination)

2.3. PRINCIPAL INVESTIGATORS AND TEST SITES

Test Site and Principal Investigator for Microscopic Evaluation	John Boyce, DVM, PhD, DACVP, DACLAM Senior Pathologist Biotechnics, a WIL Research Laboratories LLC subsidiary 310 Millstone Drive Hillsborough, NC 27278

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST SUBSTANCES AND VEHICLE

4.1.1. TEST SUBSTANCE 1 IDENTIFICATION

Test substance 1, benzyl alcohol, was received from Emerald Kalama Chemical, LLC, Kalama, WA, as follows:

Identification	Physical Description	Date of Receipt
Benzyl Alcohol Lot no. KABCN89307 Exp. date: 27 September 2010 CAS no. 100-51-6 [WIL log no. 8077A]	Clear colorless liquid	7 October 2008
Benzyl Alcohol Lot no. KABCN94301 Exp. date: 1 April 2011 CAS no. 100-51-6 [WIL log no. 8077B]	Clear colorless liquid	17 April 2009

Documentation regarding the purity and stability of test substance 1 is on file with the Sponsor and WIL Research Laboratories, LLC. Certificates of Analysis were provided by the Sponsor and are presented in Appendix B. The purity was 99.9%. Benzyl alcohol was stored at room temperature and was considered stable under this condition. Reserve samples were collected and stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. TEST SUBSTANCE 2 IDENTIFICATION

Test substance 2, benzoic acid, was received from Emerald Kalama Chemical, LLC, Kalama, WA, on 7 October 2008 as follows:

Identification	Physical Description
Benzoic Acid Lot no. KABZT87212 Re-test date: 29 July 2009 CAS no. 65-85-0 [WIL log no. 8078A]	White flakes

Documentation regarding the purity and stability of test substance 2 is on file with the Sponsor and WIL Research Laboratories, LLC. A Certificate of Analysis was provided by the Sponsor and is presented in Appendix B. The purity was 99.6%. Benzoic acid was stored at room temperature and was considered stable under this condition. A reserve sample was collected and stored in the Archives of WIL Research Laboratories, LLC.

4.1.3. VEHICLE IDENTIFICATION

The vehicle used for exposure of the control group (Group 1) was filtered air.

4.2. INHALATION EXPOSURE METHODS

Exposures were conducted using seven 7.9-L conventional nose-only exposure systems (designed and fabricated by WIL Research Laboratories, LLC) with synthetic rubber grommets in exposure ports to engage animal holding tubes. One exposure system was dedicated for each group for the duration of the study.

The following table presents the exposure system used for each group:

Group Number:	1	2[A]	3[A]	4[A]	5[A]	6[B]	7[B]
Exposure System:	1	2	3	4	5	6	7
Target Exposure Concentration (mg/m^3):	0	30	100	300	1000	2.5	12.5

[A] = Benzyl Alcohol
[B] = Benzoic Acid

Air supplied to the nose-only systems was provided from a dry compressed air source. All test atmosphere exhaust passed through a Solberg filter prior to entering the facility exhaust system, which consisted of charcoal- and HEPA-filtration.

Animals were housed in normal animal colony rooms during non-exposure hours. For the exposure each day, the animals were placed into nose-only exposure restraint tubes in the colony rooms, transported to the exposure room, placed on the nose-only system, exposed for the requisite duration, and returned to their home cages in the animal colony rooms. Animals were held in restraint tubes for approximately 20-56 minutes prior to animal exposure. Food and water were withheld during the animal exposures. Exposure methods and conditions are presented in detail in Appendix C.

4.3. TEST ATMOSPHERE GENERATION METHODS

For Exposure System 1, humidified and dilution air were added to the exposure system. Humidified air was added using a regulator and a rotameter-type flowmeter. Dry compressed air passed through a muffler-type bubbler submerged in a 2-L Erlenmeyer flask filled with deionized water to produce humidified air. Dilution air was added using a Coilhose Pneumatics regulator and a Gilmont rotameter-type flowmeter. Exposure methods and conditions are detailed in Appendix C. The following is a list of parameters used during animal exposures for the control exposure system:

Exposure System	Dilution Airflow Rate (LPM)	Humidified Airflow Rate (LPM)	Total Airflow Rate (LPM)
1	20.1	29.5	49.6

4.3.1. BENZYL ALCOHOL

A vapor/aerosol atmosphere was generated using a system which operated as follows. A syringe pump and appropriate size syringe were used to deliver test substance to an atomizer. The atomizer was comprised of a no. 2850 fluid cap and a no. 64 air cap. Using a Coilhose Pneumatics regulator, compressed air was supplied to the air port of the atomizer at a known, constant pressure to effect the atomization of the test substance.

The resulting vapor/aerosol atmosphere passed through a liquid trap prior to entering the exposure system. For Exposure Systems 2, 3, and 4, a siphon was placed in-line prior to the exposure system to reduce the concentration as needed. The siphon was controlled using a rotameter-type flowmeter. The approximate test substance delivery rates and syringe sizes are summarized in the following table:

Exposure System	Syringe Size (mL)	Test Substance Flow Rate (g/hour)
2	3	0.1 to 1.1
3	5	0.7 to 0.9
4	10	4.2
5	100	19 to 35

On 29 April 2009, the Exposure System 2 siphon rotameter-type flowmeter was replaced with a Dwyer rotameter-type flowmeter. Dilution and humidified air was added to the atmosphere prior to entering the exposure system. Dilution air was added using a Coilhose Pneumatics regulator and metered using a Gilmont rotameter-type flowmeter. Humidified air was added using a Coilhose Pneumatics regulator and metered using a Dwyer rotameter-type flowmeter. Dry compressed air passed through a muffler-type bubbler submerged in a 2-L Erlenmeyer flask filled with DI water to produce humidified air. On 30 April 2009, the siphon rotameter-type flowmeter for Exposure System 2 was moved after the humidified air. Exposure methods and conditions are detailed in Appendix C. The approximate airflows used for exposures are summarized in the following table:

Exposure System	Siphon Airflow Rate (LPM)	Atomizer Airflow Rate (LPM)	Dilution Airflow Rate (LPM)	Humidified Airflow Rate (LPM)	Total Airflow Rate (LPM)
2	0 to 9	4 to 19	0.5 to 18	16 to 40	28 to 64
3	0 to 0.1	16	0 to 12	20 to 39	39 to 59
4	1 to 3	18	6 to 20	13 to 26	40 to 57
5	NA	19	8 to 36	0 to 21	37 to 60

NA = Not applicable

4.3.2. BENZOIC ACID

A dust aerosol atmosphere of the test substance was generated using a system which operated as follows: The test substance was delivered using a Wright Dust Feeder (WDF) and controller, which fed test substance at a constant rate to a jet mill micronizer operating as a dispersion device. The WDF was equipped with a 5-cm^3 stainless steel cup. Prior to packing the test substance into the WDF cups, the test substance was sieved using a no. 25 standard sieve. The sieved test substance was packed in the cup using manual compression. Using a Coilhose Pneumatics regulator, dry compressed air was supplied to the WDF to deliver test substance to the jet mill. The WDF supply air did not add to the total airflow to the nose-only system. Using a Coilhose Pneumatics regulator, dry compressed air was supplied to 2 Ashcroft gauges with needle valves to control the airflow to the micronizing and inlet ports of the jet mill where the test substance was milled to the desired particle size. The resulting aerosol from the jet mill was delivered to the exposure system through ¾-inch ID anti-static tubing. A 28.3-L settling chamber was placed in-line between the jet mill and the exposure system to remove larger particles. A tee was placed between the settling box and exposure system to provide humidified air to the exposure system. Using a Coilhose Pneumatics regulator and a Dwyer rotameter-type flowmeter, dry compressed air passed through a muffler-type bubbler submerged in a 2-L Erlenmeyer flask filled with DI water to produce humidified air. Exposure methods and conditions are detailed in Appendix C. The following is a list

of approximate parameters used during animal exposures for the test substance exposure systems:

	Jet Mill					
Exposure System	Inlet Airflow Rate (LPM)	Micronizing Airflow Rate (LPM)	Humidified Airflow Rate (LPM)	Total Airflow Rate (LPM)	WDF Setting (Indicated)	WDF Airflow (PSI)
6	16.4	11.6	32.3	60.3	0.05-0.13	13
7	15.8	12.6	32.0	60.4	0.20-0.45	14

4.4. EXPOSURE CONCENTRATIONS

4.4.1. ACTUAL EXPOSURE CONCENTRATIONS - CONTROL AND BENZOIC ACID

Actual exposure concentrations were determined using standard gravimetric methods for the control and benzoic acid groups. Samples were collected on pre-weighed, 25-mm glass-fiber filters held in an open-faced filter holder positioned in the animal breathing zone of the nose-only exposure system. Following sample collection, the filters were re-weighed and the concentration calculated as the filter weight difference divided by the sample volume. Samples were collected at approximately 90-minute intervals for the test substance exposure systems and 1 sample was collected weekly for the control exposure system. Exposure methods and conditions are detailed in Appendix C. The following table summarizes the approximate sampling conditions:

Exposure System	Sample Flow Rate (L/minute)	Sample Time (minutes)
1	0.8	85
6	0.9 to 1.0	85
7	1.0	30 to 85

4.4.2. ANALYZED EXPOSURE CONCENTRATIONS - BENZYL ALCOHOL

Concentrations of benzyl alcohol in the exposure systems were determined at approximately 90-minute intervals using a gas chromatograph (GC). Additional samples were collected as needed. Samples were collected from the approximate animal-

breathing zone of the exposure system using a series of 2 impingers containing isopropyl alcohol (IPA) as a trapping liquid. Test atmosphere samples were pulled through the impinger sampling train. This sampling method was used to collect the test atmosphere vapor, as well as aerosol (if present). Following sample collection, the liquid in the impingers was pooled, mixed using a laboratory vortex, and manually injected into a calibrated, validated GC. Impinger samples for the presence of benzyl alcohol were not collected or analyzed for the control group. Exposure of the control group in a separate room from the benzyl alcohol exposures eliminated the possibility of exposure of the control group to benzyl alcohol. The following table lists the sampling parameters used during animal exposure.

Exposure System	IPA Volume In Each Impinger (mL)	Total IPA Volume (mL)	Sampling Time (Minutes)
2	10	20	10
3	10	20	10
4	10	20	10 to 20
5	20	40	5

4.4.3. PARTICLE SIZE DETERMINATION

Aerosol particle size determinations were conducted for Exposure Systems 5, 6, and 7 using a 7-stage cascade impactor. Pre-weighed, 25-mm glass-fiber filters were used as the collection substrates. One sample was collected weekly at approximately 1.8 LPM for 2, 300, and 60 minutes for Exposure Systems 5, 6, and 7, respectively. The filters were re-weighed and the particle size was calculated based on the impactor stage cut-offs. The aerosol size was expressed as the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). Exposure methods and conditions are detailed in Appendix C.

4.4.4. REAL TIME MONITORING - BENZOIC ACID

The benzoic acid atmospheres were monitored in real-time using a HPM-1000 light scattering aerosol photometers. The monitors were connected to an Omega RD6110 Chart Recorder to provide a continuous display of concentration. Real-time aerosol monitoring of the test atmosphere was intended to provide an index of exposure concentrations for system adjustments and was not used to define actual concentrations. Exposure methods and conditions are detailed in Appendix C.

4.5. TEST SUBSTANCE CHARACTERIZATION FOR STABILITY AND PURITY

A sample of each test substance was collected prior to the initial use for animal exposures for analysis of concentration (time-zero value). Following the final exposure, a sample of each test substance was collected from the container used for the final exposure for purity determination. The end of use percent concentration was compared to the time zero (pre-use) value for determination of stability. All analyses were conducted by the Analytical Chemistry Department, WIL Research Laboratories, LLC using a validated high performance liquid chromatography method using ultraviolet absorbance detection. Details about the methodology and results of these analyses are presented in Appendix D, and the results are summarized in Section 6.2.

4.6. TEST SYSTEM

Crl:CD(SD) rats from Charles River Laboratories, Inc., Raleigh, NC, were used as the test system for this study. This species and strain of animal is generally recognized as appropriate for inhalation studies. The Sprague Dawley rat was selected because it is a widely used strain for which significant historical control data are available. The animals were approximately 9 weeks old at the initiation of dose administration.

4.7. ORGANIZATION OF TEST GROUPS, EXPOSURE LEVELS AND TREATMENT REGIMEN

Filtered air (control group), benzyl alcohol or benzoic acid atmospheres were administered as 6-hour, nose-only inhalation exposures on a 5-day per week basis for a

period of 4 weeks (20-22 exposures for each animal) through the day prior to the scheduled necropsy. The first day of exposure was defined as study day 0.

The following table presents the study group assignment:

Group Number	Test Substance	Target Concentration Level (mg/m^3)	Number of Animals Males	Females
1	Filtered Air	0	10	10
2	Benzyl Alcohol	30	10	10
3	Benzyl Alcohol	100	10	10
4	Benzyl Alcohol	300	10	10
5	Benzyl Alcohol	1000	10	10
6	Benzoic Acid	2.5	10	10
7	Benzoic Acid	12.5	10	10

All benzyl alcohol and benzoic acid exposure concentrations were selected by the Sponsor based on known toxicity information. For benzyl alcohol, a mixed aerosol and vapor exposure atmosphere may have been present. Therefore, the exposure concentration was reported as total test substance (i.e., aerosol plus vapor).

The route of administration was inhalation exposure because this is a potential route of human exposure. Nose-only exposure methods were used to reduce the potential for dermal exposure or oral exposure resulting from grooming. The number of animals selected for this study was the minimum needed to yield statistically valid and scientifically meaningful data and to meet the objectives of the study.

4.8. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Eighty-four male and 84 female Crl:CD(SD) rats were received in good health on 31 March 2009, from Charles River Laboratories, Inc., Raleigh, NC. The animals were approximately 41 days old at receipt. Each animal was examined by a qualified technician on the day of receipt and weighed 3 days later. Each animal was uniquely identified with a subcutaneous microchip (BMDS system) in the scapular area. All animals were housed for a 22-day acclimation/pretest period. During this period, each

animal was observed twice daily for mortality and changes in general appearance or behavior.

Pretest data collection began on 3 April 2009. Individual body weights and food consumption were recorded and detailed physical examinations were performed periodically during the pretest period. Ophthalmic examination data were also recorded for pretest animals prior to the initiation of exposure. Pretest clinical observations are presented in Appendix E.

To screen animals for poor tolerance of restraint, and to limit potential effects on respiration of the novel environment/conditions of restraint, the animals were acclimated to restraint in nose-only exposure tubes by increasing the restraint time over the acclimation period. On the first, second, third, fourth, and fifth days of restraint acclimation, the animals were acclimated for 1, 2, 3, 4, and 6 hours, respectively. Animals were then acclimated for 6 hours every other day until the initiation of exposure. Following the restraint period, each animal was observed for clinical signs of injury or stress.

4.9. ANIMAL HOUSING

Upon arrival, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animal facilities at WIL Research Laboratories, LLC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Nylabones® were provided to all animals throughout the study (except during exposure or exposure acclimation periods) for environmental enrichment and to aid in maintaining the animals' oral health, and were sanitized weekly.

4.10. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), is a certified feed with appropriate analyses performed by the

manufacturer and provided to WIL Research Laboratories, LLC. Reverse osmosis-treated (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided *ad libitum* throughout the study, except during exposure or exposure acclimation periods, or any scheduled period of fasting. Municipal water supplying the facility was analyzed for contaminants according to the standard operating procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study.

4.11. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in environmentally controlled rooms. The room temperature and humidity controls were set to maintain environmental conditions of 71 ± 5°F (22 ± 3°C) and 50 ± 20% relative humidity. Room temperature and relative humidity were controlled and monitored using the Metasys® DDC Electronic Environmental control system. These data were recorded approximately hourly and are summarized in Appendix F. Actual mean daily temperature ranged from 70.4°F to 71.2°F (21.3°C to 21.8°C) and mean daily relative humidity ranged from 37.0% to 52.0% during the study. Fluorescent lighting provided illumination for a 12-hour light (0600 hours to 1800 hours)/12-hour dark photoperiod. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

4.12. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

On 13 April 2009 (9 days prior to the initiation of exposure), all available rats were weighed and examined in detail for physical abnormalities. Based on the review of all appropriate pretest data, which were collected using the WIL Toxicology Data Management System (WTDMS™), animals judged suitable for assignment to the study were selected for use in a computerized randomization procedure. A printout containing the animal numbers, corresponding body weights and individual group assignments was generated based on body weight stratification in a block design. The animals were then arranged into groups according to the printout. Individual body weights at randomization

were within ± 20% of the mean for each sex. One Group 2 (30 mg/m^3 benzyl alcohol) female was found dead in the restraint tube following the exposure conducted on study day 1 (second exposure). This animal was replaced with a suitable remaining pretest group female prior to exposure on study day 2, and the replacement animal received 20 total exposures.

Each group (Groups 1-7) consisted of 10 males and 10 females. Individual body weights ranged from 255 g to 352 g for males and from 182 g to 234 g for females at the initiation of exposure**.**

5. PARAMETERS EVALUATED

5.1. SURVIVAL

All animals were observed twice daily for mortality and moribundity, once in the morning and once in the afternoon, except on the day of scheduled necropsy.

5.2. CLINICAL OBSERVATIONS

Clinical examinations were performed prior to exposure, at the approximate mid-point during the exposure period (beginning on study day 2; third exposure), 0 to 1 hour post-exposure (designated as 1 hour post-exposure for report presentation purposes), and once daily on non-exposure days. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were conducted on all animals at least once during the pretest period, at the time of randomization and group assignment, and weekly during the exposure phase (including prior to scheduled necropsy).

5.3. BODY WEIGHTS

Individual body weights were recorded approximately weekly during the pretest period, at the time of randomization and group assignment, weekly throughout the study (including the first day of exposure), and a non-fasted body weight was collected on the day before the first scheduled day of necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded for all animals on the days of the scheduled necropsies.

5.4. FOOD CONSUMPTION

Individual food consumption was recorded weekly beginning at least 1 week prior to exposure. Food intake was calculated as g/animal/day for the corresponding body weight intervals. When food consumption could not be measured for a given interval (due to spillage, weighing error, obvious erroneous value, etc.), the appropriate interval was footnoted as "NA" (Not Applicable) on the individual tables.

5.5. CLINICAL PATHOLOGY

Blood samples for clinical pathology evaluations (hematology, coagulation, and serum chemistry) were collected from all animals on the days of the scheduled necropsies (study week 3). The animals were fasted overnight prior to blood collection. Blood was collected for hematology and serum chemistry evaluation via the retro-orbital sinus of animals anesthetized by inhalation of isoflurane. Blood was collected for coagulation parameters at the time of euthanasia via the vena cava of animals anesthetized by inhalation of isoflurane. Blood was collected into tubes containing potassium EDTA (hematology), sodium citrate (coagulation) or no anticoagulant (serum chemistry). Clinical pathology methods, procedures, and references are presented in Appendix G. Interpretation of the clinical pathology data was performed by John Boyce, DVM, PhD, DACVP, DACLAM (Appendix H). The following parameters were evaluated:

5.5.1. HEMATOLOGY AND COAGULATION

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (ProTime)
Activated partial thromboplastin time (APTT)
Reticulocyte count
  Percent (Reticulocyte)
  Absolute (Retic Absolute)
Red cell distribution width (Red CellWidth)
Hemoglobin distribution width (HGB Width)
Differential leukocyte count -
  Percent and absolute
    -Neutrophil
    -Lymphocyte
    -Monocyte
    -Eosinophil
    -Basophil
    -Large unstained cell
Platelet estimate[a]
Red cell morphology (RBC Morphology)[a]

( ) - Designates tabular abbreviation
[a] - Presented on individual tables if a manual differential was performed, and the manual data were accepted and reported instead of the automated differential data

5.5.2. SERUM CHEMISTRY

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Gamma glutamyltransferase (GlutamylTransfer)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides (Triglyceride)
Sorbitol dehydrogenase (Sorbitol 'Genase)[a]

( ) - Designates tabular abbreviation
[a] - Presented on special chemistry tables

5.6. OPHTHALMIC EXAMINATIONS

Ocular examinations were conducted on all animals during pretest (9 April 2009; study week -2) and prior to the scheduled necropsy (16 May 2009; study week 3). All ocular examinations were conducted using an indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilation with an appropriate mydriatic agent. Pretest examinations were performed by David A. Wilkie, DVM, MS, DACVO, and study week 3 examinations were performed by Brian C. Gilger, DVM, MS, DACVO (Appendix I).

5.7. ANATOMIC PATHOLOGY

5.7.1. MACROSCOPIC EXAMINATION

A complete necropsy was conducted on all animals. Animals were euthanized by isoflurane inhalation and exsanguination. The necropsies included an examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Clinical findings that were confirmed macroscopically were designated CEO (correlates with externally observed) on the individual macroscopic data

tables. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

Adrenals (2)
Aorta
Bone with marrow
  Femur with joint
  Sternum
Bone marrow smear (from femur) [a]
Brain
  Cerebrum (2 levels)
  Cerebellum with pons/medulla
Cervix
Epididymides (2) [b]
Eyes with optic nerves (2) [c]
Exorbital lacrimal glands (2)
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Harderian glands (2)
Heart
Kidneys (2)
Larynx
Liver (sections of 2 lobes)
Lungs (including bronchi, fixed by constant pressure inflation with fixative)
Lymph nodes
  Axillary (2)
  Mediastinal and bronchial (if visible)
  Mesenteric and mandibular
Mammary gland (females only) [d]
Nasal cavity with turbinates [e]
Ovaries (2) with oviducts [f]
Pancreas
Peripheral nerve (sciatic)
Peyer's patches
Pharynx
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2) [b]
Thymus
Thyroid gland with parathyroids (2) [f]
Trachea
Urinary bladder
Uterus
Vagina
All gross lesions

[a] - Not placed in formalin; not examined.
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - A corresponding section of skin was collected from the same anatomic area for males.
[e] - Following collection of the appropriate protocol-specified tissues, the entire head was removed and preserved. Following decalcification, 6 cross-sections of the nasal cavities were prepared for microscopic examination in accordance with the method described by Morgan (1991).
[f] - Examined if in plane of section and in all cases when a gross lesion of the organ was present.

5.7.2. ORGAN WEIGHTS

The following organs were weighed from all animals at the scheduled necropsy:

Adrenals	Lungs (prior to inflation with fixative)
Brain	Ovaries and oviducts
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Uterus with cervix

Paired organs were weighed together. Organ-to-final-body-weight and organ-to-brain-weight ratios were calculated.

5.7.3. SLIDE PREPARATION AND MICROSCOPIC EXAMINATION

After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides, and stained with hematoxylin and eosin.

Microscopic examination was performed on all tissues listed in Section 5.7.1. from all animals in the control, 1000 mg/m^3 benzyl alcohol, and 12.5 mg/m^3 benzoic acid groups (Groups 1, 5, and 7, respectively) at the scheduled necropsy. Gross lesions were examined from all animals. Missing tissues were identified as not found at necropsy, lost at necropsy, lost during processing or other designations as appropriate. Tissues may appear on the report tables as not examined due to the tissue not being in the plane of section, not present at trimming, etc. Microscopic examination was performed by John Boyce, DVM, PhD, DACVP, DACLAM, WIL Research Laboratories, LLC (Appendix H).

5.8. STATISTICAL METHODS

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the

control group by sex. Each mean was presented with the standard deviation (S.D.), standard error (S.E.) and the number of animals (N) used to calculate the mean. Statistical analyses were not conducted if the number of animals was 2 or less. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ±1 in the last significant figure.

Body weights, body weight changes, food consumption, clinical pathology values (except gamma glutamyltransferase), and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. Gamma glutamyltransferase values under range were assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Gamma glutamyltransferase data were subjected to the Kruskal-Wallis nonparametric ANOVA test (Kruskal and Wallis, 1952) to determine intergroup differences. If the ANOVA revealed significance ($p<0.05$), Dunn's test (Dunn, 1964) was used to compare the test substance-treated groups to the control group.

5.9. DATA RETENTION

The Sponsor has title to all documentation records, raw data, specimens, slides or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol. Data generated by the Sponsor, will be archived by the Sponsor or the Sponsor's designee.

Reserve samples of the test substances, pertinent electronic storage media, and the original final report are retained in the Archives at WIL Research Laboratories, LLC, in compliance with regulatory requirements.

6. RESULTS

6.1. CHARACTERIZATION OF EXPOSURE ATMOSPHERES

Analyses of Exposure Concentrations: Appendix C

6.1.1. NOMINAL EXPOSURE CONCENTRATIONS

The following table summarizes the overall mean nominal concentrations for each test substance exposure system:

Exposure System:	2[A]	3[A]	4[A]	5[A]	6[B]	7[B]
Mean Concentration (mg/m^3):	94	288	1284	8293	31	145
Standard Deviation:	44.4	75.5	148.1	1290.7	4.9	31.0
N:	22	22	22	22	22	22

[A]= Benzyl Alcohol, [B]= Benzoic Acid

Nominal exposure concentrations were calculated by determining the pre-exposure and post-exposure mass of the test substance for a given exposure, and dividing the difference by the total airflow rate through the exposure system. A significant portion of the generated test substance was removed using siphons or liquid traps for the benzyl alcohol groups, and using settling chambers for the benzoic acid groups, prior to the atmosphere reaching the animal exposure chambers. Because a portion of the atmospheres were removed prior to the animal exposure chambers, high mean nominal concentrations were observed compared with the actual measured exposure concentrations. For reporting purposes, the actual measured concentrations at the chamber, as described in Section 6.1.2. are considered to be the test substance concentrations to which the test system was exposed.

6.1.2. EXPOSURE CONCENTRATIONS

The following table summarizes the overall mean exposure concentrations for each exposure system:

Exposure System:	1	2[A]	3[A]	4[A]	5[A]	6[B]	7[B]
Target Concentration (mg/m^3):	0	30	100	300	1000	2.5	12.5
Actual Concentration (mg/m^3):	0	41	102	290	1072	2.5	12.6
Percent Difference from Target (%):	0	37	2.0	-3.3	7.2	0.0	0.8
Standard Deviation (mg/m^3):	0.0	12.9	16.4	24.6	163.0	0.13	0.91
Concentration (ppm)	NA	9.6	23.8	67.4	248.8	NA	NA
Standard Deviation (ppm):	NA	3.00	3.81	5.72	37.86	NA	NA
N:	4	22	22	22	22	22	22

[A]= Benzyl Alcohol, [B]= Benzoic Acid

6.1.3. PARTICLE SIZE DETERMINATION

Aerosol particle size was determined for the 1000 mg/m^3 benzyl alcohol exposure system (5) and both benzoic acid exposure systems (6 and 7). The aerosol particle size was not determined for the control group (filtered air) or for the 30, 100, or 300 mg/m^3 benzyl alcohol exposure systems. In a previous method development and validation study (Kirkpatrick, Draft) it was determined that benzyl alcohol atmospheres at concentrations less than 100 mg/m^3 did not contain aerosol particles. Additionally, due to the volatility of benzyl alcohol, and the sampling time required to obtain a measurable amount of test substance on impactor substrates, it was not possible to accurately assess the particle size of the 300 mg/m^3 benzyl alcohol exposure atmosphere, although it was determined previously that aerosol particles were present at this concentration (Kirkpatrick, Draft). It is not anticipated that the MMAD of aerosol particles in the 300 mg/m^3 benzyl alcohol exposure atmosphere would have exceeded that observed for the 1000 mg/m^3 benzyl alcohol exposure atmosphere. The following table summarizes the overall mean aerosol particle size for Exposure Systems 5, 6, and 7:

Exposure System:	5[A]	6[B]	7[B]
Mean MMAD (Microns):	3.3	2.1	2.5
Mean GSD:	2.39	3.66	3.03
N:	4	4	4

[A]= Benzyl Alcohol, [B]= Benzoic Acid

6.2. ANALYTICAL CHEMISTRY

Test Substance Purity And Stability Report: Appendix D

Benzyl alcohol and benzoic acid samples were collected and analyzed prior to use for animal exposures (101% pure and 99.8% pure, respectively) and after use for animal exposures 42 days later (99.5% pure and 101% pure, respectively). Comparison of these purity data indicated that the test substances were stable for the duration of use on the study.

Observed purity values greater than 100% are accounted for by acceptable variability in the analytical method used to assess test substance purity.

6.3. SURVIVAL

Summary Data: Table 1, Table 2, Table 3, Table 4

Individual Data: Table A1, Table A2, Table A3, Table A4

There were no test substance-related deaths. One Group 2 female (no. 8307; 30 mg/m^3 benzyl alcohol) was found dead in the restraint tube following the exposure conducted on study day 1 (second exposure). Based on the lack of clinical signs of toxicity in any test substance-treated group, this death was considered related to poor tolerance to restraint and was not considered test substance-related.

All other animals survived to the scheduled necropsy.

6.4. CLINICAL OBSERVATIONS

Summary Data: Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12

Individual Data: Table A3, Table A4, Table A5, Table A6, Table A7, Table A8, Table A9, Table A10, Table A11, Table A12

There were no test substance-related clinical observations. All clinical findings in the test substance-treated groups were noted with similar incidence in the control group, were limited to one or two animals, were not noted in an exposure concentration-related manner, and/or were common findings for laboratory rats of this age and strain.

6.5. BODY WEIGHTS

Summary Data: Table 13, Table 14, Table 15, Table 16, Table 17, Table 18; Figure 1, Figure 2

Individual Data: Table A13, Table A14, Table A15, Table A16, Table A17, Table A18

Body weights were unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared.

6.6. FOOD CONSUMPTION

Summary Data: Table 19, Table 20

Individual Data: Table A19, Table A20

Food consumption was unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared.

6.7. CLINICAL PATHOLOGY

6.7.1. HEMATOLOGY AND COAGULATION

Summary Data: Table 21, Table 22

Individual Data: Table A21, Table A22

Pathology Report: Appendix H

Hematology parameters were unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared, and all group mean values were within the WIL historical control reference ranges (version 2.8).

6.7.2. SERUM CHEMISTRY

Summary Data: Table 23, Table 24, Table 25, Table 26

Individual Data: Table A23, Table A24, Table A25, Table A26

Pathology Report: Appendix H

Serum chemistry parameters were unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared, and all group mean values were within the WIL historical control reference ranges (version 2.8).

6.8. OPHTHALMIC EXAMINATIONS

Summary Data: Table 27, Table 28, Table 29, Table 30

Individual Data: Table A27, Table A28, Table A29, Table A30

Ophthalmic Examination Report: Appendix I

No ophthalmic lesions indicative of toxicity were observed in any of the test substance-treated groups. All findings observed were typical in prevalence and appearance for laboratory rats of this age and strain.

6.9. ANATOMIC PATHOLOGY

6.9.1. MACROSCOPIC EXAMINATION

Summary Data: Table 31, Table 32

Individual Data: Table A31, Table A32

Pathology Report: Appendix H

There were no test substance-related macroscopic findings at the scheduled necropsy. All macroscopic findings noted were considered to be spontaneous and/or incidental in nature and unrelated to test substance administration.

6.9.2. ORGAN WEIGHTS

Summary Data: Table 33, Table 34

Individual Data: Table A33, Table A34, Table A35, Table A36, Table A37, Table A38

Pathology Report: Appendix H

There were no statistically significant test substance-related alterations in final body weight or organ weights. However, non-statistically significant lower mean final body weights in the males exposed to either 300 or 1000 mg/m^3 benzyl alcohol resulted in nonadverse, statistically significant, higher mean epididymide weights relative to final body weights but not relative to brain weights. All group mean values were within the WIL historical control reference range (means ± 2 standard deviations; version 2.8).

6.9.3. MICROSCOPIC EXAMINATION

Summary Data: Table 35, Table 36

Individual Data: Table A31, Table A32

Pathology Report: Appendix H

There were no test substance-related microscopic findings. All findings observed were consistent with normal background lesions in clinically normal rats of the age and strain used on this study and were considered spontaneous and/or incidental in nature and unrelated to test substance administration.

7. CONCLUSIONS

Based on the results of this study, 6-hour nose-only inhalation exposure to benzyl alcohol at mean exposure concentrations of 41, 102, 290 and 1,072 mg/m^3 or exposure to benzoic acid at target exposure concentrations of 2.5 and 12.6 mg/m^3 for 4 weeks (minimum of 20 exposures) was well-tolerated with no effects at any exposure level. The no-observed-effect-level (NOEL) and no-observed-adverse-effect level (NOAEL) were considered to be 1,072 mg/m^3 for benzyl alcohol and 12.6 mg/m^3 for benzoic acid.

8. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jason M. Roper, PhD — 8 FEB 2010 — Date
Senior Toxicologist, Inhalation Toxicology
Study Director

Report Prepared By:

Jana L. Nazelrod, BS — 8 Feb 2010 — Date
Study Analyst

Report Reviewed By:

Daniel T. Kirkpatrick, PhD, DABT — 8 Feb 2010 — Date
Director, Inhalation Toxicology

for Charlene A. Weygandt, BS — 8-Feb-2010 — Date
Lead Analyst and Scientific Advisor,
Study Analysis and Reports

Study Personnel:

Susan C. Haley, BS	Manager, Clinical Pathology
Sally A. Keets, AS	Senior Operations Manager, Vivarium
Carol A. Kopp, BS, LAT	Manager, Gross Pathology and Developmental Toxicology Laboratory
Teresa D. Morris, BS	Senior Operations Manager, Toxicology
Theresa M. Rafeld, CPhT	Group Manager, Formulations Laboratory
Michael A. Safron, AS, HT (ASCP)^{CM}	Manager, Histology
Robert A. Wally, BS, RAC	Manager, Reporting and Regulatory Technical Services

9. QUALITY ASSURANCE UNIT STATEMENT

9.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
22-Apr-2009	Test Article Exposure	22-Apr-2009	19-May-2009	S.Power
07-May-2009	Post-Dose Observations	08-May-2009	23-Jun-2009	R.Rohr
18-May-2009	Blood Collection and Analysis	18-May-2009	23-Jun-2009	J.Dieterly
18-May-2009	Necropsy	18-May-2009	23-Jun-2009	J.Dieterly
28-May-2009	Trimming of Tissues	28-May-2009	23-Jun-2009	R.Rohr
10-Jul-2009, 14-Jul-2009	Study Records (A-1)	14-Jul-2009	20-Aug-2009	C.Heifner
22-Jul-2009, 23-Jul-2009	Study Records (I-1)	24-Jul-2009	20-Aug-2009	S.Power
23-Jul-2009	Study Records (I-2)	24-Jul-2009	20-Aug-2009	S.Power
24-Jul-2009	Study Records (I-3)	24-Jul-2009	20-Aug-2009	S.Power
24-Jul-2009, 27-Jul-2009	Study Records (C-1)	27-Jul-2009	20-Aug-2009	S.Power
27-Jul-2009	Study Records (N-1)	27-Jul-2009	20-Aug-2009	S.Power
27-Jul-2009	Study Records (N-2)	27-Jul-2009	20-Aug-2009	S.Power
27-Jul-2009, 28-Jul-2009	Study Records (H-1)	28-Jul-2009	20-Aug-2009	S.Power
28-Jul-2009	Study Records (P-1)	28-Jul-2009	20-Aug-2009	S.Power
28-Jul-2009, 29-Jul-2009	Study Records (Ex-1)	05-Aug-2009	23-Sep-2009	S.Power
29-Jul-2009, 30-Jul-2009, 03-Aug-2009, 04-Aug-2009	Study Records (Ex-2)	05-Aug-2009	23-Sep-2009	S.Power
31-Jul-2009, 03-Aug-2009, 04-Aug-2009	Study Records (Ex-3)	05-Aug-2009	23-Sep-2009	S.Power

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
05-Aug-2009	Draft Report (Pathology Appendix)	06-Aug-2009	23-Sep-2009	S.Power
06-Aug-2009	Draft Report (Inhalation Appendix)	06-Aug-2009	23-Sep-2009	S.Power
06-Aug-2009, 07-Aug-2009, 10-Aug-2009	Draft Report (without Inhalation and Pathology Appendices)	10-Aug-2009	23-Sep-2009	S.Power
10-Aug-2009	Study Records (Ex-3, Supplemental)	10-Aug-2009	23-Sep-2009	S.Power
18-Aug-2009, 19-Aug-2009	Draft Report (Analytical Chemistry)	19-Aug-2009	23-Sep-2009	C.Heifner

This study was inspected in accordance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792), the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC, and the Sponsor's protocol and protocol amendments, with the following exception. The data located in Appendix B (Certificates Of Analysis) were the responsibility of the Sponsor. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. Review of the protocol and protocol amendments (if applicable) as well as a yearly internal facility inspection are conducted by the WIL Quality Assurance Unit. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data, the retention samples, and the final report will be stored in the Archives at WIL Research Laboratories, LLC, or another location specified by the Sponsor.

9.2. APPROVAL

This study was inspected according to the criteria discussed in Section 9.1.

Report Audited By:

Heather L Johnson
for: Carrie A. Heifner, BA
Associate Compliance Specialist

8 Feb 2010
Date

Steven P. Power
Steven P. Power
Compliance Specialist

08 February 2010
Date

Report Released By:

Heather L Johnson
Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

8 Feb 2010
Date

10. REFERENCES

Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* **1964,** *6(3),* 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* **1964,** *20,* 482-491.

Kirkpatrick, D.T. An Inhalation Exposure Method Development and Validation Study of Aerosolized Benzyl Alcohol, Benzaldehyde and Benzoic Acid (Study No. WIL-703001). WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* **1952**, *47*, 583-621.

Morgan, K. T., Approaches to the identification and recording of nasal lesions in toxicology studies, *Toxicologic Pathology*, *19*(4) (Part 1) **1991**, 337-351.

National Research Council. *Guide for the Care and Use of Laboratory Animals,* Institute of Laboratory Animal Resources, Commission on Life Sciences; National Academy Press: Washington, DC, **1996**.

Snedecor, G.W.; Cochran, W.G. One-Way Classifications; Analysis of Variance. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, **1980**; pp 215-237.

11. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 2.5** states that the consulting ophthalmologist will be David A. Wilkie, DVM, MS, DACVO. Due to a scheduling conflict, the end-of-study ophthalmic examinations were conducted by Brian C. Gilger, DVM, MS, DACVO.
- **Protocol Section 7.1** states that animals will be acclimated to restraint in nose-only exposure restraint tubes for 6 hours on the fifth day of restraint acclimation. Females were kept in the restraints for up to 6 minutes outside the allowable time range on the fifth day of acclimation.
- **Protocol Sections 7.4.1 and 7.4.3** state that animals will be submitted for necropsy on the day following the twentieth exposure. Due to a 3-day stagger start in the necropsy schedule, some animals were euthanized on the days following the twenty-first or twenty-second exposures.
- **Protocol Section 7.5** states that animal exposure airflow rate, temperature, and humidity for all benzyl alcohol exposure systems will be documented at approximately 60-minute intervals. On 30 April 2009, the above-mentioned parameters were not documented between 2 hours, 30 minutes and 4 hours, 30 minutes into exposure.
- **Protocol Section 7.5** states that for each day's exposure, animals will be held in restraint tubes for a minimum of 25-30 minutes prior to the initiation of exposure. On 22 May 2009, animals in the benzyl alcohol-treated groups were acclimated for only 20 minutes prior to the initiation of exposure.
- **Protocol Section 7.5** states that the average relative humidity of the exposure atmosphere will be 30% to 70%. For Exposure Systems 2 and 3, relative humidities were as low as 28% and 23%, respectively. For Exposure System 5, the relative humidity was as high as 91%.
- **Protocol Section 7.7.2** states that impinger samples will be collected with the impinger placed in an ice bath. During method development, it was determined that there was no significant benefit to sampling on ice.
- **Protocol Section 7.7.2** states that samples for the test substance exposure systems will be collected at approximately 90-minute intervals. For the benzyl alcohol exposure systems, additional samples were collected between the scheduled samples as needed and ranged from a minimum of 57 minutes to a maximum of 136 minutes.

- **Protocol Section 7.7.3** states that aerosol particle size determination will be conducted once per week for all benzyl alcohol groups. Particle size was only determined for the 1000 mg/m^3 exposure atmosphere. During a previous method development and validation study (Kirkpatrick, Draft), a calibrated HPM-1000 was used as an aerosol monitoring device and detected no aerosol in the 30 or 100 mg/m^3 exposure atmospheres. Aerosol particles were detected in the 300 mg/m^3 exposure system at a concentration of ≤ 79 mg/m^3. Due to the volatility of benzyl alcohol, and the sampling time required to obtain a measureable amount of test substance on the impactor substrates, it was not possible to measure the particle size of the 300 mg/m^3 benzyl alcohol exposure atmosphere.

- **Protocol Section 8.3** states that a clinical observation will be conducted prior to exposure and 0 to 1 hour following exposure. The following animals did not receive an observation at the appropriate interval:

Animal Number	Sex	Group	Study Day	Clinical Observation Not Received
8303	Female	3	1	Prior to exposure
8232	Male	5	3	Prior to exposure
8279	Female	7	3	Prior to exposure
8301	Female	6	4	Prior to exposure
8249	Male	5	4	0 to 1 hour post-exposure
8282	Female	4	9	0 to 1 hour post-exposure
8246	Male	3	16	0 to 1 hour post-exposure

- **Protocol Section 8.11.2** states that lungs will be weighed. At the time of necropsy on 18 May 2009, the lung weight for female no. 8292 (Group 5) was not recorded.

- **Protocol Section 8.11.3** states that microscopic examination will be performed on tissues for all animals that die spontaneously. Female no. 8307 (Group 2) was replaced on study day 1 after an apparent accidental death. The tissues for this animal were not processed to slide or examined microscopically. Necropsy data will be maintained in the study records, but not presented in the final report.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: May 19, 2010

SUBJECT: Concentration of Use Benzyl Alcohol, Benzoic Acid, Sodium Benzoate and Potential Additions

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use
Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, Calcium Benzoate*, Magnesium Benzoate*, Potassium Benzoate*, Benzyl Benzoate*¶

Ingredient	Product Category	Concentration of Use
Benzyl Alcohol	Baby shampoos	0.3%
Benzyl Alcohol	Baby lotions, oils, powders and creams	0.3%
Benzyl Alcohol	Other baby products	0.004%
Benzyl Alcohol	Bath oils, tablets and salts	0.009-0.9%
Benzyl Alcohol	Bubble baths	0.001-0.3%
Benzyl Alcohol	Eyebrow pencil	0.00002-0.2%
Benzyl Alcohol	Eyeliner	0.000008-0.8%
Benzyl Alcohol	Eye shadow	0.00003-0.4%
Benzyl Alcohol	Eye lotion	0.3-0.6%
Benzyl Alcohol	Eye makeup remover	0.000009-0.5%
Benzyl Alcohol	Mascara	0.00003-0.7%
Benzyl Alcohol	Colognes and toilet waters	0.4-2%
Benzyl Alcohol	Perfumes	0.04-3%
Benzyl Alcohol	Powders (dusting and talcum)	0.04-0.05%
Benzyl Alcohol	Sachets	2%
Benzyl Alcohol	Other fragrance preparations	0.02-0.9%
Benzyl Alcohol	Hair conditioners	0.002-1%
Benzyl Alcohol	Hair sprays (aerosol fixatives)	0.0003%
Benzyl Alcohol	Permanent waves	0.0002%
Benzyl Alcohol	Rinses (noncoloring)	3%
Benzyl Alcohol	Shampoos (noncoloring)	0.006-1%
Benzyl Alcohol	Tonics, dressings and other hair grooming aids	0.0008-1%
Benzyl Alcohol	Other hair preparations (noncoloring)	0.005-2%
Benzyl Alcohol	Hair dyes and colors (all types requiring caution statement and patch testing)	2-10%
Benzyl Alcohol	Hair tints	0.0004%
Benzyl Alcohol	Hair bleaches	0.00007-4%
Benzyl Alcohol	Other hair coloring preparations	10%
Benzyl Alcohol	Blushers (all types)	0.00005-0.0005%

Benzyl Alcohol	Face powders	0.0001-0.02%
Benzyl Alcohol	Foundations	0.0006-1%
Benzyl Alcohol	Leg and body paints	0.05-0.4%
Benzyl Alcohol	Lipstick	0.02-0.9%
Benzyl Alcohol	Makeup bases	0.0002%
Benzyl Alcohol	Other makeup preparations	0.00002%
Benzyl Alcohol	Basecoats and undercoats (manicuring preparations)	0.00002%
Benzyl Alcohol	Cuticle softeners	0.2%
Benzyl Alcohol	Nail creams and lotions	0.00006-0.09%
Benzyl Alcohol	Nail polish and enamel	0.002-0.5%
Benzyl Alcohol	Nail polish and enamel removers	0.0009-0.3%
Benzyl Alcohol	Dentifrices (aerosol, liquid, pastes and powders)	0.00002-0.2%
Benzyl Alcohol	Mouthwashes and breath fresheners (liquids and sprays)	0.000006%
Benzyl Alcohol	Bath soaps and detergents	0.005-0.9%
Benzyl Alcohol	Deodorants (underarm)	0.03-0.5%
Benzyl Alcohol	Feminine hygiene deodorants	1%
Benzyl Alcohol	Other personal cleanliness products	0.009-0.09%
Benzyl Alcohol	Aftershave lotions	0.0002-0.5%
Benzyl Alcohol	Preshave lotions (all types)	0.02%
Benzyl Alcohol	Shaving cream (aerosol, brushless and lather)	0.0001-0.02%
Benzyl Alcohol	Shaving soaps (cakes, sticks, etc.)	0.04%
Benzyl Alcohol	Other shaving preparations	0.00005-0.4%
Benzyl Alcohol	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00003-1%
Benzyl Alcohol	Depilatories	0.003-0.3%
Benzyl Alcohol	Face and neck creams, lotions and powders	0.04-1%
Benzyl Alcohol	Body and hand creams, lotions and powders	0.02-0.7%
Benzyl Alcohol	Moisturizing creams, lotions and powders	0.0008-1%
Benzyl Alcohol	Night creams, lotions and powders	0.06-0.6%
Benzyl Alcohol	Paste masks (mud packs)	0.0002-0.7%
Benzyl Alcohol	Skin fresheners	0.5%
Benzyl Alcohol	Other skin care preparations	0.003-0.009%
Benzyl Alcohol	Suntan gels, creams and liquids	0.0005%

Benzyl Alcohol	Indoor tanning preparations	0.0002%
Benzyl Alcohol	Other suntan preparations	0.0009%
Benzoic Acid	Baby lotions, oils powders and creams	0.000002%
Benzoic Acid	Bath oils, tablets and salts	0.000003-0.08%
Benzoic Acid	Eyebrow pencil	0.1%
Benzoic Acid	Eyeliner	0.00005-0.03%
Benzoic Acid	Eye shadow	0.2%
Benzoic Acid	Eye lotion	0.005-0.2%
Benzoic Acid	Eye makeup remover	0.2%
Benzoic Acid	Mascara	0.002%
Benzoic Acid	Colognes and toilet waters	0.000003%
Benzoic Acid	Perfumes	0.000003-0.05%
Benzoic Acid	Powders (dusting and talcum)	0.001%
Benzoic Acid	Hair conditioners	0.00002-1%
Benzoic Acid	Hair sprays (aerosol fixatives)	0.02-0.08%
Benzoic Acid	Rinses (noncoloring)	0.00005%
Benzoic Acid	Shampoos (noncoloring)	0.00002-0.5%
Benzoic Acid	Tonics, dressings and other hair grooming aids	0.0004-0.3%
Benzoic Acid	Hair dyes and colors (all types requiring caution statement and patch testing)	0.004%
Benzoic Acid	Hair lighteners with color	0.03%
Benzoic Acid	Other hair coloring preparations	0.01%
Benzoic Acid	Face powders	0.000005%
Benzoic Acid	Foundations	0.00005-0.2%
Benzoic Acid	Lipstick	0.003-0.3%
Benzoic Acid	Makeup bases	0.6%
Benzoic Acid	Cuticle softeners	0.2%
Benzoic Acid	Nail creams and lotions	0.0005%
Benzoic Acid	Mouthwashes and breath fresheners (liquids and sprays)	0.005-2%
Benzoic Acid	Bath soaps and detergents	0.000005-0.5%
Benzoic Acid	Deodorants (underarm)	0.03-0.5%
Benzoic Acid	Other personal cleanliness products	0.000003-0.2%

Benzoic Acid	Aftershave lotions	0.00005-0.1%
Benzoic Acid	Shaving cream (aerosol, brushless and lather)	0.06-0.2%
Benzoic Acid	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000005-5%
Benzoic Acid	Depilatories	0.008-0.09%
Benzoic Acid	Face and neck creams, lotions and powders	0.0003-0.2%
Benzoic Acid	Body and hand creams, lotions and powders	0.0006-1%
Benzoic Acid	Moisturizing creams, lotions and powders	0.00005-0.2%
Benzoic Acid	Night creams, lotions and powders	0.07-0.3%
Benzoic Acid	Paste masks (mud packs)	0.0002-0.2%
Benzoic Acid	Skin fresheners	0.2%
Benzoic Acid	Other skin care preparations	0.0006-0.2%
Benzoic Acid	Suntan gels, creams and liquids	0.00005%
Benzoic Acid	Other suntan preparations	0.2%
Sodium Benzoate	Baby shampoos	0.3%
Sodium Benzoate	Baby lotions, oils, powders and creams	0.3%
Sodium Benzoate	Other baby products	0.5-0.9%
Sodium Benzoate	Bath oils, tablets and salts	0.3-1%
Sodium Benzoate	Bubble baths	0.004-0.3%
Sodium Benzoate	Eyeliner	0.0001-0.3%
Sodium Benzoate	Eye shadow	0.1-0.2%
Sodium Benzoate	Eye lotion	0.02-0.3%
Sodium Benzoate	Eye makeup remover	0.2%
Sodium Benzoate	Mascara	0.00001%
Sodium Benzoate	Other eye makeup preparations	0.4%
Sodium Benzoate	Perfumes	0.0001-0.05%
Sodium Benzoate	Hair conditioners	0.000005-1%
Sodium Benzoate	Hair sprays (aerosol fixatives)	0.3%
Sodium Benzoate	Permanent waves	0.1%
Sodium Benzoate	Rinses (noncoloring)	0.3-1%
Sodium Benzoate	Shampoos (noncoloring)	0.00001-0.6%
Sodium Benzoate	Shampoos (noncoloring)	0.1-0.5%
Sodium Benzoate	Other hair preparations (noncoloring)	0.003%

Sodium Benzoate	Hair dyes and colors (all types requiring caution statement and patch testing)	0.008-0.5%
Sodium Benzoate	Hair bleaches	0.4%
Sodium Benzoate	Other hair coloring preparations	0.2%
Sodium Benzoate	Blushers (all types)	0.02%
Sodium Benzoate	Face powders	0.3%
Sodium Benzoate	Foundations	0.2%
Sodium Benzoate	Leg and body paints	0.2-0.5%
Sodium Benzoate	Lipstick	0.002%
Sodium Benzoate	Makeup bases	0.0002%
Sodium Benzoate	Cuticle softeners	0.001%
Sodium Benzoate	Nail polish and enamel	0.00001%
Sodium Benzoate	Dentifrices (aerosol, liquid, pastes and powders)	0.003-0.6%
Sodium Benzoate	Mouthwashes and breath fresheners (liquids and sprays)	0.3-0.6%
Sodium Benzoate	Other oral hygiene products	0.001%
Sodium Benzoate	Bath soaps and detergents	0.0004-0.5%
Sodium Benzoate	Deodorants (underarm)	0.5%
Sodium Benzoate	Other personal cleanliness products	0.005-0.6%
Sodium Benzoate	Aftershave lotions	0.001-0.002%
Sodium Benzoate	Shaving creams (aerosol, brushless and lather)	0.1-0.3%
Sodium Benzoate	Other shaving preparations	0.3%
Sodium Benzoate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000001-0.6%
Sodium Benzoate	Depilatories	0.00005-0.2%
Sodium Benzoate	Face and neck creams, lotions and powders	0.002-0.4%
Sodium Benzoate	Face and neck sprays	0.5%
Sodium Benzoate	Body and hand creams, lotions and powders	0.0001-1%
Sodium Benzoate	Moisturizing creams, lotions and powders	0.0004-0.3%
Sodium Benzoate	Night creams, lotions and powders	0.009-0.3%
Sodium Benzoate	Paste masks (mud packs)	0.0004-0.5%
Sodium Benzoate	Other skin care preparations	0.003-0.2%
Sodium Benzoate	Suntan gels, creams and liquids	0.0002%
Sodium Benzoate	Other suntan preparations	0.003%

Calcium Benzoate	Face powders	0.002%
Calcium Benzoate	Foundations	0.003%
Calcium Benzoate	Suntan gels, creams and liquids	0.004%
Potassium Benzoate	Cuticle softeners	0.0003%
Potassium Benzoate	Bath soaps and detergents	0.0002%
Benzyl Benzoate	Bath oils, tablets and salts	0.007-2%
Benzyl Benzoate	Bubble baths	0.0005%
Benzyl Benzoate	Eye lotion	0.0006-0.002%
Benzyl Benzoate	Perfumes	0.5-4%
Benzyl Benzoate	Powders (dusting and talcum)	0.3%
Benzyl Benzoate	Other fragrance preparations	0.002%
Benzyl Benzoate	Hair conditioners	0.009-2%
Benzyl Benzoate	Hair sprays (aerosol fixatives)	0.006%
Benzyl Benzoate	Shampoos (noncoloring)	0.3-2%
Benzyl Benzoate	Tonics, dressings and other hair grooming aids	0.05-4%
Benzyl Benzoate	Other hair preparations (noncoloring)	0.01%
Benzyl Benzoate	Hair dyes and colors (all types requiring caution statement and patch testing)	0.5-2%
Benzyl Benzoate	Other hair coloring preparations	0.07%
Benzyl Benzoate	Face powders	0.001-0.02%
Benzyl Benzoate	Foundations	0.0001-0.005%
Benzyl Benzoate	Leg and body paints	0.002%
Benzyl Benzoate	Lipstick	0.000005-0.2%
Benzyl Benzoate	Nail creams and lotions	0.2%
Benzyl Benzoate	Nail polish and enamel	0.8%
Benzyl Benzoate	Dentifrices (aerosol, liquid, pastes and powders)	0.0002%
Benzyl Benzoate	Bath soaps and detergents	0.006-0.4%
Benzyl Benzoate	Deodorants (underarm)	0.006-0.6%
Benzyl Benzoate	Other personal cleanliness products	0.0014-0.2%
Benzyl Benzoate	Aftershave lotions	0.001-0.4%
Benzyl Benzoate	Preshave lotions (all types)	0.0008%
Benzyl Benzoate	Shaving cream (aerosol, brushless and lather)	0.0001-0.4%

Benzyl Benzoate	Shaving soaps (cakes, sticks, etc.)	0.02%
Benzyl Benzoate	Other shaving preparations	0.05%
Benzyl Benzoate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.009-0.02%
Benzyl Benzoate	Depilatories	0.007%
Benzyl Benzoate	Face and neck creams, lotions and powders	0.009-0.6%
Benzyl Benzoate	Body and hand creams, lotions and powders	0.006-0.6%
Benzyl Benzoate	Body and hand sprays	0.007%
Benzyl Benzoate	Night creams, lotions and powders	0.004-0.007%
Benzyl Benzoate	Other skin care preparations	0.02%
Benzyl Benzoate	Indoor tanning preparations	0.004%
Benzyl Benzoate	Other suntan preparations	0.008%

*Under consideration for addition to the report on Benzyl Alcohol, Benzoic Acid and Sodium Benzoate.
¶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 2010
Table prepared May 19, 2010

Memorandum

To: CIR Expert Panel Members and Liaisons

From: Monice M. Fiume *MMF*
Scientific Analyst/Writer

Date: June 28, 2010

Subject: Re-review Summaries

The following 2 re-review summaries from the April meeting have been prepared for your approval:

1. Polyquaternium-7
2. Quaternium-22

SNF had stated that they were preparing a data package for submission to CIR that would include use data on Polyquaternium-7 and test and safety data on its monomers. To date, these data have not been received.

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Polyquaternium-7

CONCLUSION: In 1995, the Cosmetic Ingredient Review (CIR) Expert Panel stated that polyquaternium-7 was safe as used in cosmetic formulations.[1] The Expert Panel reviewed information available since that assessment,[2,3] along with updated frequency and concentration of use information. The Expert Panel determined to not initiate a rereview of the safety of polyquaternium-7 and confirmed the existing conclusion.

DISCUSSION: The use of polyquaternium-7 in cosmetic formulations has increased greatly, from 138 reported uses in 1994[1] to 975 uses in 2010.[4] Concentration of use was not reported to the Food and Drug Administration (FDA) in 1994, nor is it reported to FDA currently. In response to a survey conducted by the Personal Care Products Council, industry reported current use concentrations of 0.009-5% for polyquaternium-7[5] (Table 1).

The Panel noted that polyquaternium-7 is now used in aerosolized products, and noted the absence of inhalation toxicity data. However, in the absence of these data, the Panel determined that polyquaternium-7 can be used safely in hair sprays, because the product particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (around 38 μm) and pump hair sprays (>80 μm) is large compared to respirable particle sizes (≤10 μm). Polyquaternium-7 is now also used in leave-on type products and products that are applied to the eye. The Panel was satisfied that data in the report supported the safety of these uses.

In the original safety assessment, the Expert Panel acknowledged the presence of acrylamide as an impurity in polyquaternium-7. An extrapolation using the current use concentration and the greatest amount of acrylamide impurity given in the original report confirmed that the amount of residual acrylamide was not of concern. The Expert Panel confirmed that polyquaternium-7 is safe as used in cosmetic formulations.

1. Andersen FA (ed.). Final report on the safety assessment of Polyquaternium-7. *J Am Coll Toxicol*. 1995;14:(6):476-484.

2. Food and Drug Administration. 21 CFR 176.170. Diallyldimethylammonium chloride with acrylamide (CAS Reg. No. 26590-05-6). http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?FR=176.170&CFRPart=&FRSearch=. Website accessed Nov. 22, 2009.:

3. Gallo, R., Basso, M., Voltolini, S., and Guarrera, M. Allergic contact dermatitis from laureth-9 and polyquaternium-7 in a skin-care product. *Contact Dermatitis*. 2001;45:(6):356-357.

4. Food and Drug Administration (FDA). Frequency of use data for Polyquaternium-7. FDA Database. Updated Feb 18. *Received Mar 2010 in response to an FOIA request.* 2010.

5. Personal Care Products Council (Council). Concentration of use of Polyquaternium-7. Industry survey. 2010. Unpublished data received from Council. (Feb 25.) 1 p.:

6. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2009. Washington, DC: FDA.

Table 1. **Current and historical uses of polyquaternium-7.**

Product	Frequency of Use 1994 (# in category)[1]	Frequency of Use 2010[4] (# in category-2009)[6]	Conc. Of Use 1994[1]	Conc. Of Use (%) 2010[5]
Baby Shampoos	2 (19)	7 (56)	NA	NR
Other Baby Products	1 (23)	8 (143)	NA	0.04
Bath Oils, Tablets, and Salts	NR	NR (313)	NR	0.009
Bubble Baths	2 (214)	15 (169)	NA	0.05-0.4
Other Bath Preparations	4 (132)	14 (234)	NA	NR
Eye Shadow	NR	2 (1215)	NR	NR
Mascara	NR	6 (499)	NR	NR
Other Fragrance Preparation	2 (136)	1 (566)	NA	NR
Hair Conditioner	16 (614)	31 (1226)	NA	0.01-0.3
Hair Spray (aerosol fixatives)	NR	10 (312)	NR	NR
Permanent Waves	4 (387)	1 (69)	NA	0.07-5
Rinses (non-coloring)	1 (58)	2 (33)	NA	0.2
Shampoos (non-coloring)	37 (852)	234 (1361)	NA	0.04-1
Tonics, Dressings, and Other Hair Grooming Aids	19 (563)	34 (1205)	NA	0.2-3
Other Hair Preparations	3 (376)	21 (807)	NA	0.2-3
Hair Dyes and Colors (req. caution stmts)	NR	16 (2393)	NR	0.04
Hair Shampoos (coloring)	3 (15)	1 (40)	NA	NR
Hair Color Sprays (aerosol)	NR	NR (7)	NR	0.02
Bath Soaps and Detergents	26 (343)	292 (1665)	NA	0.09-.3
Other Personal Cleanliness Products	3 (321)	198 (792)	NA	0.08-0.2
Aftershave Lotion	1 (229)	3 (367)	NA	0.2
Shaving Cream	4 (147)	2 (122)	NA	0.09
Shaving Soap	NR	1 (10)	NR	NR
Cleansing	8 (746)	51 (1446)	NA	0.02-1
Face and Neck (excl. shave)	NR	3 (1583)	NR	0.06-0.08
Body and Hand (excl. shave)	NR	9(1744)	NR	0.3
Moisturizing	NR	3 (2508)	NR	NR
Skin Fresheners	NR	1 (259)	NR	NR
Other Skin Care Preps	1 (790)	8 (1308)	NA	0.4
Other Suntan Preparations	1 (61)	1 (62)	NA	NR
TOTAL	**138**	**975**	**NA**	**0.009-5**

NR-not reported as used in that category
NA – concentration of use data not reported at that time

Quaternium-22

CONCLUSION: In 1995, the Cosmetic Ingredient Review (CIR) Expert Panel stated that quaternium-22 is safe in the present practices of use.[1] The Expert Panel reviewed information available since that assessment[2,3] along with updated frequency and concentration of use information. The Expert Panel determined to not initiate a rereview of the safety of quaternium-22 and confirmed the existing conclusion.

DISCUSSION: Quaternium-22 is reported to function as an antistatic agent, film former, and hair conditioning agent.[4] Reported use has decreased from 80 reported uses in 1994[1] to 58 uses in 2010,[5] but the types of use have generally remained the same. Concentration of use was not reported to the Food and Drug Administration (FDA) in 1994, nor is it reported to FDA currently. In response to a survey conducted by the Personal Care Products Council, industry reported current use concentrations of 0.06-2% for quaternium-22[6] (Table 1).

The Panel noted that uaternium-22 is now used in aerosolized products, and noted the absence of inhalation toxicity data. However, in the absence of these data, the Panel determined that quaternium-22 can be used safely in hair sprays, because the product particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (around 38 μm) and pump hair sprays (>80 μm) is large compared to respirable particle sizes (≤10 μm)

1. Andersen FA (ed.). Final report on the safety assessment of Quaternium-22. *J Am Coll Toxicol*. 1995;14 (6):485-497.

2. Le Coz, C. J., Leclere, J. M., Arnoult, E., Raison-Peyron, N., Pons-Guiraud, A., Vigan, M., and Members of Revidal-Gerda. Allergic contact dermatitis from shellac in mascara. *Contact Dermatitis*. 2002;46:(3):149-152.

3. Scheman, A. J. Contact allergy to quaternium-22 and shellac in mascara. *Contact Dermatitis*. 1998;38:(6):342-343.

4. Gottschalck TE and Bailey JE. International Cosmetic Ingredient Dictionary and Handbook. 13 *ed*. Washington, DC: Personal Care Products Council, 2010.

5. Food and Drug Administration (FDA). Frequency of use data for Quaternium-22. *FDA Database.* 2010. Feb 18:

6. Personal Care Products Council (Council). Concentration of use of Quaternium-22. Industry survey. 2010. Unpublished data received from Council. (Feb 25.) 1 p.:

7. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2009. Washington, DC: FDA.

Table 1. **Historical and current concentration of use data for quaternium-22.**

Product	**Frequency of Use – 1994 (# in category)[1]**	**Frequency of Use – 2010[5] (# in category)[7]**	**Conc. Of Use (1994)[1]**	**Conc. Of Use (%) (2010)[6]**
Bubble Baths	2 (208)	NR (169)	NA	0.5
Other Bath Preparations	3 (111)	NR (234)	NA	NR
Eyeliner	NR	1 (754)	NR	NR
Mascara	34 (178)	17 (499)	NA	0.06-1
Hair Conditioner	4 (597)	5 (1226)	NA	1
Hair Spray (aerosol fixatives)	1 (294)	1 (312)	NA	NR
Hair Straighteners	NR	NR (178)	NR	0.3
Permanent Waves	NR	NR (69)	NR	0.2
Shampoos (non-coloring)	17 (845)	7 (1361)	NA	0.6
Tonics, Dressings, and Other Hair Grooming Aids	2 (494)	3 (1205)	NA	0.06-0.7
Other Hair Preparations	2 (356)	3 (807)	NA	0.5
Hair Dyes and Colors (req. caution stmts)	NR	NR (1458)	NR	2 (1% after dilution)
Hair Bleaches	NR	NR (149)	NR	2
Bath Soaps and Detergents	5 (335)	7 (1665)	NA	0.09-2
Other Personal Cleanliness Products	1 (296)	NR (792)	NA	NR
Cleansing Skin Care Preparations	1 (702)	5 (1446)	NA	0.2
Face and Neck Creams, Lotions, and Powders	NR	NR (1583)	NR	0.6
Moisturizing	1 (806)	7 (2508)	NA	NR
Paste Masks (mud packs)	NR	1 (441)	NR	NR
Other Skin Care Preparations	1 (745)	1 (1308)	NA	0.06
No. of uses listed under trade name	6	NR	NA	NR
TOTAL	**80**	**58**	**NA**	**0.06-2**

NR – not reported as used in that category
NA – concentration of use data not reported at that time