Final Report of the Safety Assessment of Niacinamide and Niacin[1]

Niacinamide (aka nicotinamide) and Niacin (aka nicotinic acid) are heterocyclic aromatic compounds which function in cosmetics primarily as hair and skin conditioning agents. Niacinamide is used in around 30 cosmetic formulations including shampoos, hair tonics, skin moisturizers, and cleansing formulations. Niacin is used in a few similar product types. The concentration of use of Niacinamide varies from a low of 0.0001% in night preparations to a high of 3% in body and hand creams, lotions, powders and sprays. Niacin concentrations of use range from 0.01% in body and hand creams, lotions, powders and sprays to 0.1% in paste masks (mud packs). Both ingredients are accepted for use in cosmetics in Japan and the European Union. Both are GRAS direct food additives and nutrient and/or dietary supplements. Niacinamide may be used in clinical treatment of hypercholesteremia and Niacin in prevention of pellegra and treatment of certain psychological disorders. Both ingredients are readily absorbed from skin, blood, and the intestines and widely distribute throughout the body. Metabolites include N^1-methylnicotinamide and N^1-methyl-4-pyridone-3-carboxamide. Excretion is primarily through the urinary tract. While Niacinamide is more toxic than Niacin in acute toxicity studies, both are relatively non-toxic. Short-term oral, parenteral, or dermal toxicity studies did not identify significant irreversible effects. Niacinamide, evaluated in an in vitro test to predict ocular irritation, was not an acute ocular hazard. Animal testing of Niacinamide in rabbits in actual formulations produced mostly non-irritant reactions, with only some marginally irritating responses. Skin irritation tests of up to 2.5% Niacinamide in rabbits produced only marginal irritation. Skin sensitization tests of Niacinamide at 5% during induction and 20% during challenge were negative in guinea pigs. Neither cosmetic ingredient was mutagenic in Ames tests, with or without metabolic activation. Niacinamide and Niacin at 2 mg/ml were negative in a chromosome aberration test in Chinese hamster ovary cells, but did produce large structural chromosome aberrations at 3 mg/ml. Niacinamide induced sister chromatid exchanges in Chinese hamster ovary cells, but Niacin did not. Under certain circumstances, Niacinamide can cause an increase in unscheduled DNA synthesis in human lymphocytes treated with UV or a nitrosoguanidine compound. Niacinamide itself was not carcinogenic when administered (1%) in the drinking water of mice. No data on the carcinogenic effect of Niacin were available. Niacinamide can moderate the induction of tumors by established carcinogens. Niacinamide in combination with streptozotocin (a nitrosourea compound) or with heliotrine (a pyrrolizidine alkaloid), produced pancreatic islet tumors. On the other hand, Niacinamide reduced the renal adenomas produced by streptozotocin; and intestinal and bladder tumors induced by a preparation of bracken fern. Niacinamide evaluated in in vitro test systems did affect development, but Niacinamide reduced the reproductive/developmental toxicity of 2-aminonicotinamide-amino-1,3,4-thiadiazole hydrochloride and urethane. Clinical testing of Niacinamide produced no stinging sensation at concentrations up to 10%, use tests produced no irritation at concentrations up to 5%, and a 21-day cumulative irritation test at concentrations up to 5% resulted in no irritancy. Niacinamide was not a sensitizer, nor was it a photosensitizer. The CIR Expert Panel considered that Niacinamide and Niacin are sufficiently similar from a toxicologic standpoint to combine the available data and reach a conclusion on the safety of both as cosmetic ingredients. Overall, these ingredients are non-toxic at levels considerably higher than would be experienced in cosmetic products. Clinical testing confirms that these ingredients are not significant skin irritants, sensitizers or photosensitizers. While certain formulations were marginal to slight ocular irritants, other formulations were not. Niacinamide, while not carcinogenic alone, can modulate the induction of tumors by certain established carcinogens. The Panel noted that the doses in these studies are high relative to the low concentrations at which Niacinamide is used in cosmetic formulations. In neither case (tumor protection or tumor promotion) are these findings considered relevant to the use of Niacinamide at its current low concentrations of use in cosmetics. Both ingredients were considered safe as used in cosmetics.

INTRODUCTION

This report assesses the safety of Niacinamide (CAS No. 98-92-0) and Niacin (CAS No. 59-67-6) as cosmetic ingredients. These cosmetic ingredients function primarily as hair and skin-conditioning agents. Niacinamide and Niacin are the terms used in the *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger et al. 2000). Another common term for Niacinamide is Nicotinamide and Niacin is often referred to as Nicotinic Acid. The terms Nicotinic Acid and Nicotinamide are recommended by the International Institute of Nutrition Sciences and by the American Institute of Nutrition. These terms are used by the Federation of American Societies for Experimental Biology (FASEB) in their evaluation of the health aspects of Niacin and Niacinamide (FASEB 1979). Throughout this report, the terms Niacinamide and Niacin will be used.

CHEMISTRY

Description

Niacinamide is the heterocyclic aromatic amide that conforms to the structure shown in Figure 1a, and Niacin, a
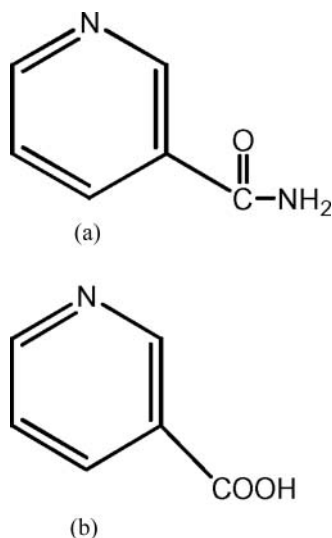

FIGURE 1
Chemical structures for Niacinamide and Niacin (Wenninger et al. 2000). (a) Niacinamide and (b) Niacin.

heterocyclic aromatic, conforms to the structure shown in Figure 1b (Wenninger et al. 2000). Synonyms include nicotinamide, nicotinic acid amide, vitamin PP, vitamin B$_3$, 3-pyridinecarboxylic acid amide, benicot, aminicotin, ui-nicotyl, dipegyl, nicamindon, nicotilamide, pelonin amide, amide PP, and nicofort (Informatics 1974).

Niacinamide is part of nicotinamide adenine dinucleotide (NAD) and functions as a component of coenzyme NAD. Most animals cannot manufacture this compound in amounts sufficient to prevent nutritional deficiency and it therefore must be supplemented through dietary intake (National Library of Medicine 2000).

Niacin is a water-soluble vitamin of the B complex occurring in various animal and plant tissues. Synonyms include nicotinic acid, nicacid, nicangin, niconacid, nicotinipca, nicyl, P.P. factor, pellagra preventive vitamin, anti-pellagra vitamin, pyridine-3-carboxylic acid, pyridine-B-carboxylic acid, 3-pyridinecarboxylic acid, akotin, nicobid, nico-400, and nicotinex (Informatics 1974).

Niacin is required by the human body for the formation of coenzymes NAD and NADP (nicotinamide adenine dinucleotide phosphate) and has pellagra-curative, vasodilating, and antilipemic properties (National Library of Medicine 2000). The compound pyridine 3-carboxylic acid, also known as Niacin or vitamin PP, is designated as Nicotinic Acid (Darby et al. 1975).

Chemical and Physical Properties

The chemical properties and synonyms of these water-soluble cosmetic ingredients are given in Table 1 (Niacinamide) and Table 2 (Niacin).

Impurities

Niacinamide—The official monograph of Niacinamide in the United States Pharmacopeia (USP) (Committee of Revision of the USP, 1995) states that pharmaceutical preparations should contain not less than 98.5% and not more than 101.5% of $C_6H_5NO_2$, calculated on the dried basis, and limits heavy metals to not more than 0.003%.

Niacin—The official monograph of Niacin in the USP (Committee of Revision of the USP 1995) states that pharmaceutical preparations should contain not less than 99% and not more than 101% of $C_6H_6N_2O$, calculated on a dried basis. A limitation of heavy metals is not more than 0.002%, chloride not more than 0.02% and sulfate not more than 0.02%.

Analytical Methods

Niacinamide and Niacin have been analyzed by infrared and ultraviolet spectroscopy (Committee of Revision of the USP 1995). Gas-liquid chromatography was utilized after converting Niacin and Niacinamide to ethyl nicotinate and *N*-ethylnicotinamide (Prosser and Sheppard 1968).

Ultraviolet Absorption

The absorption spectrum of Niacinamide was measured using a Unicam SP.800 spectrophotometer with a standard 1 cm path length. Niacinamide solutions at 0.001%, 0.01%, and 0.1% were prepared in ethanol. Absorbance values were reported at the 262 nm peak (using the 0.001% concentration data) and at

TABLE 1
Chemical and Physical Properties of Niacinamide

Chemical description	Colorless needles or white crystalline powder; odorless with a bitter taste	Lewis 1993
Molecular weight	122.14	Lewis 1993
Octanol/water partition coefficient	log −0.37	Unilever 1998a
Empirical formula	$C_6H_6N_2O$	Informatics 1974
Density	1.40	Lewis 1993
Solubility	Soluble in water, ether, and glycerin	Lewis 1993
Melting point	129°C	Lewis 1993
Boiling range	150–160°	National Toxicology Program (NTP) 2000

TABLE 2
Chemical and Physical Properties of Niacin

Chemical description	Colorless needles or white crystalline powder with a slight odor	Lewis 1993
Molecular weight	123.12	Lewis 1993
Octanol/water partition coefficient	0.63	Bronaugh and Stewart 1985
Water solubility	16.7 g/L	Bronaugh and Stewart 1985
Octanol solubility	10.5 g/L	Bronaugh and Stewart 1985
Empirical formula	$C_6H_5NO_2$	Informatics 1974
Density	1.473	Lewis 1993
Solubility	Soluble in water and alcohol; insoluble in most lipid solvents	Lewis 1993
Melting point	236°C	Lewis 1993
Boiling range	Sublimes	NTP 2000

two positions (300 and 320 nm) on the edge of the peak (using the 0.01% concentration data). The 0.1% solution did not yield useful data. The samples tested did not absorb in the visible region. The absorption coefficient at each wavelength was extrapolated to a 1% concentration to yield the following: 350 at 262 nm, 9 at 300 nm, and 1 at 320 nm. The authors concluded that there was negligible absorption of solar ultraviolet radiation and, therefore, that the photochemical reactions in sunlight were unlikely to be significant (Unilever 1998e, 2001).

USE

Cosmetic

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, Niacinamide and Niacin both function as hair conditioning agents and skin conditioning agents - miscellaneous in cosmetics (Wenninger et al. 2000).

The Food and Drug Adminstration (FDA) provided frequency of use data for Niacinamide and Niacin as a function of product category (FDA 2001) and the industry has reported the current concentration at which these ingredients are used, also as a function of product category (CTFA 2001). These data are presented in Table 3.

The first line of Table 3 shows that, of the 84 eye makeup remover products reported to FDA to be on the market, two contain Niacinamide, but that a current concentration of use is not available. Line two indicates that, of the 636 eye makeup remover products reported to FDA to be on the market, only two were reported to contain Niacinamide, and that the range of current use concentrations is between 0.05 and 0.1%. Further down the table, it can be seen that there are 827 face and neck creams, lotions, powders and sprays reported to FDA as being on the market, but none were reported to FDA as containing Niacinamide, yet direct information from industry indicates Niacinamide is used in this category at concentrations between 0.001 and 0.5%.

The current concentration of use data may be compared to historical data in which Niacinamide was reported to be used at a maximum concentration range in wrinkle smoothing products of between 1.0 and 5.0%. Most concentrations were less than 0.1% (FDA 1984). Historical data for Niacin show two bath preparations in the 5.0–10.0% range and one skin care preparation in the 0.1 to 1.0% range (FDA 1984).

The use of neither Niacinamide nor Niacin is restricted in Japan (Japan Ministry of Health, Labor, and Welfare 2000). Neither ingredient is restricted in any way under the rules governing cosmetic products in the European Union (European Commission 2002).

Non-Cosmetic

Niacinamide is listed in the Code of Federal Regulations (CFR) as a GRAS direct food additive (21 CFR 184.1535) and as a GRAS nutrient and/or dietary supplement (21 CFR 182.5535).

Niacin is listed as a GRAS direct food additive (21 CFR 184.1535) and as a GRAS nutrient and/or dietary supplement (21 CFR 182.5535) in the Code of Federal Regulations. Niacin is also used as a food additive in frozen desserts, cereal flours and related products, and macaroni and noodle products (21 CFR 135.115, 137.21, and 139.21). The current recommended daily intake (RDI) for Niacin is 20 mg (21 CFR 101.9); no separate RDI is established for Niacinamide.

Niacin is used to treat psychiatric disorders such as schizophrenia, anxiety, depression, and chronic alcoholism and in the treatment of pellagra, a disease with characteristic neuropsychiatric, dermatological, and gastrointestinal manifestations (Ban 1971).

GENERAL BIOLOGY

Absorption

Human Studies

Feldman and Maibach (1970) reported that 11.08% of the total applied dose of Niacinamide was absorbed after application to the ventral forearm of humans over 5 days (approximately

TABLE 3
Frequency and Concentration of Use Data for Niacin and Niacinamide

Category (Number of Formulations in Category Reported to FDA, 2001)	Number of formulations containing Niacinamide or Niacin (FDA 2001)	Current concentration of use (CTFA 2001)
Niacinamide		
Eye Makeup Remover (84)	2	—
Hair Conditioners (636)	2	0.05–0.1%
Rinses, Non-coloring (40)	1	—
Shampoos, Non-coloring (860)	5	0.05–0.2%
Tonics, Dressings, and Other Hair Grooming Aids (549)	7	0.05%
Other Hair Preparations (276)	4	—
Skin Cleansing (653)	6	0.005%
Face and Neck Creams, Lotions, Powders and Sprays (827)	—	0.001–0.5%
Body and Hand Creams, Lotions, Powders and Sprays (827)	—	0.04–3%
Moisturizing (769)	15	0.1%
Night Creams, Lotions, Powders and Sprays (188)	8	0.0001%
Paste Masks—mud packs (269)	—	0.005%
Eye Lotion (18)	1	—
Other Skin Care Preparations (692)	10	—
2001 Total uses/concentration ranges for Niacinamide	61	0.0001–3%
Niacin		
Bubble Baths (200)	1	—
Hair Conditioners (636)	1	—
Shampoos, Non-coloring (860)	1	—
Body and Hand Creams, Lotions, Powders and Sprays (827)	—	0.01%
Paste Masks—mud packs (269)	—	0.1%
Other Skin Care Preparations (715)	—	0.07%
Indoor Tanning Preparations (68)	—	0.06%
2001 Total uses/concentration ranges for Niacin	3	0.01–0.1%

2–3% of the dose of Niacinamide absorbed per day). In this experiment, ^{14}C-labeled Niacinamide was dissolved in acetone and applied to a 13 cm^2 area of skin, i.e., 4 μg/cm^2. The area was washed 24 h after application and this procedure was repeated for 4 consecutive days. Urine was collected for 5 days and the concentration of ^{14}C-labeled Niacinamide was determined. The results were corrected for extra-renal excretion which was determined in a separate experiment in which an intravenous dose of Niacinamide (4 μg/cm^2) was administered and the fraction excreted in urine was calculated. The results of these studies demonstrate that Niacinamide absorption continues for up to 5 days after topical application, with the maximum absorption rate occurring between 48 h and 72 h.

Franz (1975) determined Niacinamide absorption in vitro through full-thickness excised human abdominal skin (obtained at autopsy) mounted in flow-through diffusion cells. In this study, Niacinamide was dissolved in acetone and applied at a dose of 4 μg/cm^2. The absorption of Niacinamide into the receptor fluid at 24 h was calculated to be 28.8% of the starting dose.

Buccal absorption was studied by a method involving circulation of a pre-incubated buffered solution of Niacin and Niacinamide in the human mouth for 5 min. The samples were analyzed by spectrophotometry. Buccal absorption rates were linear with respect to initial concentration between 2 and 10 mM. The authors stated that absorption was sodium-dependent and suggested carrier-mediated transport (Evered et al. 1980).

The in vitro absorption of Niacinamide from a wide variety of complex matrices was investigated using split thickness human cadaver skin (Procter and Gamble 1998a). Non-occluded topical doses of Niacinamide (2–20%), dissolved in various product types including moisturizers, foundations and lipsticks, were applied to skin explants and the rate of penetration was measured for up to 48 h. The integrity of the tissue samples was assessed using the ^{3}H$_2$O permeation method. Samples yielding water flux less than 1.2 mg/cm^2 were considered acceptable and those with fluxes greater than 3 mg/cm^2 were discarded.

The skin explants were mounted on modified Franz cells with a nominal surface area of 0.79 cm^2. The receptor solution,

Dulbecco's phosphate buffered saline, was stirred and maintained at 37°C in temperature-controlled aluminum blocks, yielding a skin surface temperature of 30–32°C.

The rate of penetration was found to be inversely associated with the dose, although the absolute amount of Niacinamide transported across the skin increased with starting concentration of Niacinamide. At 24 h, approximately 1–3% of the starting dose was measured in the receptor fluid after application of an oil/water emulsion containing Niacinamide. At 48 h, approximately 10% and 6% Niacinamide was absorbed from formulae containing 2% and 10%, respectively. In addition, these studies found that the rate of penetration of Niacinamide was relatively independent of the vehicle (Procter & Gamble 1998a).

Human and Animal Comparison Studies

The in vitro percutaneous absorption of Niacin was studied in flow-through diffusion cells using skin from male Fischer 344 rats and humans. After the application of Niacin to the epidermal surface of unoccluded full-thickness rat skin, the absorption of Niacin across the skin and into the receptor fluid at 72 h reached $5.7 \pm 0.6\%$ of the applied dose ($2.4 \ \mu g/cm^2$). In the same procedure with human skin, the absorption of Niacin across the skin and into the receptor solution at 72 h was significantly less than with rat skin, reaching $0.7 \pm 0.2\%$ of the applied dose ($2.1 \ \mu g/cm^2$). Occlusion of the skin surface with teflon caps significantly enhanced the percutaneous absorption of Niacin although the effect was not uniform (Hotchkiss et al. 1992).

Animal Studies

Bergmann and Wislicki (1953) conducted a study in cats, dogs, rats, and rabbits to determine the pharmacokinetics of Niacinamide. Doses of 1000 mg/kg were given to rabbits ip and orally, doses of 2000 mg/kg were given to rabbits orally. Doses of 500 mg/kg iv were given to cats and dogs. Rats were dosed at 750 mg/kg sc and 1000 mg/kg ip. The blood concentration of Niacinamide in cats and dogs was high initially and decreased linearly over several hours. The blood concentration of Niacinamide in rabbits increased over several hours and plateaued. No data were given for the rats. The authors concluded that Niacinamide is rapidly absorbed and distributed throughout the extracellular fluid.

An in vitro system, using everted sacs of rat small intestine, were used to study the transfer of mixtures of Niacin and Niacinamide from mucosal to serosal side and in the reverse direction. Transfer in each direction occurred at approximately equal rates, suggesting that absorption of these compounds is by passive diffusion (Turner and Hughes 1962).

In vitro studies using everted sacs of the small intestine of adult golden hamsters strongly suggested the absorption of Niacin and Niacinamide is by passive diffusion (Spencer and Bow 1964).

Unilever (1998b) conducted a study in which [14C]Niacinamide was incorporated into an oil-in-water (o/w) skin cream and into a 30% (w/w) soap base and applied to the skin of female Colworth Wistar rats. The final concentration of Niacinamide in the soap solution was approximately 0.3% (w/v) and was 1% (w/w) in the skin cream. Application of the skin cream and soap paste was made to rat skin at approximately 20 mg/cm^2. The cream was carefully massaged over 10 cm^2 of skin for up to 5 min before covering with polythene-lined occlusive protective patches.

The rats were placed in metabolism cages for 48 h during which time all excreta was collected. At 48 h, the animals were killed and the patch, carcass, and treated area of skin were assayed for 14C. Up to 32% 14C was recovered in excreta and in the carcasses from rats treated with skin cream containing [14C]Niacinamide and up to 30% from those treated with soap paste (Unilever 1998b).

In a number of experiments, Unilever (1998c) investigated the percutaneous absorption of radiolabeled Niacinamide in pig skin using two methods of dosing, one with 10% aqueous ethanol as the vehicle under occluded infinite dose conditions, the other with a skin cream as the vehicle under non-occlusive infinite dose conditions. In addition, [Fructose-1-^{3}H(N)]-sucrose (1 mCi, 20Ci/mmol) was used as an internal standard to demonstrate the integrity of the pig skin. A stock [14C]Niacinamide solution was prepared by dissolving the solid [14C]Niacinamide (0.7 mg) in 50 μl 50% (v/v) aqueous solution. Fresh pig skin was excised and washed in 0.9% saline. The hair was clipped and the subcutaneous fat and muscle were removed. Discs of skin 1.5 cm in diameter were cut out and mounted into flow-through diffusion cells, epidermis uppermost. The receptor solution was pumped through the cells at a flow rate of approximately 2.3 ml/h.

The test solution for experiment 1 was prepared by placing 5.7 μl [14C]Niacinamide stock solution in a vial and removing the solvent in a stream of nitrogen. To this 16 μl of [^{3}H]sucrose solution (as received) were added and the solvent removed in a stream of nitrogen. The [14C]Niacinamide and sucrose were then reconstituted in 2 ml 10% (v/v) aqueous ethanol. The test cream used in experiment 2 was prepared by adding 20 μl [14C]Niacinamide and 50 μl [^{3}H]sucrose (as received) to 1 g skin cream (containing 1% w/w Niacinamide) heated to 70°C in a water bath. The sample was homogenized and allowed to cool. In experiment 1, each cell was dosed with 100 μl test solution and the cells occluded. Five aliquots of each dose equivalent were counted as the dose standards and were used to calculate the exact dose.

Three dose standards were prepared for the second experiment. These were prepared by taking 10 μl of cream into a positive displacement pipette, weighing the pipette, discharging the pipette into a volumetric flask and then re-weighing the pipette so the amount of cream dosed could be calculated. Each cell was then dosed by the following procedure. A 10 μl aliquot of cream was taken up into the positive displacement pipette and the pipette weighted. The cream was discharged onto the surface of the skin and the pipette re-weighed. A glass rod was weighed which was used to spread the cream evenly over the skin surface. The rod was then re-weighed and the actual dose

applied was calculated. The mean dose applied was 7.9 mg and the cells were left unoccluded. Hourly fractions were collected from each cell for 24 h.

The pieces from each skin sample were pooled in counting vials, LiquiScint was added and the whole samples were counted. The disposition of ^{14}C and ^{3}H (as a percentage of the applied dose) and the flux of these through the membranes were calculated. When dosed in an ethanolic solution under occlusion, 7.52% of the applied dose of 3.5 μg was recovered in the receptor solution over 24 h. This equates to 0.26 μg of [^{14}C]Niacinamide. The steady state was reached at approximately 21 hours with a flux of 0.06 μg/cm^2/h. When the skin cream was used as the vehicle, 5.89% penetrated. This was equivalent to 4.81 μg total Niacinamide (81.7 μg dosed onto the skin). Steady-state was again reached at 21 hours with a flux of 1 μg/cm^2/h.

If the Niacinamide associated with the stratum corneum and epidermis/dermis was assumed to be bio-available, then a total of 14.40% of the dose was absorbed in this experiment. The penetration of the [Fructose-1-3H(N)]-sucrose was shown to be within the normal range over the first few hours of both experiments. The results indicated that Niacinamide has a moderate capability to penetrate skin, whether dosed in an aqueous ethanolic solution or within the commercial cream formulation. It was debatable whether the test substance associated with the stratum corneum and inner skin (after the upper skin surface had been washed) is bio-available.

If the [^{14}C]Niacinamide associated with the stratum corneum and epidermis/dermis is assumed to be bio-available, then a total of 10.87% of the dose was absorbed in experiment 1 and 14.4% in experiment 2. The solubility of Niacinamide in water is 100 g/100 ml, so the dermis should not be the limiting factor to penetration of this substance through the skin. Instead, poor solubility of Niacinamide in the lipid rich stratum corneum is likely to be the greatest barrier to penetration (Unilever 1998c).

Distribution
Human Studies
Horsman et al. (1993) conducted a study to determine the pharmacokinetics of Niacinamide in humans in order to have data to compare with mouse pharmacokinetic and radiosensitization data, with the goal of predicting the extent of enhancement of ionizing radiation that could result from clinically achievable levels of Niacinamide. Six healthy male human volunteers orally ingested increasing doses of up to 6 g Niacinamide in capsule form on an empty stomach. HPLC (high performance liquid chromatography) analysis of blood samples showed peak plasma concentrations, typically within 45 min after ingestion, which were linearly dependent on the ingested dose. The peak plasma concentration at the highest dose was 159.5 (144.5–174.4) μg/ml, which is also the concentration that produced maximal radiosensitization in mice. The authors concluded that 6 g Niacinamide should produce radiosensitization in humans.

Other data in the report relevant to Niacinamide pharmacokinetics included the elimination half-life (T^1/$_2$) and area under the curve (AUC). These were found to increase with drug dose, although the increases were non-linear. The mean T^1/$_2$ values were 2.7 h, 5.9 h, and 8.1 h after taking 1, 3, or 6 g of Niacinamide, respectively. Headache, dizziness, nausea, and vomiting were common side effects in the six volunteers. There did not appear to be any relationship between the ingested dose and the observed side effects (Horsman et al. 1993).

Kaanders et al. (1997) administered Niacinamide daily as a liquid formulation to head and neck cancer patients receiving a 5- to 7-week course of radiotherapy. Niacinamide was administered orally 1.5 h before irradiation. The daily dose was 80 mg/kg bw to a maximum of 6 g. A dose reduction to 60 mg/kg was introduced for patients with severe side-effects. Blood samples were drawn and Niacinamide concentrations were determined. On the first treatment day serial samples were obtained followed by daily samples at the time of irradiation during the first and last full weeks of the treatment. Side-effects of Niacinamide were monitored.

In all patients, peak concentrations greater than 700 nM/ml could be obtained 0.25–3 h after drug intake. During the first week of treatment, plasma concentrations at the time of irradiation were adequate in 82% of the samples. Nausea, with or without vomiting, occurred in 65% of patients. Tolerance improved after a 25% reduction of the dose in six of seven patients but plasma concentrations at the time of irradiation decreased below 700 nM/ml in four out of six patients. Other Niacinamide side effects included gastrointestinal symptoms, flushing, dizziness, sweating, fatigue, and headache. The most powerful single predictor for severe Niacinamide toxicity was the mean of the plasma concentration measured at the time of irradiation during the first week (Kaanders et al. 1997).

Animal Studies
The organ distribution and metabolic fate of Niacinamide were investigated in male C3H mice administered aqueous solutions of ^{13}N-Niacinamide via tail vein injections (Tominaga et al. 1986). The authors stated that ^{13}N-Niacinamide is transported throughout the body, mainly by simple diffusion, where part of the tracer is metabolized into hydrophilic compounds, i.e., ^{13}N-NAD, etc., and trapped. Most radioactivity was found in the small intestine, where the level continuously increased for 30 min after administration, despite a rapidly decreasing blood level. The authors suggest that this phenomenon is due to redistribution of compounds occurring after the first phase of transfer from the blood, and is probably the result of bile duct excretion of the tracer and its metabolites.

Pregnant JCL:ICR mice received a single intraperitoneal injection of 0.18 μCi of [carbonyl-^{14}C]Niacinamide/g of bw on day 9 of gestation. Mice were killed 0.5, 1, 3, 6, or 12 h after injection. Radioactivity was detected in the maternal organs, placenta, and fetus shortly after injection, and decreased gradually. The time to reduce the radioactivity in each organ by a factor of two was approximately 6 h. However, there were large differences in the radioactivity in each organ. Levels of radioactivity

in the placenta and fetus were about 16 and 5 times higher than in the maternal blood, respectively. Small amounts of nicotinamide adenine dinucleotide (NAD^+) were detected in the placenta and fetus shortly after injection. Nicotinamide adenine dinucleotide phosphate ($NADP^-$) was mainly found in the liver at 12 h after injection. Niacin was not detected (Gotoh et al. 1988).

Horsman et al. (1993) reported pharmacokinetic data from mice injected with Niacinamide in the radiosensitization studies described earlier. CDF1 mice with C3H mammary carcinomas were used. Analysis of the blood and tumor samples taken from mice injected intraperitoneally with 100–1000 mg/kg. Niacinamide in plasma was linear with injected dose over the range of concentrations studied. The elimination $T^1/_2$ in plasma exhibited a small dose-dependent increase over the concentration range, although the tumor concentration remained unchanged.

Unilever (1998d) studied the penetration and distribution of a skin cream containing 3% Niacinamide in an in vitro procedure using pig skin. A skin cream with 1% Niacinamide was tested for comparison. Two experiments were planned, one to study the penetration of Niacinamide through the skin (penetration) and another to study the distribution of Niacinamide within the skin (distribution). A third study (penetration) was done to measure penetration after it was discovered that sample radioactivity was lost overnight from a receptor solution that had not been sterilized. Microbial action was presumed to be the cause. Data, therefore were adjusted by measuring the loss of radioactivity from receptor solutions spiked with radioactively labeled Niacinamide.

In all experiments, after allowing the skin to thaw to room temperature, discs of skin 1.7 cm in diameter were mounted into flow-through diffusion cells, epidermis uppermost. The diffusion cells were placed in a heated cell holder and receptor solution pumped through them at a flow rate of approximately 2.6 ml/h. Diffusion cells were dosed with weighed aliquots of test preparation (10 mg) and the cells left unoccluded. The preparation was carefully spread over the skin surface using a round ended glass rod.

Half of the diffusion cells were dosed with the test skin cream containing 3% Niacinamide and the other half with the 1% formulation. In all experiments, hourly fractions were collected from the dosed cells. In the distribution experiment, five diffusion cells were taken after 8 h (one piece of control skin, two dosed with 1% formulation and two dosed with the 3% formulation) and the remaining diffusion cells were taken at 24 h. The skin was taken for slicing or microautoradiography.

The adjusted mean penetration (% of applied dose) of Niacinamide through whole pig skin from the 1% formulation was 5.33% with a SD (standard deviation) of 1.37. The adjusted mean penetration (% of applied dose) from the 3% formulation was 1.94% (SD 0.62). The adjusted total mean penetration values were 11.74 μg/cm^2 (SD 3.22) and 13.16 μg/cm^2 (SD 3.83) for the 1% and 3% formulations, respectively.

While complete data on radioactivity as a function of slice were not provided, the report states that the distribution of Niacinamide in the skin was primarily in the first two skin slices and decreased rapidly with increasing depth. At the 8 hour time point, the total amount of Niacinamide present in the basal layer (slices 4 and 5, 75–125 μm depth) was 320 ng for the 1% formulation and 819 ng for the 3% formulation. At 24 hours basal levels of the 1% and 3% formulations had fallen to 120 ng and 185 ng, respectively. In the 3% formulation slice profile, the authors reported some localization of radioactivity at a depth of 400–600 μm within the skin, which is the depth at which the sebaceous glands are located. Only low levels of activity were detectable in the sections prepared for microautoradiography and there was no localization in the sebaceous glands, although the levels of radioactivity were below those normally used in microautoradiography (Unilever 1998d).

Metabolism

Human Studies

Reddi and Kodicek (1953) reported that, after doses of 100 mg of Niacin (total dose of 200 mg), the urinary excretion of alkali-hydrolysable Niacin derivatives and of N^1-methylnicotinamide increased from 6.0 to 14.6 mg and from 2.8 to 5.7 mg after 3 h in four human subjects, respectively.

Chromatographic results showed the major metabolite in the urine to be nicotinuric acid, forming 92–99% of the alkali-hydrolysable derivatives. Niacinamide (1–4%) was the other metabolite. There was no free Niacin in the urine except in one subject who had intense flushing of the skin soon after ingesting the Niacin. In these four subjects, there was a large increase in the excretion of N^1-methylnicotinamide, which varied from 6.9 to 16.6 mg/3 h. The small rise in the tertiary nicotinyl derivatives (0.9 to 1.8 mg) was solely due to Niacinamide, since no other nicotinyl compound could be detected on the paper chromatograms. Urine from undosed subjects, averaged 0.53 mg N^1-methylnicotinamide for a period of 3 h. The total content of tertiary alkali-hydrolysable derivative of Niacin ranged from 0.2 to 0.3 mg within 3 h (Reddi and Kodicek 1953).

N^1-Methyl-4-pyridone-3-carboxamide was detected on chromatograms of plasma extracts after oral administration of Niacinamide to two human subjects (Abelson et al. 1963).

Nakagawa et al. (1969) studied the conversion of tryptophan to Niacin using a basal diet. The subjects, six women, were given supplements of tryptophan or Niacin in experimental periods lasting five days each. In the first study period, the diet contained 0 mg Niacin and supplements of 500, 750, 1250, or 1750 mg tryptophan. In the second study period, the diet contained 250 mg tryptophan and 0, 8.3, 12.5, 20.8, or 29.2 mg Niacin. These levels of Niacin were chosen to correspond to the levels of tryptophan in an initial study, assuming a conversion of 60:1. Total nitrogen was kept constant at about 11.4 g per day by isonitrogenous substitution of glycine. Determinations were made of 24-h urinary excretions of total nitrogen, urea, creatinine, pyridone, N-methylnicotinamide, free Niacin, xanthurenic acid, and kynurenic acid.

On the diets with no Niacin, excretion of Niacin metabolites did not rise markedly until the tryptophan supplement reached 1250 mg. In the second study period, excretion of Niacin metabolites rose markedly with the addition of 8.3 mg Niacin. The concentration of blood pyridine nucleotides did not change during the second period. By comparing the excretion of Niacin metabolites in the two periods, the point at which a rapid rise was obtained, 1250 mg tryptophan or 8.3 mg Niacin, gave a ratio of 150:1 tryptophan to Niacin. At higher concentrations of tryptophan or Niacin (1650 and 22 mg, respectively) a ratio of 75:1 was found (Nakagawa et al. 1969).

Human and Animal Comparison Studies

Chang and Johnson (1961) injected (intra-muscular) one male Rhesus monkey (4 yr old, 18 lb) with 3.5 mg ^{14}C-carboxyl labeled Niacin (plus 17.2 mg of non-labeled Niacin) and gave non-labeled Niacin 100 mg orally to each of four male volunteers. Urine from the human subjects was spiked with ^{14}C-labeled N-methyl-4-pyridone-5-carboxamide and N-methyl-2-pyridone-5-carboxamide as tracers.

In the human male, normal excretion of N-methyl-2-pyridone-5-carboxamide was between 1.4 and 4.4 times that of N-methyl-4-pyridone-5-carboxamide. Ingestion of the bolus of Niacin caused only a slight increase in excretion of N-methyl-4-pyridone-5-carboxamide, while the increase in excretion of N-methyl-2-pyridone-5-carboxamide was marked. In the monkey, no comparison with normal levels was made; the only observation reported was that the excretion of N-methyl-2-pyridone-5-carboxamide was much higher than N-methyl-4-pyridone-5-carboxamide; 6–10 times higher, depending on the day (Chang and Johnson 1961).

Animal Studies

Leifer et al. (1951) injected nine female CF1 mice, seven male Sprague-Dawley rats, and six hamsters intraperitoneally with 20 mg/kg bw [^{14}C]Niacin. Approximately 3, 4, and 7% of the injected dose was exhaled as $^{14}CO_2$ in the 24 hours following injection into mice, rats, and hamsters, respectively. A female dog was also injected intravenously with 20 mg/kg bw of [^{14}C]Niacin and [^{14}C]Niacinamide. Less than 1% of either compound was exhaled as $^{14}CO_2$ in the 24 hours post-injection. Urinary metabolites of [^{14}C]Niacin 12 to 24 h post-injection in all species were N^1-methylnicotinamide, nicotinuric acid, Niacin, N^1-methyl-6-pyridone-3-carboxylamide, Niacinamide, and an unidentified metabolite.

Preiss and Handler (1957) stated that Niacin is readily absorbed from all portions of the intestinal tract and from parenteral sites. Subsequently it is converted into Niacinamide and distributed to all tissues. Niacin and Niacinamide function as constituents of two co-enzymes: coenzyme I, diphosphopyridine nucleotide (DPN) or nicotinamide adenine dinucleotide (NAD), and coezyme II, triphosphopyridine nucleotide (TPN) or nicotinamide adenine dinucleotide phosphate (NAPH). These coenzymes are the main hydrogen carriers in the metabolism of carbohydrates, amino acids, and fats, and are also important in the synthesis of adrenal cortical hormones from acetyl coenzyme A, the conversion of lactic acid to pyruvic acid and the dehydrogenation of ethyl alcohol.

Chang and Johnson (1959) injected (ip) 65 young male Sprague-Dawley rats (about 150 g each) with ^{14}C-carboxyl labeled Niacin, collected urine and isolated and identified radiolabeled metabolites. They identified N-methyl-4-pyridone-5-carboxamide as a major normal metabolite of Niacin in the rat, along with N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide, and Nicotinamide.

Quinn and Greengard (1966) stated that N^1-methyl-4-pyridone-3-carboxamide is a major metabolite of Niacin and Niacinamide which has been found to be synthesized from N^1-methylnicotinamide. The authors stated that the biosynthesis is catalyzed by a soluble enzyme system present in the liver.

Lee and Gholson (1969) detected 6-hydroxynicotinamide and 6-hydroxynicotinic acid as urinary metabolites by comparison of ultraviolet, infrared, and mass spectra following intraperitoneal injections of [^{14}C]Niacin or [^{14}C]Niacinamide into rats.

Collins and Chaykin (1972) administered 0.9 μM [^{14}C]Niacin or [^{14}C]-Niacinamide to female C3HeB/FeJ mice (total number not stated). Administration was primarily by intraperitoneal injection, although administration by stomach tube or direct injection into the stomach or intestine with the pyloris tied was done in some cases. Urine samples were collected prior to killing (5 to 90 min after administration). Tissue extracts and urine were analyzed by chromatography.

In all tissues except the liver, and to some extent the intestine, Niacinamide was a more effective precursor of DPN or nicotinamide adenine dinucleotide (NAD) than was Niacin, when injected intraperitoneally. However, in the liver and intestine the labeled Niacin produced a transient considerable labeling of DPN. Niacinamide in the liver following Niacin injection was greater than after Niacinamide injection. Following the intragastric administration of Niacin, the concentrations of Niacin and Niacinamide in the blood changed with the liver and intestine serving as centers for the conversion of Niacin to Niacinamide. It was also shown that deamidation of Niacinamide to Niacin was not required for the absorption of Niacinamide from the digestive tract (Collins and Chaykin 1972).

Vargas and Jenden (1996) injected male Sprague-Dawley rats subcutaneously in the interscapular region with 1, 5, and 10 mM/kg bw Niacinamide. There were seven rats in each of the 5 and 10 mM/kg groups and 5 animals in a saline control group. The number of animals in the 1 mM/kg group was not reported, nor were any data provided on the effect of that treatment. The rats were killed and determinations of cisternal cerebrospinal fluid (CSF) concentrations of choline and acetylcholine and N^1-methylnicotinamide (NMN) were made.

CSF choline concentrations peaked 2 h after Niacinamide administration and were accompanied by increases in striatal, cortical, hippocampal, and plasma choline concentrations. The increase in the concentration of CSF choline were both time- and

dose-dependent. Brain tissue concentrations of acetylcholine were not altered by Niacinamide treatment. The enzymatic formation of [^{3}H]NMN in the rat brain was evaluated by incubating aliquots of rat brain cytosol with unlabelled Niacinamide and the methyl donor [^{3}H]S-adenosylmethionine. High performance liquid chromatography and radio-chemical detection demonstrated the [^{3}H]NMN was specifically formed by a brain cytosol enzyme. The production of [^{3}H]NMN was dependent on exogenous Niacinamide and could be prevented by denaturing the cytosol (Vargas and Jenden 1996).

Excretion

Human Studies

Perlzweig et al. (1950) gave two human subjects oral doses (500 mg) of Niacinamide daily for 5 days. After one week, they were given 224 mg of N^1-methylnicotinamide daily for three days. One week later, the subjects received a single dose of pyridone. During the last three days of dosing, the subjects excreted between 190 and 280 mg of N^1-methylnicotinamide, 230 to 280 mg of 2-pyridone, and 4.5 to 7.5 mg of Niacin. On the day before dosing, the same subjects fed a normal diet excreted 6 to 9 mg N^1-methylnicotinamide, 10 to 12 mg of 2-pyridone, and about 1 mg of Niacin. It was estimated that on an average diet, about 80% of the ingested Niacinamide was excreted in these forms.

The authors stated that similar studies have been conducted using multiple species. Results were similar to the human data for dogs, rat and pigs, but not for rabbits, guinea pigs, calves, goats, and sheep. The authors suggested that carnivores and omnivores utilize methylating mechanisms to dispose of excess Niacin, but herbivores use other pathways (Perlzweig et al. 1950).

Animal Studies

Reddi and Kodicek (1953) fed rats two diets (a low-protein maize and a synthetic casein) in separate groups (8 animals per group). The maize diet was deficient in Niacin while the casein diet contained normal amounts of Niacin. Urinary excretion was measured. Rats given the maize diet deficient in Niacin excreted a small amount of N^1-methyl-nicotinamide, Niacin, and Niacinamide, while those fed the normal casein diet excreted daily 0.24 mg N^1-methylnicotinamide and 0.04 mg total tertiary nicotinyl compounds per rat. Both deficient and normal rats fed the casein diet excreted Niacin and Niacinamide in equal proportions.

After dosing with 2.5 mg Niacin, the major urinary excretion products in deficient rats were N^1-methylnicotinamide and nicotinuric acid; in normal animals, the major urinary excretion products in descending order were N^1-methylnicotinamide, nicotinuric acid, Niacin, and Niacinamide. Deficient and normal rats dosed with 2.5 mg Niacinamide excreted N^1-methylnicotinamide, Niacinamide, but no nicotinuric acid (Reddi and Kodicek 1953).

Petrack et al. (1966) noted that when large doses (500 mg/kg bw) of Niacin or Niacinamide were given by intraperitoneal injection to female Sprague-Dawley rats, the compounds were excreted largely unchanged in the urine. However, at low doses (5 mg/kg bw), N^1-methylnicotinamide was the main excretory product of Niacinamide. Small amounts of Niacinamide and pyridone derivatives were also excreted. In case of injected Niacin, about equal amounts of N^1-methylnicotinamide and nicotinuric acid were excreted alone with small quantities of Niacin, Niacinamide, nicotinamide N-oxide, and pyridone derivatives.

Shibata et al. (1990) determined the urinary excretion of Niacinamide and its metabolites in the mouse, guinea pig, and hamster. Male CD-1 (ICR) mice, male Hartley guinea pigs, and male hamsters (five of each) were housed in metabolic chambers and fed a commercial nonpurified diet. On day 42, the animals were injected intraperitoneally with 500 mg/kg bw Niacinamide. The metabolites of Niacinamide before and after injection into all three species are listed in Table 4. In the guinea pig and hamster urine, deaminated metabolites predominated after injection of Niacinamide.

As part of a percutaneous absorption study, Unilever (1998b) reported the rate and route of excretion of intraperitoneally administered [^{14}C]Niacinamide (1 ml containing 33.84 μg) in female Colworth Wistar rats. The main route of excretion was via the urine (45.98%) with the low fecal ^{14}C (2.6%) and ^{14}CO$_2$ (2.39%) levels indicating only minor involvement of these routes. The retention of ^{14}C in tissues throughout the body reflected the widespread endogenous distribution of Niacinamide.

Effect on Carbohydrate and Lipid Metabolism

Human Studies

Altschul et al. (1955) reported that administration of large doses (1 or 4 g daily) of Niacin lowered serum concentrations of cholesterol in man. No details were provided.

Parsons and Flinn (1957) conducted a study in which hypercholesteremic patients (14 male and 10 female with an average pretreatment cholesterol level of 270 mg per 100 cc) initially were given doses of 3 g Niacin daily for 12 weeks. Then, if the blood cholesterol was normal (less than 250 mg per 100 cc) the same dose was continued. In patients whose cholesterol levels remained high, the daily dose was increased to 4.5 and later to 6 g. After 30 weeks, Niacinamide was substituted in a dose equal to the most effective dose of Niacin.

Prompt and sustained reduction of blood cholesterol concentrations were obtained in nearly all of the patients. There were no serious side-effects. Nearly all patients experienced a flush which occurred briefly after each dose for the first one to seven days of administration. Pruritus frequently accompanied the flush and often persisted a few days longer. Liver function tests, determinations of blood nonprotein nitrogen concentratons, and urinalysis revealed no abnormality in 19 patients after 8 to 32 weeks of therapy (Parsons and Flinn 1957).

TABLE 4
Niacinamide Metabolites Before and after Intraperitoneal Injection (Shibata et al. 1990)

Species	Metabolite	Percentage	
		Before injection	After injection
Mouse	Nicotinamide N-oxide	35%	79.7%
	N^1-methyl-2-pyridone-5-carboxamide	20%	11.2%
	Niacinamide	18%	—
	N^1-methylnicotinamide	16%	4.9%
	N^1-methyl-4-pyridone-3-carboxamide	11%	6.3%
Guinea Pig	N^1-methyl-2-pyridone-5-carboxamide	80%	—
	N^1-methylnicotinamide	11%	—
	Niacinamide	3%	—
	N^1-methyl-4-pyridone-3-carboxamide	3%	—
	Nicotinamide N-oxide	3%	—
	Niacin	—	7.7%
	Nicotinuric acid	—	79.5%
Hamster	Niacinamide	44%	—
	N^1-methyl-2-pyridone-5-carboxamide	21%	—
	Nicotinamide-oxide	15%	—
	N^1-methylnicotinamide	10%	—
	N^1-methyl-4-pyridone-3-carboxamide	10%	—
	Niacin	—	50.4%
	Nicotinuric acid	—	26.3%

Gurian and Adlersberg (1959) administered Niacin (3 g daily) orally to 3 groups of patients: 1) 3 patients with normal serum lipid levels; 2) 4 patients with hypercholesteremia or hyperlipemia, or both; and 3) 3 patients with diabetes mellitus.

The 3 g doses significantly lowered serum lipid fractions in both normal and those with elevated serum lipids. Niacin consistently and significantly diminished carbohydrate tolerance in non-diabetic patients. Patients with adult-onset type of diabetes mellitus had no significant effects on diabetic control after Niacin treatment. Side effects noted were cutaneous flushing, pruritus, nausea and vomiting (Gurian and Adlersberg 1959).

Chazin (1960) gave patients with cholesterol levels above 250 mg Niacin for three months. All patients started on 250 mg Niacin three times a day. Two weeks later the dosage was doubled, and in another two weeks it was doubled again. No patient received more than 3 g of Niacin daily. Fifteen patients received the drug for three months, one took it for four months, and four took it for five months. Total cholesterol and cholesterol esters, blood urea nitrogen, alkaline phosphatase or thymol turbidity, complete blood count, and urinalysis were repeated every month and before the study began.

In all patients, there was a lowering of blood cholesterol which was statistically significant. When the initial cholesterol was higher, the effect was greater. Higher doses of Niacin correlated to lower cholesterol concentrations. Reported side effects during the study were hot flushes, pruritus, diarrhea, and leg cramps (Chazin 1960).

Balasse and Neef (1973) studied ten female subjects 17 to 34 years of age with body weights 95 to 190 percent of ideal weight. Three normal and three obese subjects fasted for 15 h (overnight) and 4 obese subjects starved for 10 or 11 days. All had normal oral glucose tolerance before the study. 1-^{14}C-glucose was then infused for 3 h in an antecubital vein at the constant rate of 0.15 μCi/kg·h. Each subject was studied in a control state and during the administration of Niacin which was injected as iv pulses of 100 or 150 mg every 20 minutes, the first of which was given 30 minutes before the start of the glucose infusion. In the controls, saline injections were used.

All the nonstarved and two of the starved subjects demonstrated decreased free fatty acid concentrations in plasma and an increase in the rates of turnover and oxidation of glucose in plasma over the 3 h study period. These changes were not noted in the other two starved subjects (Balasse and Neef 1973).

Animal Studies

In perireral adipose tissue from rat, both noradrenaline and theopylline stimulated the glycerol-release and increased the content of cyclic AMP (adenosine mono-phosphate) in the tissue as well as the incubation medium. Niacin blocked or reduced the submaximal lipolytic effect of both noradrenaline and theophylline; the effect of theophylline was most sensitive to Niacin inhibition. Niacin concentrations that almost completely blocked the lipolytic actions of noradrenaline or theophylline,

also blocked the effect on cyclic AMP (Andersson et al. 1973).

Magide and Myant (1974) reported a study in which the effect of single and multiple doses of Niacin on the serum lipids of two immature male Rhesus monkeys (*Macaca mulatta*) was observed. Each monkey received a single subcutaneous injection of 250 mg/kg Niacin, and in a separate experiment received 150 mg/kg doses of Niacin subcutaneously for 7 days.

The single injections decreased the serum free fatty acid and triglyceride concentration in the two monkeys. The repeated injections led to a substantial decrease in serum cholesterol concentrations and was accompanied by a decrease in hepatic synthesis of cholesterol. However, there was no change in the fecal excretion of endogenous steroids. Each monkey then received an intravenous injection of 50 μCi of [4-^{14}C]-cholesterol emulsified in its own plasma. The rate of cholesterol synthesis, estimated from the fecal excretion of endogenous steroids and from the serum specific radioactivity curve, was such that a moderate degree of inhibition would have accounted for the observed decrease in the amount of circulating cholesterol during Niacin treatment. Neither monkey had any signs of discomfort when given a single injection of Niacin. Repeated injections of Niacin produced a moderate increase in the serum activity of aspartate transaminase in both monkeys, but no increase in alkaline phosphatase. Liver biopsies from both monkeys appeared normal when examined by light microscopy (Magide and Myant 1974).

Cell and Tissue Toxicity

Unilever (1998f) assessed the toxicity of Niacinamide to cultured macrophages using mouse peritoneal macrophages harvested and cultured for three days in the presence of Niacinamide at 10, 100, 250, 500, or 1000 μg/ml. Changes in morphology, the ability to phagocytose particulate carmine, and the dehydrogenase activity of the cells were measured. Niacinamide was virtually non-toxic to cultured macrophages. At 1 mg/ml, slight toxic effects could be observed, but the cells were still capable of ingesting particulate carmine.

Hoorens and Pipeleers (1999) reported that Niacinamide, at concentrations of 2 and 5 mM/l, prevented hydrogen peroxide-induced necrosis of human pancreatic beta cells and protected rat beta cells against necrosis by streptozotocin or hydrogen peroxide. Single beta cells from pancreatic islets were used. Niacinamide failed to protect human and rat pancreatic beta cells against apoptosis induced by a combination of the cytokines interleukin-1β, interferon-γ, and tumor necrosis factor-α. In the rat, Niacinamide (2 to 20 mM/l) induced apoptosis of pancreatic beta cells; this effect was not observed in the human.

Tissue necrosis in an abdominal island pedicle skin flap treated with Niacinamide was examined by Collins et al. (1989). A 7 × 7 cm island pedicle skin flap ligating the left inferior neurovascular pedicle was created on 50 male Sprague-Dawley rats. Animals received intraperitoneal saline injections or intraperitoneal Niacinamide (25, 50, 100, or 200 mg) injections for 16 days (14 days preoperatively and 2 days postoperatively).

Fourty-eight hours postoperatively, each animal received 25 mg of Fluorescein via the tail vein. The area of necrosis was visualized and quantified as % viable. The % viable skin flaps were saline −58.8%, 25 mg Niacinamide −68.6%, 50 mg Niacinamide −82%, 100 mg Niacinamide −80.8%, and 200 mg Niacinamide −86%.

Radiosensitization

Peters et al. (1997) studied radiosensitivity after Niacinamide administration in murine carcinoma NT, a rapidly growing, poorly differentiated adenocarcinoma, carcinoma NT. Other parameters were measured, including tumor interstitial fluid pressure (IFP) and transient vessel non-perfusion. Carcinoma NT was transplanted into female CBA mice intradermally over the sacral region of the back.

Niacinamide doses of 500 and 1000 mg/kg significantly reduced tumor IFP within 20 min of administration, with recovery to control values by 6–80 min. The percentage of previously non-perfused vessels that became perfused 20 min after administration of 1000 mg/kg Niacinamide significantly exceeded the percentage that became perfused within 20 min in the absence of Niacinamide. By 90 min after the administration, the differential effect was abolished. However, 1000 mg/kg Niacinamide radiosensitized the NT carcinoma 80 min after administration (Peters et al. 1997).

ANIMAL TOXICOLOGY

Acute Toxicity
Oral

The oral LD50 of Niacin in mice was reported to be around 4300 mg/kg and, in rats, around 5100 mg/kg bw. Niacinamide was stated to be twice as toxic, with reported oral LD50s in mice and rats of ∼2100 mg/kg and ∼2700 mg/kg, respectively. Death occurred within 12 to 36 hours after the animals received lethal doses. The authors suggested that the rather slow and non-characteristic symptoms following lethal doses suggests that the lethal effect of high doses of Niacinamide was caused by the osmotic pressure of the 10% solution of the compound rather than a specific or selective effect since an injection of the same volume of an equimolar concentration of sodium chloride (4%) produced death with similar signs (Unna 1939).

Parenteral

Chen et al. (1938) listed the Niacin intravenous delivery LD values seen in Table 5.

Unna (1939) also reported that the subcutaneous LD50 of Niacin in mice was around 5100 mg/kg and, in rats, around 7000 mg/kg bw. Niacinamide was stated to be twice as toxic via this route of administration, with reported LD50s in mice and rats of ∼2900 mg/kg and ∼3500 mg/kg, respectively.

Brazda and Coulson (1946) stated (no study details provided) that Niacin and Niacinamide LD50s are 5000 and 1680 mg/kg, respectively, in rats administered subcutaneous injections.

TABLE 5
Niacin iv LD Values (Chen et al. 1938)

Species	Measurement	LD (mg/kg)
Mouse	LD0	4000
Mouse	LD100	4500
Rat	LD40	3000
Rat	LD60	3500
Guinea pig	LD0	3000
Guinea pig	LD60	3500

Bergmann and Wislicki (1953) reported LD50s after iv injection of 1620 mg/kg Niacinamide and after ip injection of 1800 mg/kg Niacinamide in mice. The authors concluded that the closeness of the iv and ip values in their study and that of Brazda and Coulson (1946) suggested that Niacinamide is rapidly absorbed into circulating blood.

Adult female Swiss albino and Charles River mice were used in a study by Bederka et al. (1975). The LD25 after intraperitoneal injection of Niacin to approximately 160 mice was 3.1 g/kg bw. The LD25 after intraperitoneal injection of Niacinamide to the same number of animals was 1.9 g/kg bw. Death generally occurred between 10 and 72 h after injection.

Informatics (1974) listed the LD values in rats seen in Table 6. In each case, 5 animals were exposed to Niacin.

Dermal

In the rabbit, a dermal LD50 (24 h covered contact) for Niacinamide was reported as > 2 g/kg bw (BIBRA International 1998).

Short-Term Toxicity
Oral

Handler and Dann (1942) reported a decreased growth rate in male Vanderbilt strain rats fed diets containing 10% casein and large amounts of Niacin or Niacinamide daily. The rats weighed 48 to 54 g at the onset of the study and received diets containing

TABLE 6
Niacin LD Values in Rats (Informatics 1974)

Route	Measurement	LD (mg/kg bw)
Intraperitoneal	LD0	700
	LD16	820
	LD50	1080
	LD84	1400
	LD100	1500
Subcutaneous	LD0	2000
	LD16	2050
	LD50	2550
	LD84	3125
	LD100	3200

1% or 2% (1.0 or 2.0 g/kg bw) Niacinamide or Niacin for 30 or 40 days. Each feeding group consisted of six rats and most experiments were repeated three times. Growth rate was decreased to a greater degree with Niacinamide than with Niacin. A slight decrease in growth rate was noted at the 2% level with Niacin, but not at 1%. Fatty liver formation was noted at both Niacin feeding levels. Almost complete growth inhibition occurred in animals fed 1% Niacinamide.

In separate studies (Handler 1944), rabbits and guinea pigs were placed on the same diets as the rats in the above study. Niacinamide in the diets of both the rabbits and guinea pigs was non-toxic. Toxicity in the rat may have been due to the synthesis and excretion of N^1-methylnicotinamide.

Cava et al. (1959) administered 600 mg of cholesterol, 500 mg of Niacin, or both to male albino rabbits (11 animals in each group) daily in their feed for 3 months. A control group received only stock rabbit chow supplemented with calf-meal pellets. Hypercholesteremia and the degree of atheromatosis were less in the Niacin + cholesterol group than in rabbits receiving cholesterol without Niacin. Niacin did not influence the concentration of serum cholesterol nor the cholesterol-induced accumulation of lipids in the parenchyma of the liver. No adverse effects were noted in animals fed the Niacin but no cholesterol.

Horger and Gerheim (1958) fed groups of 12 male rats Niacinamide in their diet for a period of 8 to 12 weeks. At 0.1% of the diet (100 mg/kg bw per day), Niacinamide caused no significant change in the growth rate; at 0.2%, growth rate was enhanced; but at 0.4%, a marked inhibition of growth rate resulted.

Rikans et al. (1964) studied weanling male albino rats in each of four feeding groups: high fat with or without added Niacinamide (0.1% of diet, 100 mg/kg/day bw); low fat with or without added Niacinamide. This amount of Niacinamide was an excess intake. There were ten animals per group. Two control groups, one fed a high fat and the other fed a low fat diet, containing adequate quantities of Niacin, were included. Blood samples were collected for pyridine nucleotide determinations on the twenty-first and forty-second day of the experiment. The animals were killed on day 44 and the livers analyzed. Results indicated that excess Niacin enters the metabolic pathways to produce increased concentrations of pyridine in the blood and liver and increased levels of fat in the liver. Excess fat was found in the livers only when the high fat diet was combined with an excess intake of Niacinamide.

In a review of Niacinamide toxicity, BIBRA International (1998) reported the results of a 28-day feeding study, in which Niacinamide 215 or 1000-mg/kg bw was supplied in the diet of rats. The animals were observed for 6-weeks following cessation of the treatment and then were necropsied. No toxic effects were observed at the low dose, 215-mg/kg bw. However, some effects occurred in rats fed the high dose, 1000-mg/kg bw. These were considered reversible and included slight liver enlargement, increased activity of certain liver enzymes, reductions in spleen and total body weight, reduced food consumption and red blood cell formation.

Parenteral

Sun et al. (1986) fed male Sprague-Dawley rats a basal diet containing 12% vitamin-free casein for 2–3 days before random assignment to multiple diet treatments. Selected rats were injected intraperitoneally with Niacinamide (60 mg/ml per 100 g bw) and control animals were injected with saline alone.

In the first experiment, four rats were placed into each of three groups. One group received the basal diet and the saline injection, one group received the basal diet plus the Niacinamide injection, and the third group was fed the basal diet plus a 0.1% choline supplement. After 18 days, the rats were placed in metabolic chambers for a 48 h urine collection and then killed.

In the second experiment, 40 rats were divided into 4 diet groups: (1) basal diet, (2) basal +0.075% choline, (3) basal +0.20% carnitine, and (4) basal +0.40% carnitine. One-half of the rats in each diet group were injected daily with Niacinamide and the other with saline alone. The experiment lasted 3 weeks and at termination a 48 h urine specimen and tissues were collected.

In the third experiment, 70 rats were divided into seven diet groups: (1) basal, (2) basal +0.40% L-methionine, (3) basal +0.05% choline, (4) basal +0.10% choline, (5) basal +0.40% carnitine, (6) basal +0.80% histidine, and (7) basal +15 μg/kg vitamin B_{12}+ 3 mg folic acid. One-half of the rats were injected daily with Niacinamide and the other half with saline. The experiment lasted for 4 weeks and urine and tissue samples were collected. Creatine in the skeletal and heart muscle, urinary creatine, and total lipids in the liver were determined.

In all experiments, the Niacinamide-treated groups had higher liver lipids, lower weight gains and the creatine content of the heart and skeletal muscle were lower than in the saline-injected control groups. Choline and methionine prevented these alterations, depending on the level of supplementation. No consistent responses among carnitine, histidine, or folic acid plus vitamin B_{12} treated rats were observed (Sun et al. 1986).

Yourick et al. (1992) studied the effect of short-term Niacinamide administration on sulfur mustard-induced microvesication. Male hairless guinea pigs were exposed to sulfur mustard by vapor cup. Niacinamide (750 mg/kg bw, 3 ml/kg in saline) or saline was administered as either a 30 min pretreatment, or a 30 min pretreatment and 6, 24, and 48 h treatment doses after sulfur mustard exposure. Each data point was described as the mean of seven to eight animals.

Niacinamide given as a 30 min pretreatment did not reduce the degree of microvesication 72 h after sulfur mustard compared to controls. However, Niacinamide given as a 30 min pretreatment and at 6, 24, and 48 h after sulfur mustard exhibited a 28% reduction in microvesication 72 h after sulfur mustard exposure. Skin NAD+ content at 72 h after sulfur mustard was depleted by $\sim$53% in the saline and Niacinamide treated groups. Niacinamide did not reduce the degree of erythema at 48 or 72 h (Yourick et al. 1992).

Dermal

Unilever (1998g) conducted a study in which Niacinamide in aqueous surfactant solutions, at doses equivalent to 0.5 to 50 mg per treatment, was applied once daily for four weeks to the skin of C57BL mice. Five concentrations of Niacinamide (0.01, 0.03, 0.1, 0.3, and 1.0 g/ml) plus a vehicle control were studied: 5 male and 5 female mice were dosed per level. The highest concentration was the maximum soluble and the lowest concentration was the consumer-use level. Dobanol 25 sulfate (0.5% w/v) was added to reduce surface tension and allow satisfactory spreading. The schedule of treatments was 0.05 ml once per day, 5 days per week for 4 weeks. The mice were examined daily and any evidence of skin irritation or ill health was noted, as was any change in color from pink to black (due to hair growth) and frequency of clipping. The mice were weighed weekly. At the end of the test, the mice were subjected to necropsy, blood was taken for hematological or biochemical analysis, and appropriate tissues were prepared for microscopic examination.

There were no signs of ill-health in any of the mice; all mice survived to the end of the test. The body weights of the mice were normal. There were no differences attributable to treatment. Under the conditions of this study, Niacinamide was not an irritant to mouse skin, even at the highest concentration. In addition, body weight, organ weight, and results of hematological and biochemical tests did not show any obvious adverse effects. There were no macroscopically adverse effects, although some microscopic changes were recorded in the treatment animals only. Dermal extra-follicular melanin was recorded in seven males and one female (16% of the treated total) and lymph node pigment in ten males (20% of the treated total), but there was no dose response (Unilever 1998g).

Subchronic Toxicity
Dermal

Unilever (1998h) also conducted a study in which Niacinamide in aqueous surfactant solutions, at dose levels of 0.0819-4.65M, was applied once daily for five days per week for thirteen weeks to the clipped dorsal skin of C57BL mice. Five dose levels of Niacinamide were used: 56.8% (4.65M), 30% (2.46M), 10% (0.819M), 3% (0.246M), and 1% (0.0819M). A solvent control was included. There were ten animals of each sex per dose level. The schedule of testing was 0.1 ml once per day, 5 days per week for 13 weeks. Dobanol 25 sulfate at 0.5% (w/v) was used as a solvent in distilled water. The interscapular skin and mid-dorsal skin of the mice were treated. Skin condition, frequency of hair growth and body weight were recorded. At the end of the test period, the mice were subjected to necropsy and blood was taken for hematological or biochemical analysis.

The number of mice entering hair growth, and the patterns of hair growth were not influenced by treatment. Under the conditions of this study, Niacinamide was not an irritant to mouse skin, even at the highest concentration. In addition, body weight and the weights of most organs were not affected by treatment. Liver weights of the females were slightly increased at the higher doses

of Niacinamide. Most clinical biochemistry analyses showed no effect attributable to treatment, but transaminase activity was markedly increased in some mice receiving the higher dose levels. Blood chemistry assays showed no difference between treatment and control animals (Unilever 1998h).

Photoprotection/Phototoxicity

Gensler (1997) applied Niacinamide to the shaved backs and ears of 40 mice at a concentration of 40 μmol in 200 μl of acetone twice weekly. Treatment started two weeks before UV treatments and continued throughout the experiment. Nicotinamide was administered approximately 30 min after UV treatment. Control mice received the solvent only. UVB irradiation consisted of five weekly 30 min exposures to six FS40 Westinghouse fluorescent sunlamps. Mice received approximately 6.2×10^5 J/m^2 in a passive transfer assay for immunosuppression and 1.09×10^6 J/m^2 in the photocarcinogenicity study.

In the passive transfer assay, splenocytes from UV-irradiated mice treated with Niacinamide did not transfer enhanced tumor growth rate to recipient mice. Application of Niacinamide to UV-irradiated mice reduced skin tumor incidence from 75% to 42.5% (p = 0.016). The topical Niacinamide prevented the immunosuppression and skin tumor induction by UVB irradiation (Gensler 1997).

In a photoirritancy study by Unilever (1998j), Albino Wistar rats were treated with 1% Niacinamide in a skin cream which was applied without dilution. Thirty weanling rats (18 males and 12 females) were used in this study. The number of rats in each litter was equal to the required number of treatment groups and the rats were no more than 29 days old at the start of testing. One animal from each litter was allocated to each treatment group. Each rat in a group of ten (group A) had a single dose of 0.1 ml of the test substance spread across the clipped dorsal skin and twenty minutes later was exposed to light from a Blacklamp (10T). A control group of ten rats (group B) received the test substance in the same manner but without radiation and a second control group of ten rats (group C) received irradiation followed by application of the test substance. The treatment sites were examined under standard lighting conditions at 3, 6, 24, 48, and 72 h after the end of treatment. At each examination, reactions were assessed for erythema, edema, cracking, scaling, dryness, hemorrhage, ulceration, scabbing, and exudation. The Sign Test (binomial distribution test) was used to determine the statistical significance between treatment groups. Marginal differences between pairs were further analyzed by the Wilcoxon Matched Pairs Signed Ranks Test.

Statistical analyses of the scores indicated that there was no significant difference between the groups A, B, and C. Under the conditions of this study, the skin cream containing 1% Niacinamide was not phototoxic (Unilever 1998j).

Skin Sensitization/Skin Irritation

Unilever (1998k) conducted a study in which 7 New Zealand White rabbits were intradermally injected with a series of low concentrations of Niacinamide, sodium lauryl sulfate (SLS), and saline control. Niacinamide at 0.1, 0.25, 1, and 2.5% dissolved in saline, SLS at 0.02, 0.005, 0.1, and 0.5%, and saline were given in aliquots of 0.1 ml at different sites on the clipped dorsum of each rabbit. The size and nature of the reactions were assessed under a uniform white light at 24 and 48 h after injection. The longest and shortest diameters of each reaction and the necrotic center, if any, were measured. The appearance of each reaction was graded on a scale of faint pink to deep pink and the color of the necrotic sites were noted. Pairs of reactions to two treatment solutions were compared directly on each animal and the Sign Test was carried out to determine the significance of the differences.

Niacinamide produced a slight irritation in three out of seven animals at 2.5%, with a mean reaction size of 2.43 mm at 24 h, and no visible response at 1, 0.25, and 0.1%. The level of response to all concentrations of Niacinamide was less than to 0.5 and 0.1% SLS. At 0.5% SLS, distinct to fairly well developed irritation and slight necrosis was seen in all seven animals with a mean reaction size of 20.29 mm at 24 h. Under the conditions of this study, 2.5% Niacinamide was only marginally irritating to rabbit skin. The overall level of irritation was significantly less than that produced by SLS (Unilever 1998k).

Unilever (1998l) assessed the skin sensitization potential of Niacinamide in guinea pigs. Preliminary to the sensitization test, irritation tests were carried out in guinea pigs to determine concentrations of the test substance for induction and challenge of sensitization. Eight previously untreated guinea pigs (4M and 4F) were injected intradermally in the clipped and shaved flanks with 0.1 ml aliquots of a range of concentrations of the test substance in physiological saline. Twenty-four hours later, the skin was examined for erythema, and edema.

The concentration which produced slight but perceptible irritation with no edema was selected as the injection induction concentration (IIC). Aliquots of 0.1 ml, using a range of concentrations of the test substance in distilled water, were applied in small circular areas to the shaved flanks of four previously untreated guinea pigs. Twenty-four hours later the skin was examined for erythema and the highest concentration which caused no irritation was selected as the application challenge concentration (ACC).

As a result of the preliminary studies, the concentrations selected for the sensitization test on Niacinamide were 5% for the ICC and 20% for the ACC. In the sensitization test, ten guinea pigs were used in the test group and aliquots of 0.1 ml of the test substance at 2.5 times the ICC (i.e. 12.5%) were injected intradermally in each animal at four sites which overlay the two axillary and two inguinal lymph nodes. Fourteen days later, each animal was challenged intradermally in one flank and topically in the other with 0.1 ml Niacinamide at the respective ICC and ACC (challenge A1). Twenty-four hours later, the reactions were scored.

As there was no evidence of sensitization, the induction procedure was repeated and the animals were challenged two weeks later (challenge B1). A confirmatory challenge with control

animals was made a week later (challenge B2). At the challenge with controls, four previously untreated animals of the same sex and similar weight to the test animals were treated intradermally and topically on opposite flanks with 0.1 ml of Niacinamide at the ICC and ACC. Reactions were examined under constant artificial daylight. Each injection reaction was given a total score based on size (two largest diameters), erythema and edema. Individual reactions were considered positive when their total score was significantly greater then the average total score for control reactions. Application reactions were scored on a 0 to $++++$ (no reaction to deep pink erythema with edema, with or without necrosis) scale and individual reactions were considered positive if they were $+$ or greater and there were no erythema reactions in controls.

At 24 hours after the first challenge (A1) with 5% Niacinamide intradermally and 20% Niacinamide applied topically, none of the ten animals showed a positive response. At the second and confirmatory challenges (B1 and B2), again with 5% and 20% Niacinamide respectively, none of the animals showed a positive response (Unilever 1998l).

Ocular Irritation

Low-volume eye tests (LVETs) were conducted in New Zealand White rabbits with solutions of Niacinamide dissolved in water at 15% and 25% w/v (Procter and Gamble 1997). The maximum average score (MAS) for the 15% and 25% aqueous solutions of Niacinamide was 0.67 and the median number days for the eyes to clear of all effects was 1.0. The authors concluded that Niacinamide does not constitute an acute ocular hazard.

Unilever (1998i) assessed the eye irritation potential of two skin creams each containing 1% Niacinamide in rabbits by a method similar to and based on that prescribed in the United States Federal Hazardous Substances Labeling Act. The test substances were creams each containing 1% Niacinamide were tested as supplied at 100% concentration. Two groups of six New Zealand White rabbits (eight weeks old) were used. Prior to treatment, the corneal thickness of each eye to be treated was measured with a slit lamp.

Each test substance was applied to one eye of each rabbit and the subsequent responses assessed by macroscopic observation and by measurement of corneal swelling. Observations were continued after treatment, until the eyes were considered to be of normal appearance. The test substance (0.1 ml) was applied to one eye of each of the six rabbits in the test groups by gently pulling the lower eyelid away from the eyeball and placing the test substance in the conjunctival sac; the other eye was used as an untreated control. Each animal was assessed for conjunctival damage before it was returned to its cage. Further examinations were carried out for corneal, conjunctival, and iridial reactions and for pannus at 24 h and up to 48 h after treatment. The eyes were also examined with a slit lamp before treatment and at daily intervals afterwards and corneal thickness measured.

One skin cream caused slight conjunctival reaction in one animal. This lesion had healed within 24 h. The remaining five were unaffected. The other skin cream caused slight conjunctival reactions in three animals and these lesions healed within 48 h. The three remaining animals were unaffected. Under the conditions of this study, the first skin cream was virtually non-irritating to rabbit eyes and the second skin cream was marginally irritating (Unilever 1998i).

The ocular irritancy potential of Niacinamide was evaluated in vitro using the Tissue Equivalent Assay (Procter and Gamble 2000c and 2000d). This assay utilized cultures of stratified human keratinocytes to assess the potential ocular irritancy of test articles. The conversion of MTT (3[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide) to a blue formazan precipitate is used to assess cellular metabolism and, ultimately, cell viability, after exposure to a test article for various times. The duration of exposure resulting in a 50% decrease in MTT conversion in the test article treated human cell constructs is determined as the t_{50}.

In studies with Niacinamide, the t_{50} results for the 10% Niacinamide-containing formulations were 3.3 h and 11.2 h (Procter and Gamble 2000c) and for a 3.5% Niacinamide-containing moisturizer, the t_{50} was 20.5 h (Procter and Gamble, 2000d). All formulations were classified as mild or innocuous to slight ocular irritants. For comparison, the positive control, 0.3% Triton X-100 (Octoxynol-9), gave a t_{50} reading of 19.6 minutes (Procter and Gamble 2000c and 2000d).

GENOTOXICITY

Niacinamide (Litton Bionetics, Inc. 1977a) and Niacin (Litton Bionetics, Inc. 1977b) were tested at 5, 0.5, 0.05, 0.005, and 0.0005% w/w or v/v in *Saccharomyces cerevisiae*, strain D4 and *Salmonella typhimurium* strains TA 1535, 1537, 1538, 98, and 100 with and without metabolic activation. Liver homogenates were prepared from the following mammalian species: ICR random bred adult male mice, Sprague-Dawley adult male rats, and *Macaca mulatta* adult male monkeys. The positive controls used in direct and activation assays were: methylnitrosoguanidine, ethylmethanesulfonate, 2-nitrofluourene, quinacrine mustard, dimethylnitrosamine, 2-acetylaminofluorene, 8-aminoquinoline, 2-aminoanthracene. Neither Niacinamide nor Niacin exhibited mutagenic activity in any of the assays used in these studies.

Utakoji et al. (1979) demonstrated that Niacinamide can produce an increase in sister chromatid exchanges (SCEs) in Chinese hamster ovary cells in culture. Oikawa et al. (1980) extended that work by postulating that inhibitors of poly(ADP-Rib) polymerase such as Niacinamide may induce SCEs. The authors confirmed the earlier finding that Niacinamide did produce an increase in SCEs, but that Niacinamide was not as effective as benzamide or m-aminobenzamide. A linear relationship was reported between SCEs and poly(ADP-Rib) polymerase inhibition. Incidental to examining this hypothesis, these authors reported that 3 mM Niacin did not induce SCEs.

Sims et al. (1981) studied the effect of 2 mM Niacinamide on unscheduled DNA synthesis on resting human lymphocytes.

In cells treated with UV irradiation or with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), Niacinamide caused a two-fold stimulation of unscheduled DNA synthesis and retarded the rate of NAD^+ lowering caused by these treatments. Niacinamide also reduced the burst of poly(ADP-ribose) synthesis caused by MNNG treatment. The effect of Niacinamide on unscheduled DNA synthesis was shown to be independent of protein or polyamine synthesis.

Miwa et al. (1981) examined the effects of Niacinamide and Niacin (20mM) on unscheduled DNA synthesis in normal human lymphocytes and in peripheral lymphocytes of a patient with xeroderma pigmentosum, with or without 20 J/m² UV radiation at 254 nm. Unscheduled DNA synthesis was measured by the incorporation of [³H]-thymidine (Thd). In the absence of UV radiation, Niacinamide and Niacin had little effect on [³H]-Thd incorporation. In the presence of UV radiation, Niacinamide produced a 6.3 fold increase in [³H]-Thd incorporation (compared to UV radiation alone), and Niacin produced only a 1.1 fold increase, which was probably not different from UV alone.

Barra et al. (1982) reported a study of the impact of Niacinamide on the action of bleomycin, a known carcinogen. After a 30 min incubation with 200 μg/ml bleomycin, [³H]dThd incorporation into DNA was stimulated during a subsequent 30 min incubation with hepatocytes of BUF rats but was decreased in HTC (hepatoma) cells of BUF rats. A dose of 20 mM Niacinamide and iso-nicotinamide caused an approximately 50% inhibition of total [³H]dThd incorporation in HTC cells. Significant inhibitory effects of 20 mM Niacinamide and isonicotinamide on unscheduled DNA synthesis were observed after preincubation of hepatocytes and HTC cells with bleomycin. When the effects of bleomycin on DNA structure were assessed fluorometrically with ethidium bromide after mild alkaline incubation, Niacinamide and isonicotinamide did not significantly affect the damage resulting from bleomycin alone. Niacinamide and isonicotinamide also prohibited the proliferation of HTC cells, but the effects were not additive with the effect of bleomycin.

In a study by Lawson (1983), a single dose of Niacinamide (350 mg/kg bw, intraperitoneally) given 3 h after a single dose of the carcinogen N-nitrosobis (2-oxopropyl) amine (BOP) (10 mg/kg bw, subcutaneously) stimulated the repair of DNA induced by BOP in the hamster pancreas (male Syrian golden hamsters). DNA damage was measured by alkaline elution 24 h after dosing with BOP. Niacinamide given up to 1 h before BOP was ineffective. The rate of unscheduled DNA synthesis was not stimulated by Niacinamide.

An Ames test (Ishidate et al. 1984) using *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98, with and without metabolic activation, and a chromosome aberration test using a Chinese hamster fibroblast cell line were carried out on both Niacin and Niacinamide. At a maximum dose of 50 mg/plate and 2 mg/ml, Niacinamide was negative in both the Ames and chromosome test, respectively. In the Ames and chromosome test, Niacin tested negative at maximum concentrations of 10 mg/plate and 2 mg/ml, respectively. However, in a later study, Ishidate et al. (1988) reported that Niacinamide at concentrations of 3 mg/ml (25 mM) induced large structural chromosome aberrations in vitro in Chinese hamster ovary cells.

Kjellen et al. (1986) measured the effects of Niacinamide on ADP-ribosyl transferase activity (ADPRT), unscheduled DNA synthesis (UDS), NAD^+- and ATP-pools and cytotoxicity in γ-irradiated human mononuclear leukocytes. The data were presented as a percentage of the untreated control at 37°C.

In the presence of 2 mM Niacinamide, the γ-induced UDS level at 37°C increased approximately 70%, and the γ-induced UDS with Niacinamide was higher over the whole temperature range (37–46°C) compared to γ-induced UDS without Niacinamide. The radiation-induced increase in ADPRT activity was inhibited by 2 mM Niacinamide to about 50% and remained at about that level over the entire temperature range tested. The NAD^+ level increased ∼50% in the presence of 2 mM Niacinamide at all temperatures tested except 46°C. The authors concluded that Niacinamide had no effect on the ATP pool (Kjellen et al. 1986).

In a study of ornithine decarboxylase (ODC) activity and DNA synthesis rates by Rosenberg et al. (1986), four groups of eight male Fischer 344 rats were each injected intraperitoneally three times with water or 500 mg/kg bw of Niacinamide (0.1 ml) in water. The rats were injected 4, 12, and 24 h before they were killed. The control animals received a water injection at all time intervals, but the other three groups received an injection at either the 4, 12, or 24 h times and a water injection at the other time intervals. ODC was measured. Four groups of sixteen Fisher 344 rats were given 0, 0.67, 6.7, and 30 mM Niacinamide in the drinking water. Eight animals per group were killed after 7 days and eight after 28 days on Niacinamide, and the ODC activity was determined. DNA synthesis was measured after the chronic and acute exposures.

Four hours after an intraperitoneal injection of 500 mg/kg Niacinamide, there was a decrease in kidney ODC activity, followed by a substantial increase by 24 h. Rats exposed to 0, 0.67, 6.7, and 30 mM Niacinamide in their drinking water for 7 and 28 days also exhibited a statistically significant increase in kidney ODC activity, but the rate of DNA synthesis was unaffected (measured by the incorporation of [³H]thymidine into DNA and % labeled proximal tubule nuclei) and the total amount of DNA/kidney was also unaffected (Rosenberg et al. 1986).

Weitberg (1989) studied the ability of Niacin to protect human lymphocytes in vivo against oxygen radical-induced DNA strand breakage. NAD^+ concentrations increased in lymphocytes to nearly 5 times baseline levels in human volunteers ingesting Niacin (100 mg/day) for 8 weeks.

Strand breaks decreased proportionately to NAD^+ concentrations over this time period in lymphocytes exposed to oxygen radicals. After 8 weeks of supplementation with Niacin, radical-treated lymphocytes incubated for 24 h had significantly less DNA damage compared to non-treated controls (Weitberg 1989).

Riklis et al. (1990) studied the effects of addition of Niacinamide (1 mM, 3 mM, and 5 mM concentrations) on DNA repair capacity following γ- and germicidal ultraviolet radiation in both repair-proficient and repair-deficient cell lines. The cells used were K-1735 mouse melanoma non-metastatic C1-11 cells (mouse melanoma), XP 2 OSA cells (xeroderma pigmentosum), and Hep-2 cells originating from a human carcinoma of the larynx. XP cells are highly sensitive to germicidal UV radiation because of DNA repair deficiency, but less sensitive to γ radiation.

The addition of Niacinamide at any concentration did not cause modification of the repair ability of the XP cells after germicidal UV radiation. Exposure to these cells to γ radiation resulted in a certain level of repair capacity, and the presence of Niacinamide caused a marked increase of the repair capacity, with a maximum effect at 3 mM Niacinamide. Higher concentrations of Niacinamide caused a reduction in the repair capacity to a level similar to that of the control cells lacking the additional Niacinamide.

The mouse melanoma cells had a high repair capacity following γ-irradiation. Additional Niacinamide caused an increase in repair capacity with a maximal increase obtained at 3 mM, while higher concentrations reversed the effect. The mouse melanoma had a low capacity for repair of germicidal UV radiation damage and the addition of Niacinamide at different concentrations caused further decrease in repair capacity. Hep-2 cells had a high capacity for repair of DNA damage following exposure to both γ-irradiation and germicidal UV radiation. The addition of 3 mM Niacinamide caused a two-fold increase in the DNA repair capacity after exposure to both the γ-irradiation and germicidal UV radiation (Riklis et al. 1990).

Zheng and Olive (1996) used the alkaline comet assay to measure both tumor hypoxic fraction and DNA strand break rejoining kinetics in individual cells from tumors and tissues of C3H/HeN mice exposed to ionizing radiation and Niacinamide.

The percentage of hypoxic cells in SCCVII murine squamous cell carcinoma decreased from 18.4 to 4.4% in mice injected with 200 mg/kg bw Niacinamide 30 min before irradiation. At higher doses (500 and 800 mg/kg) Niacinamide increased the half-time of strand break rejoining in tumor, thymus, spleen, bone marrow, and testis from 10–20 min to 40–80 min. Cells with extensive breaks appeared 24 h after treatment with Niacinamide and radiation alone.

For most tissues, damage was more consistent with necrosis than with apoptosis. The percentage of heavily damaged cells was dependent on tissue type, time after radiation, radiation dose, Niacinamide dose, and sequence of administration. In SCCVII tumors, Niacinamide enhanced radiation-induced cell killing primarily in cells close to vasculature, but in tumors clamped before irradiation, 500 mg/kg Niacinamide did not increase the number of cells killed (Zheng and Olive, 1996).

Niacinamide was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100. The tests were conducted on agar plates in the presence and absence of a preparation made from livers of male Sprague-Dawley rats treated with Aroclor 1254. The co-factors required for mixed-function oxidase activity were also present in the preparation. Under the conditions of this study, Niacinamide was not mutagenic or toxic up to a dose level of 3.3 mg per plate in any of the tested strains (Unilever, 1998m).

CARCINOGENICITY

There were no reported studies of the carcinogenicity of Niacin. Only one study (Toth, 1982) evaluated the carcinogenicity of Niacinamide alone. All other reports address some aspect of the carcinogenicity of Niacinamide in combination with other agents.

Roe (1964) studied the effect of Niacinamide on a known carcinogen in mice, 9,10-dimethyl-12-benzanthracene (DMBA). Niacinamide was administered in the drinking water to mice of both sexes (120 animals) for 4 weeks ($\sim$ 250 mg/kg bw per day). A single application of DMBA on the skin was followed, after a three week interval, with weekly paintings of croton oil for 15 weeks. Niacinamide had no effect on the development of skin tumors in response to DMBA application.

Rakieten et al. (1971) reported results of a study in which streptozotocin, a naturally occurring nitrosourea, was given to rats in combination with Niacinamide. Groups of male Holtzman rats were given a single 50 mg/kg iv dose of streptozotocin or two 350 mg/kg ip doses of Niacinamide at 3 h intervals. Other rats were given the same doses of Niacinamide 10 minutes before and 180 minutes after the streptozotocin dose. Control rats received only the streptozotocin vehicle.

Pancreatic islet tumors occurred in 18/28 rats treated with both streptozotocin and Niacinamide. The earliest tumor was detected on day 226. Only 1/26 rats treated with streptozotocin alone developed a tumor and that tumor first appeared on day 543. The last animals were sacrificed on days 547–551. No tumors were detected in the 27 rats receiving Niacinamide alone or in the 26 control rats. The authors concluded that Niacinamide appeared to act as a cocarcinogen in the production of pancreatic islet tumors (Rakieten et al. 1971).

Rakieten et al. (1976) further reported that streptozotocin-produced renal adenomas in the rat can be reduced by treatment with Niacinamide. Male Holtzman rats received either 50 mg/kg streptozotocin iv; 50 mg/kg streptozotocin with 350 mg/kg doses of Niacinamide given 10 minutes before and 3 h after; 350 mg/kg doses of Niacinamide given 3 h apart; or the streptozotocin vehicle alone.

Renal adenomas occurred in 20/26 rats receiving streptozotocin, but in only 5/28 rats receiving both streptozotocin and Niacinamide. Tumors in the rats receiving streptozotocin alone appeared earlier (day 165) than in rats receiving streptozotocin and Niacinamide (day 281). No renal adenomas appeared in either the Niacinamide (27 rats) or control (26 rats) groups. The authors concluded that the renal oncogenic activity of streptozotocin is significantly decreased by Niacinamide (Rakieten et al. 1976).

Schoental (1977) described previous studies which found that the pyrrolizidine alkaloid, heliotrine, in combination with Niacinamide produces pancreatic islet tumors, and presented further work investigating the effect of Niacinamide on diethyl-nitrosamine (DEN) carcinogenesis. Niacinamide was given ip to pregnant Wistar rats at doses of 350–500 mg/kg 10 minutes before and 3 h after each dose of DEN (100 mg/kg). Another group received DEN alone and another group receiving neither DEN nor Niacinamide served as a control.

There was one pancreatic islet tumor in the DEN group and one in the DEN/Niacinamide group. In the DEN group there were 6/9 animals with liver tumors, 1/9 with kidney tumors, and 4/9 with nasal tumors. These compare with 3/9 with liver tumors, 5/9 with kidney tumors, and 4/9 with nasal tumors in the DEN/Niacinamide group. No tumors were seen in the contol group. The authors concluded that more kidney tumors appeared to develop in the rats that had received Niacinamide + DEN compared to DEN alone (Schoental 1977).

Pamukcu et al. (1981) exposed 84 male and female albino rats, divided into four groups, to a diet containing a preparation of bracken fern (0.33 g/g of basic diet), Niacinamide (5 mg/g of basic diet), both (at the same concentrations), or neither. Niacinamide alone did not produce any tumors. Intestinal tumors developed in 88% of the male and 91% of the female rats receiving a preparation of bracken fern in the diet. Bladder tumors developed in 75% of the male and 73% of the female rats receiving a preparation of bracken fern in the diet. The Niacinamide plus the bracken fern preparation reduced the incidence of intestinal tumors to 45% in the male rats and 50% in the female rats, and of bladder tumors to 45% in males and 31% in females. The authors concluded that Niacinamide had a protective effect.

Toth (1982) studied the carcinogenic effect of Niacinamide itself. A solution of 1% Niacinamide was administered in the drinking water of Swiss albino mice (50 male and 50 female) for life that were six weeks of age at the beginning of the experiment. An untreated control group of 100 male and 100 female mice were observed until death. All animals were killed if in poor condition or were allowed to die and complete necropsies were performed. The average daily consumption of the 1% Niacin drinking water was 6.6 ml for the females and 10.0 ml for males. The treatment had no substantial effect on survival rates when compared to untreated controls and had no carcinogenic effect (Toth, 1982).

Pour and Lawrence (1984) studied the effect of Niacinamide on the induction of pancreatic ductal adenomas and carcinomas by N-nitrosobis(2-oxopropylamine) (BOP) in Syrian golden hamsters. The hamsters, males and females, were divided into 6 groups. Group 1 received a single injection of BOP, 10 mg/kg bw subcutaneously. Groups 2, 3, and 4 were given Niacinamide (350 mg/kg intraperitoneally) once 10 min before and once 3 h after the same dose of BOP, streptozotocin (30 mg/kg) alone, or BOP plus streptozotocin in combination, respectively. Group 5 received two doses of Niacinamide three hours apart (same dose); and group 6 received saline. The animals were checked periodically during the first 3 weeks and twice daily thereafter. Moribund hamsters were killed and necropsied immediately. Surviving hamsters were killed 52 weeks after the BOP injection and were subjected to complete necropsies.

Hamsters in groups 2, 3, 5, and 6 did not develop ductal-ductular carcinomas, whereas 5 of 60 animals in group 1 and 4 of 43 animals in group 4 did. Niacinamide did not affect either the incidence or multiplicity of ductular proliferation, whereas it inhibited ductular adenoma development only in hamsters treated with BOP alone, but not in BOP plus streptozotocin animals (Pour and Lawrence, 1984).

Rosenberg et al. (1985) conducted a tumor promoter experiment in male F-344 rats ($\sim$150 g body weight) treated with Niacinamide as the tumor promoter. All rats were subject to a 70% partial hepatectomy and divided into 5 groups. One group (10 rats) received an ip injection of DEN (25 mg/kg) in water 24 h post-hepatectomy. Another group (10 rats) received only a water ip injection, followed 2 weeks later with the addition of 30 mM Niacinamide to their drinking water. A third group (15 rats) received the DEN injection, followed 2 weeks later with the addition of 30 mM Niacinamide to their drinking water. A fourth group (10 rats) was identical to the third group except that the concentration of Niacinamide was only 6.7 mM. Presumably the fifth group received only a water injection, although the study does not report any results for this group. Daily Niacinamide consumption was estimated as 366 mg/kg/day (average weight is 0.25 kg). The study was terminated at 20 months and surviving animals sacrificed.

The group which received DEN only developed renal tubular cell tumors in 1/20 kidneys examined. The group receiving only Niacinamide developed no renal tumors in the 20 kidneys examined. There were tumors in 13/22 kidneys examined in the DEN/Niacinamide (30 mM) group (note—apparently 4 animals in this group died before the study termination). Tumors developed in 5/18 kidneys examined in the DEN/Niacinamide (6.7 mM) group (note—apparently 1 animal in this group died before study termination). The authors stated that, while data from the control group supports the conclusion that Niacinamide is not a rat carcinogen, in rats initiated with diethyl-nitrosamine, Niacinamide appeared to act as a kidney tumor promoter. The authors indicated that the mechanism underlying this effect is unclear (Rosenberg et al. 1985).

Gotoh et al. (1988) evaluated the effect of Niacinamide on urethane-induced lung tumorigenesis in mice. A single subcutaneous injection of urethane (1.0 mg/g) was given to 28-day-old female JCL:ICR mice. The mice were also fed a powdered diet containing 0 (31 animals), 1% (51 animals), and 2.5% (54 animals) Niacinamide for 10 days. The diet was changed and weighed every day to determine the amount of Niacinamide consumed by the mice. The mice were killed 5 months after the urethane treatment. Gross pathological lesions, especially tumors were examined.

Most of the tumors induced were in the lungs. Lung tumor frequency, defined as the average number of tumor nodules per

lung, increased with the dose of urethane, whereas the increase in tumor incidence, defined as the percentage of tumor-bearing mice among survivors leveled off at urethane concentrations above 0.2 mg/g bw in earlier experiments. Urethane-initiated lung tumorigenesis was significantly inhibited by post-treatment with Niacinamide in the diet. Inhibiting effects increased with doses of Niacinamide, resulting in 35% and 62.8% inhibition at dietary concentrations of 1 and 2.5% Niacinamide, respectively (Gotoh et al. 1988).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In Vitro

Teratogenic and nonteratogenic compounds, including Niacin, were tested for their ability to interfere with normal growth and differentiation of mouse N1E–115 neuroblastoma cells. A substance that had introduced the appearance of neurites in more than 5% of cells, a level significantly above control values under these conditions, was scored as positive. Control cells were cultured in medium containing serum. The Niacin toxic dose was $>1 \times 10^{-2}$ M and the no effect dose was 1×10^{-2} M. There was no interference with growth and differentiation (Mummery et al. 1984).

Niacinamide (5 μM) had no detectable stimulation or inhibition on cleavage stage development or morula/blastocyte formation in hamster embryos (McKiernan 2000). In another study, Tsai and Gardner (1994) reported that mouse zygotes cultured in an amino acid free medium supplemented with (1) minimal essential medium B-group vitamins (8.2 μM Niacinamide) had decreased cleavage rates and (2) Ham's F-10 medium B-group vitamins (5.0 μM Niacinamide) had both decreased cleavage rates and reduced morphological development. These authors concluded that Niacinamide inhibits mouse embryo development in culture and reduces embryo viability.

Animal

The teratogenic potential of Niacinamide was studied by Bionetics Research Labs, Inc. (1968). Pregnant BL6 mice were given 61 mg/kg bw Niacinamide administered subcutaneously in DMSO (dimethyl sulfoxide) daily beginning on the 6th day and continuing through the 14th day of pregnancy. The mice were sacrificed on the 18th day of gestation and the following effects were studied; implantation and/or fetal mortality, fetal weight, volume of amniotic fluid, fetal development, maternal weight gain, and maternal liver weight. There was a reduction in the amount of amniotic fluid per fetus and maternal liver weight but no significant effects on the mouse fetuses were seen.

Effect on Reproductive/Developmental Toxicity Induced by Other Agents

A 30-mg i.p. dose of Niacinamide given to mice (approx. 900 mg/kg bw) on gestation day 6 had no adverse effect on development. Niacinamide had no effect on the developmental toxicity of sulfadiazine given i.p. as 12 mg of the sodium salt on day 6, and as 0.8% of the diet on the 6th and 7th day of gestation (Bass et al. 1951).

Chamberlain (1967) studied the effects of 50–100 mg/kg i.p. of Niacinamide at various stages of pregnancy in rats and found no effects. When given 2 h before, simultaneously, or within 1 h after single injection of 8 mg/kg 2-aminonicotinamide (on gestation day 15), Niacinamide reduced the incidence of cleft palate. When given 12–24 h before or 2–72 h after 2-aminonicotinamide, there was no protective effect.

Beaudoin (1973) reported that injection of 50-mg Niacinamide (approximately 250 mg/kg, assuming 200 g body weight) into the peritoneal cavity of female Wistar rats on gestation day 11 had no adverse effects, and reduced the teratogenic and lethal effects of 2-amino-1,3,4-thiadiazole hydrochloride.

Gotoh et al. (1988) studied the effects of Niacinamide on urethane-induced teratogenesis. Pregnant JCL:ICR female mice received a single subcutaneous injection of urethane (1 mg/g bw) at 14:00 h on day 9 of gestation. Immediately after urethane treatment, 5 doses of Niacinamide at 0 (18 animals), 0.1 (17 animals), 0.3 (18 animals), or 0.5 (18 animals) mg/g bw were given intraperitoneally at 6 h intervals.

In other experimental groups, 5 doses of Niacinamide (0.5 mg/g) were similarly injected during a period of 24–48 h or 48–72 h after urethane treatment. Urethane (1.0 mg/g) or the highest dose (0.5 mg/g) Niacinamide were given to pregnant mice using the same time schedule.

Pregnant JCL:ICR mice were also fed a powdered diet containing Niacinamide at 0 (18 animals), 0.5% (19 animals), 1% (19 animals), 3% (12 animals), or 5% (11 animals) concentration during a period 0–48 h after a single injection of urethane (1.0 mg/g) on gestation day 9.

Niacin (1% concentration) was also given i.p. (0.5 mg/g bw) or orally after the same urethane treatment. As a control, the highest dose of Niacinamide (5% in diet) was given to pregnant mice (number not stated) without urethane treatment using the same time schedule. The pregnant mice were killed on day 18. After hysterectomy, implants, early deaths, late deaths, and living fetuses were recorded. Living fetuses were weighed and examined for external and skeletal malformations.

Urethane induced significant numbers of malformations. The predominant types of induced malformations were polydactyly, cleft palate, and tail anomalies. The incidence of urethane-induced malformation was significantly suppressed by Niacinamide given intraperitoneally immediately after urethane treatment. Inhibiting effects increased with increasing doses of Niacinamide. Polydactyly and tail anomlies were markedly reduced by post-treatment with Niacinamide, while cleft palate was not.

The antiteratogenic effects of Niacinamide were still significant when it was given during the period 24-48 h after urethane, but not at 48–72 h after. Urethane malformations were also suppressed by the post-treatment with low doses of Niacinamide in the diet. However, the inhibiting effects of dietary Niacinamide at higher doses were less than those at lower doses.

The inhibiting effects of Niacinamide increased almost linearly at low dose range. Actual intake of Niacinamide was 1, 1.6, 4.5, and 4.7 mg/g bw for 48 h when the diet containing Niacinamide was given at concentrations of 0.5, 1, 3, and 5%, respectively. High doses of Niacinamide, given both in the diet and intraperitoneally, resulted in a slight decrease in body weight gain of pregnant mice, but there were no differences in the body weights of live fetuses between all Niacinamide-treated groups and controls.

The highest doses of Niacinamide given in the diet (5%) and intraperitoneally (0.5 mg/g) were not teratogenic. When Niacin was given to pregnant mice after urethane treatment, there were no significant differences in the incidence of malformations from the urethane-treated controls.

To further study the effect of Niacinamide on spontaneous malformations, four intraperitoneal injections of Niacinamide (0.5 mg/g) were given to pregnant CL/Fr mice daily at 14:00 h from gestation day 7 to day 10. Pregnant CL/Fr mice were also fed powdered diet containing Niacinamide at a concentration of 0.5% or 1% during the period of gestation day 7 to day 10. The powdered diet containing Niacinamide was given at 14:00 h on gestation day 7 and stopped at 14:00 day 10. The diet was weighed and changed every day. About 30% of the CL/Fr fetuses developed cleft palates and lips spontaneously. The mice were killed on day 17 and gross anomalies, especially cleft lips and palates, were examined.

Cleft palates and lips, which developed spontaneously in the CL/Fr mice, were suppressed by dietary Niacinamide (0.5%). The level of inhibition was 38.5%. However, the data were not consistent. Higher doses of Niacinamide (1% in diet) did not suppress malformations. The Niacinamide injections from gestation day 7 to day 10 did not suppress spontaneous malformations (Gotoh et al. 1988).

CLINICAL ASSESSMENT OF SAFETY

The Select Committee of the FASEB in their evaluation of Niacin and Niacinamide as food ingredients concluded that, "there is no evidence in the available information on Niacin or Niacinamide that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future" (FASEB 1979).

Goldsmith (1958) reviewed the studies linking both dietary Niacin and tryptophan to pellegra and estimated the minimal daily human dietary requirement for Niacin. The author noted that tryptophan is effective in the diet because it is converted to Niacin in humans. Diets which result in Niacin deficiency furnished 3.4 to 5.4 mg of Niacin and 151 to 207 mg of tryptophan daily. The author went on to calculate (assuming 60 mg of tryptophan $\equiv$ 1 mg Niacin) that the "niacin equivalent" of the above diets ranged from 5.9 to 8.8 mg. Diets which were not deficient had "niacin equivalents" between 7.4 and 10.6 mg. The author estimated the normal dietary need in humans to be around 9 mg daily.

Dermal Irritation

Unilever (1998n) examined a skin cream with 1% Niacinamide and 5% potassium lactate and a skin lotion with 1% Niacinamide in skin irritation studies. The panel of volunteers was comprised of 22 healthy adults (11 males and 11 females). Both a negative and positive control (both skin creams) were used in this study. Two rows of 'Al-test' patches were placed onto 5 cm wide Leukosilk tape, thus providing four site patches.

At the time of treatment, 40 μl of each test and control substance were applied to filter paper discs on the patch. Each filter paper disc held one test or control substance. Patches were then prepared to provide random treatment. The patches were applied to the outer surface of the upper arm, on the side of the biceps. Patches were removed 23 h after the first application. Sites were individually wiped with tissues moistened with distilled water and dried. A mark was made along the lower edge of the 'Al-test' square for each site.

Assessments were taken an hour after patch removal. Immediately after assessment, a fresh patch was applied to each treatment site. Any sites that were judged as having an unacceptable degree of reaction were not treated for the second 23 h period. The second patches were removed in the same manner as the first patches. Sites were assessed one hour after removal (the 48 h assessment) and 25 h after removal (the 72 h assessment). Erythema and dryness were assessed visually under artificial daylight and recorded. Other assessment parameters, such edema, wrinkling, glazing, and vesicles were also assessed and recorded as comments by the assessor. All assessments were made according to a scale where 0 = no reaction to 4 = strong reactions. Erythema at grade 3 or above was considered too great for any further treatment.

Using the Steel's Test, the positive control produced significantly more erythema than the negative control at the 48 h assessment. The levels of dryness elicited by all of the substances were low during the whole study. At the 48 h assessment, the test skin cream produced more erythema than the test lotion. Using the Sign test, this difference was statistically significant. The test cream elicited higher levels of erythema than the negative control although the difference was not statistically significant. The negative control and the test skin lotion elicited similar low levels of erythema.

There were a total of 25 reactions which reached a grade of 3 or more by the 48 h assessment; two to the negative control, 16 to the positive control, six to the test skin cream, and one owing to a reaction to the tape. At the 72 h assessment, an additional three reactions reached a score of 3 or greater; one to the test skin cream and two to the test skin lotion. Under the conditions of this study, the skin cream containing 1% Niacinamide and 5% potassium lactate produced higher levels of erythema than the skin lotion with 1% Niacinamide. Using the Sign test, the difference was statistically significant. The skin lotion produced low levels of irritation similar to that produced by the negative control (Unilever, 1998n).

Unilever (1998o) tested the skin irritation potential of a skin cream containing 3% Niacinamide on 32 healthy adults (13 males and 19 females). The patches were applied to the outer surface of the upper arm, on the side of the biceps. Two rows of 'Al-test' patches were placed onto 5 cm wide Leukosilk tape, thus providing four site patches. At the time of treatment, 40 μl of each test and control substance was applied to filter paper discs on the patch. Each filter paper disc held one test or control substance. The patches were removed 23 h after the first application. The sites were individually wiped with tissues moistened with distilled water and dried.

Assessments were taken an hour after patch removal (the 24 h assessment). Immediately after assessment, a fresh patch was applied precisely to each treatment site. Any sites that were judged as having an unacceptable degree of reaction (grade 3 or higher) were not treated for the second 23 h period. The second patches were removed in the same manner as the first. The sites were assessed one hour after removal (the 48 h assessment) and 25 hours after removal (the 72 h assessment). Erythema and dryness were assessed visually under artificial daylight and recorded. Other assessment parameters, such as edema, wrinkling, glazing, and vesicles were also assessed and recorded as comments by the assessor. Reaction grades ranged from 0 (no reactions) to 4 (strong or outstanding reactions).

One panelist experienced a sensory reaction requiring the patch to be removed after approximately six hours. The erythema and dryness data for this panelist were not included in the analyses. Using the Steel's test, the positive control produced significantly more erythema than the negative control at the 48 h assessment. The level of irritation elicited by each material was ranked as: positive control > skin cream with 3% Niacinamide > negative control. The levels of dryness elicited by all the materials were low during the whole study. Under the conditions of this study, a skin cream with 3% Niacinamide produced marginally more erythema than the negative control (Unilever 1998o).

Unilever (1998p) assessed the skin irritation potential of a skin cream containing 1% Niacinamide in humans using a simulated use test. A skin cream formulation, marketed with a history of safe use and routinely used as a standard, was tested as supplied for comparison. The test substance and standard were applied to matched skin sites (inner face of the arm, between the elbow crease and point of the elbow) six times daily for 21 days. Each volunteer administered his or her own treatment. Skin condition was assessed on day 1 before treatment began, and throughout the test. The panel comprised of 22 adults (10 male and 12 female). Each volunteer was provided with two containers of cream each labeled according to which arm the test cream or standard should be applied and a card giving assessment times and providing space for each panelist to record the number of treatments achieved each day. Each panelist was asked to attempt six treatments each day. The test substance was applied equally to each arm. Skin condition was assessed before treatment began and early on days 3, 5, 8, 11, 15, and

19 of the test and during the recovery period on days 23 and 24. The condition of the skin in the treated areas was classified according to the definitions: 0 = no reaction; 1 = slight reaction, barely perceptible; 2 = distinct reaction, well-developed redness of a part of the site; 3 = well-developed reaction of the whole site; 4 = strong reaction, red or "burn like" color. The number of reactions at each grade associated with the test substance and standard were compared. Skin reactions caused by the test substance were compared with those caused by the standard in the same person. The results were analyzed statistically by the Sign Test and marginal differences were analyzed by Wilcoxon's Matched-Pairs Signed-Ranks Test. The median grade of erythema for both test substance and the standard was determined throughout the test.

Following a comparison of reactions in matched pairs of treatment sites, there were no significant differences at any time. The median number of treatments for the whole panel was 5.81. Under the conditions of this test, the skin cream containing 1% Niacinamide was not significantly more irritating than the standard tested for comparison (Unilever 1998p).

In a follow-up study, the skin irritation potential of 5% Niacinamide was assessed in humans using a simulated use test. The test substance was Niacinamide at 5% in distilled water and the negative control was distilled water alone. Material was applied to matched skin sites (the inner face of the arm, between elbow crease and point of elbow) with roll ball applicators six times daily for 21 days. Each panelist administered his/her own treatment.

Skin condition was assessed on day 1 before treatment began and early on days 3, 5, 8, 11, 15, and 19 of the test, and days 22 and 25 of the recovery period. The panel was comprised of 23 adults (11 male and 12 female). The condition of the skin in the treated areas was classified according to the definitions provided in the previous study by Unilever. The analysis of results was also conducted in the same manner as the previous study by Unilever. The median grade of erythema for both test substance and standard was 'slight' throughout the test. On day 19, one panelist had a well-developed erythema and distinct dryness too great for further treatment.

Following a comparison of reactions in matched pairs of treatment sites, there were no significant differences at any occasion. The median number of treatments for the whole panel was 5.81. Under conditions of this study, 5% Niacinamide in distilled water was not a significant irritant (Unilever 1998q).

Unilever (1998r) investigated the skin stinging potential of a skin cream containing 1% Niacinamide and 5% potassium lactate and a skin lotion containing 1% Niacinamide in 23 panelists by application to the nasolabial fold (one substance to each side). The panel was comprised of 23 healthy adults (8 male and 15 female). Of this panel, 14 panelists were previously categorized as "stinger," two were categorized as "inconsistent stinger" and seven were categorized as "non-stinger." Before the treatment phase began, the panel was balanced for stinging category and sex. The assessment of stinging was carried out on a

self assessment basis by means of a questionnaire. The panelists recorded the appropriate level of stinging (none, slight, moderate, or severe) at each of the four time intervals; immediate (<10 sec), 2.5 min, 5 min, and 8 min after application. Panelists were asked to record any other sensations in a similar manner and describe the sensation, e.g. itch, tingle, or burn. The condition of the nasolabial folds was assessed prior to application, immediately after washing off the substances and 24 h after application. Erythema and dryness were assessed visually under artificial daylight and were recorded; other parameters such as edema, wrinkling, glazing, and vesicles were also assessed and recorded as comments by the assessor.

During the eight minute treatment, if an individual panelist experienced a severe sensory effect then both substances were washed off immediately. These responses would continue to be recorded and were included in the analysis. Three panelists experienced a slight stinging response; one at 2.5, 5, and 8 min to the skin lotion, the second to the skin lotion at 2.5 and 5 min, and the third to the skin cream immediately, 2.5 and 8 min after application. Two of the three were previously identified as "stingers" in the pre-screen study, the remaining panelist was classified as a "non-stinger." Two other panelists experienced one episode of stinging during the eight min application, one to each of the test substances. None of the remaining 18 panelists experienced any stinging during the eight min application. Grades of reaction (erythema and dryness) were similar for both substances. There was a slight increase in grades from the initial assessment to the eight min assessment, but grades generally returned to pre-treatment values by the 24 h assessment.

The differences between the reactions elicited by the two test substances were not statistically significant. A total of 11 comments were made by the panelists or assessor during the study which were attributed to six of the panelists. Of these, four comments concerned the skin lotion and seven to the skin cream. Under the conditions of this study, both the skin cream and skin lotion elicited similar low levels of reaction. Neither the cream nor lotion showed any significant potential to produce stinging during the test period (Unilever 1998r).

In a follow-up study, the skin stinging potential of two skin creams containing 1% and 3% Niacinamide was investigated by application of the creams to the nasolabial fold (one substance to each side). The panel was comprised of 24 healthy adults (11 male and 13 female). In a pre-screening study, 13 panelists were classified as "stingers," three were classified as "inconsistent" and eight were categorized as "non-stingers." The study procedure and methods are explained in the previous Unilever study.

Only three panelists experienced any stinging sensation. One panelist reported slight stinging at 2.5, 5, and 8 min to the skin cream with 1% Niacinamide. Another panelist experienced slight stinging to the skin cream with 1% Niacinamide at only 2.5 min. The third panelist reported slight stinging to the skin cream with 3% Niacinamide at 5 min only. All three panelists were previously identified as "stingers." None of the remaining

TABLE 7
pH Measurements of Niacinamide Solutions used in Sting Potential Studies (Unilever, 1998t)

Niacinamide concentration (%)	pH	
	Before test	After test
1.0	6.06	6.04
2.5	6.18	6.15
5.0	6.96	6.92
10.0	6.15	6.10

21 panelists experienced any stinging during the eight minute application.

Grades of reaction for both erythema and dryness were similar for both substances. There were slight increases in the grades from the initial assessment at the eight minute mark, but grades generally returned to pre-treatment levels by the 24 hour assessment. Under the conditions of this study, the skin creams with 1% and 3% Niacinamide did not show a potential to produce stinging and no evidence of irritation was observed (Unilever 1998s).

Unilever (1998t) conducted further studies to evaluate the skin stinging potential of Niacinamide as a function of concentration were done in humans using a sensory perception or 'sting' test. The test substance, Niacinamide, was made to a clear colorless solution at concentrations of 1, 2.5, 5, and 10%. The pH of the treatment solutions were determined before and after treatment as seen in Table 7.

Distilled water was used as a negative control. A cotton wool bud soaked in the test substance was rubbed briskly over the nasolabial fold of one side of the face. The opposite side was treated in a similar manner with distilled water. Subjective assessments of stinging were made immediately 2.5, 5, and 8 min after treatment and then the substances were rinsed from the treatment sites. The average of the scores obtained at 2.5, 5, and 8 min after treatment resulted in an overall stinging assessment of none, slight, moderate, or severe.

The study consisted of four panels; each panelist had taken part in two or more previous tests using 10% lactic acid. Treatment group A (1%), comprised of 12 females and 12 males, included 5 panelists who had previously responded as 'moderate,' 1 as 'slight,' 9 as 'non,' and 9 as 'inconsistent' stingers to 10% lactic acid. Treatment group B (2.5%), comprised of 11 females and 13 males, included 6 panelists who had previously responded as 'moderate,' 4 as 'slight,' 8 as 'non' and 6 as 'inconsistent' stingers to 10% lactic acid. Treatment group C (5%), comprised of 12 females and 12 males, included 5 panelists who had previously responded as 'moderate,' 5 as 'slight,' 7 as 'non', and 7 as 'inconsistent' stingers to 10% lactic acid. Treatment group D (10%), comprised of 12 females and 12 males, included 3 panelists who had previously responded as 'moderate,' 5 as 'slight,' 8 as 'non,' and 8 as 'inconsistent' stingers to 10% lactic

acid. Erythema was assessed prior to treatment and 24 h after treatment. Stinging was assessed immediately, 2.5 min, 5 min, and 8 min after treatment.

Niacinamide at 1, 2.5, 5, and 10% in distilled water did not elicit any unacceptable stinging response in any panelist. At 1% one panelist had a moderate response, at 2.5% two had a slight response and 10% Niacinamide only elicited a slight response from one panelist. At each treatment concentration of Niacinamide, median grades of erythema were similar to distilled water. Under the conditions of this study, Niacinamide at 1, 2.5, 5, and 10% in distilled water did not have significant stinging potential to human skin (Unilever 1998t).

Procter and Gamble (1999c) evaluated two facial moisturizer products, one benchmark moisturizer (Product Code SC) and a cream containing 2% Niacinamide (Product Code SJ), for cutaneous and ocular tolerance under normal conditions of use in a 4-week study. Female subjects (n = 163) between the ages of 20 and 65 were enrolled, and 159 subjects completed the study. Eighty-one (81) subjects were randomized to use Product Code SC (79 completed) and 82 subjects to use Product Code SJ (80 completed). Subjects were all normal healthy women, ranging in age from 20 to 65, with a mean age of 44.6. Subjects were scored at baseline and after 4 weeks of regular (twice daily) product use for severity of erythema, dryness/scaling, and fissures of the face and neck, as well as with respect to the parameters of a typical slit-lamp ocular examination.

At the final dermatological evaluations, no subject showed any condition worse than slight localized erythema and/or skin dryness, and no subject showed a worsening of any condition observed at baseline. Seven subjects using Product Code SC and no subjects using Product Code SJ showed conditions of greater than trace severity on slit-lamp ocular examination at Week 4. None of the conditions represented a clinically significant observation attributable to use of the study product. This evaluation of 159 healthy normal female volunteers over a 4-week period of regular product use yielded no significant evidence of potential for cutaneous or ocular irritation of the two facial moisturizers tested (Procter and Gamble 1999c).

The potential for Niacinamide to induce skin irritation was investigated in a 21-day cumulative irritation study (Procter and Gamble 2000a). Subjects (n = 25) were exposed to various products containing 2% and 5% Niacinamide and non-niacinamide-containing moisturizing creams under standard test conditions using both semi-occlusive and occlusive patches. All of the formulations had a low irritancy potential regardless of the patch, i.e., occlusive or semi-occlusive. No relationship between Niacinamide concentration and skin irritation was demonstrated, and no significant differences were found in the irritancy potential between the formulas with and without Niacinamide. These data show Niacinamide-containing formulas to be generally well tolerated.

Information on skin irritation was generated when Procter & Gamble (1998b, 1999a, and 1999b) conducted several Human Repeat Insult Patch Tests (HRIPT) to investigate the effects of a moisturizer (oil-in-water emulsion) containing 0%, 2%, 10% and 20% Niacinamide on delayed contact hypersensitivity. In these studies, the formulations were applied neat to the skin under semi-occlusive and occlusive patch conditions, 3 times per week (24 h at a time) for 3 weeks. Following removal of the patch, the skin was graded for signs of irritation.

There were no signs of skin irritation observed in these studies. These data provide information as to the cumulative effects of Niacinamide on skin irritation, as the skin is in prolonged contact (23 h patches applied 3 times weekly for 3 weeks) with Niacinamide, under conditions that enhance penetration. From these studies, it was concluded that exposure to moisturizers containing Niacinamide at levels up to 20%, does not induce significant skin irritation (Procter & Gamble 1998b, 1999a, and 1999b).

Dermal Sensitization

Three separate HRIPT tests were conducted to investigate the effects of oil-in-water emulsions containing Niacinamide, 0, 1, 2.5 and 10 mg/cm^2 under semi-occlusive and occlusive patch conditions (Procter & Gamble 1998b, 1999a, 1999b). The number of volunteers was 100 per study. There was no evidence of the induction or elicitation of delayed type hypersensitivity in any volunteer.

Phototoxicity

There was no evidence of phototoxicity following application of a lipstick containing 2% Niacinamide or a foundation containing 5% Niacinamide under fully occlusive conditions with UVA and UVB light exposure (Procter and Gamble 1999d, 2000b). In these studies, adult volunteers (n = 12 per study) were exposed to the test articles, under occlusive patch conditions. Following 24 h of exposure, the patches were removed and one patch site was exposed to 20 Joules/cm^2 of UVA. The other patch test sites served as the non-irradiated control. Test sites were evaluated 24 and 48 hours following irradiation. The authors concluded that the formulations were not phototoxic.

Photoallergenicity

No photosensitization reactions were observed in a photoallergenicity study evaluating a foundation containing 5% Niacinamide under fully occlusive conditions (Procter and Gamble 1999e). In this study, 25 adult volunteers were exposed to the formulation, under occlusive patch test conditions twice weekly for three consecutive weeks. Twenty-four hours following each exposure, the treated site was irradiated with 3 MED's (minimal erythema dose) of UV light (UVA and UVB) as previously determined for each test subject. Following a 14-day rest period, duplicate patches containing the test material were applied to naive skin site for 24 hours. Following treatment exposure, one patch was removed and the test site was immediately irradiated with 1/2 MED of UVB (290–320 nm) 4 Joules/cm^2 of UVA (320–400 nm). An additional test site, which did not receive test

material, was irradiated as just described. The non-irradiated treatment site served as a control for possible induction of contact sensitization. All sites were evaluated 48, 72 and 96 hours following irradiation.

The authors concluded that the foundation containing 5% Niacinamide was not associated with a photoallergenic response (Procter and Gamble 1999e).

Comedogenicity

The comedogenic potential of a moisturizer containing 2% Niacinamide was evaluated on the backs of 16 subjects (Procter and Gamble 2001). Three subjects withdrew from the study for reasons unrelated to the test articles and 13 subjects completed the study. After a 4-week application to the backs of comedone-prone subjects, under occlusive conditions, the 2% niacinamide-containing cream did not produce an increase in microcomedones. Microcomedone scores were significantly less than for those of the negative control and the cream was considered non-comedogenic by the authors.

Therapeutic Application

Niacin

Niacin is given orally or topically principally to treat hypercholsteremia or related conditions. Representative studies are summarized in Table 8.

Niacinamide

An open study of high dose (no exact doses given) Niacinamide in the treatment of 13 patients with necrobiosis lipoidica was reported. These patients remained on Niacinamide therapy for 1–20 years. Of the 13 patients who remained on treatment for more than one month, eight improved. There were no reported significant side effects, particularly with respect to diabetic control, an important finding as lesions tended to relapse if treatment was stopped (Handfield-Jones et al. 1988).

Heathy male human volunteers ingested 1, 3, or 6 g of Niacinamide in capsule form on an empty stomach. Headache, dizziness, nausea, and vomiting were common side effects. There did not appear to be any relationship between the ingested drug dose and the observed side effects (Horsman et al. 1993).

Shalita et al. (1995) studied the safety and efficacy of topically applied 4% Niacinmide gel for the treatment of acne vulgaris. Thirty-eight patients applied 4% Niacinamide gel twice daily for 8 weeks. After eight weeks, 82% of the patients treated had beneficial results, a 60% decrease in papules/pustules and a 53% decrease in acne severity.

Kaanders et al. (1997) administered Niacinamide daily (80 mg/kg bw to a maximum of 6 g) as a liquid formulation to head and neck cancer patients receiving a 5- to 7-week course of radiotherapy. A dose reduction to 60 mg/kg was introduced for patients with severe side-effects. The most important side effect of Niacinamide was nausea with or without vomiting occurring in 65% of patients. Tolerance improved after a 25%

reduction of the dose in six of seven patients but plasma levels at the time irradiation fell below 700 nM/ml in four out of six patients. Other Niacinamide side effects included gastrointestinal symptoms, flushing, dizziness, sweating, fatigue, and headache.

Case Reports

Case reports of blurred vision, cystoid maculopathy, skin flushing, erethematous papules, sensation of warmth, itching, jaundice, hepatitis, abnormal liver function, hypothyroxinemia, leukopenia and thrombocytopenia, nausea, and vomiting all have been reported in the case literature (Lyman et al. 1957; Rivin 1959; Berge 1961; Winter and Boyer 1973; Sugerman and Clarke 1974; Einstein et al. 1975; Ma and Medenica 1983; Patterson et al. 1983; Clementz and Holmes 1987; Millay et al. 1988; Etchason et al. 1991; O'Brien et al. 1992; and Lawrence 1993).

SUMMARY

Niacinamide and Niacin function in cosmetics primarily as hair and skin conditioning agents. Niacinamide and Niacin are commonly called Nicotinamide and Nicotinic Acid, respectively. Both are heterocyclic aromatic compounds. Niacinamide and Niacin both play key roles in normal metabolism. Both ingredients are crystalline powders which are soluble in water and organic solvents.

USP grade Niacinamide is limited to not more than 0.003% heavy metals and USP grade Niacin is limited to not more than 0.002% heavy metals, and chloride and sulfate to not more than 0.02%. Niacinamide has an ultraviolet radiation absorption tail in the UVB region, but the peak absorption is at 262 nm.

Niacinamide is used in at least 33 cosmetic formulations ranging from shampoos and hair tonics to skin moisturzers and cleansing formulations. Niacin is used in three similar product types, one use in each. The concentration of use of Niacinamide varies from a low of 0.0001% in night preparations to a high of 3% in body and hand creams, lotions, powders and sprays. Niacin concentrations of use range from 0.01% in body and hand creams, lotions, powders and sprays to 0.1% in paste masks (mud packs). Both ingredients are accepted for use in cosmetics in Japan and the European Union.

Niacinamide and Niacin are both GRAS direct food additives and nutrient and/or dietary supplement. Niacinamide may be used in clinical treatment of hypercholesteremia and Niacin in prevention of pellegra and treatment of certain psychological disorders.

Both ingredients are readily absorbed from skin, blood, and the intestines and widely distribute throughout the body. Metabolites include N^1-methylnicotinamide and N^1-methyl-4-pyridone-3-carboxamide. Excretion is primarily through the urinary tract. Tryptophan may be metabolically converted to Niacin.

TABLE 8
Therapeutic Application of Niacin

Condition	Number of patients	Treatment	Observations	Reference
Hypercholesteremia	31 male	1 to 6 g of Niacin or Niacin combinations up to 3 years	Eighty percent of patients continued the medication for long periods without significant side effects. Side effects included nausea and vomiting, flushing, epigastric burning, jaundice, and hives.	Riven 1962
Hypercholesteremia	4 male 4 female 3 untreated	1.5 to 6 g daily oral Niacin for 1.75 to 9 years	Light microscopic findings were essentially normal in all eight treated patients and in the three untreated patients. Fine structural changes were observed by electron microscopy. In those treated, the most pronounced alteration was dilation of the endoplasmic reticulum with the formation of vesicles and sacs of various sizes and shapes. This was accompanied by a diminution of parallel arrays of rough endoplasmic reticulum. Replacement of the rough endoplasmic reticulum by vesicles with loss of ribosomal granules was observed in all biopsy specimens of treated patients. This effect was mild in one, moderate in three, and severe in four cases.	Baggenstoss et al. 1967
Hypercholesteremia	160 (male:female ratio of 4:1)	3 g daily oral Niacin; 51 patients followed for more than 5 years; 16 for more than 10 years	Flushing of the skin and pruritus were sufficiently a problem to cause discontinuation of treatment in 10 patients. Approximately 26% of patients experienced nausea and vomiting and 45% of this group discontinued the medication. Clinical evidence of active peptic ulcers developed in nine patients (3 with a previous history of duodenal ulcer disease). Hepatomegaly, jaundice, and increased serum glutamic oxalacetic transaminase and alkaline phosphatase developed in three patients, and ascites in one of them. All liver function studies returned to normal after cessation of Niacin therapy.	Charman 1972
None	16 male	420 mg oral Niacin daily	Elevated Niacin concentrations in serum (over 80 g/100 ml) induced an acute rise in bilirubin to 1–3 mg/100 ml serum. After treatment for 1 week in humans with 0.5 g Niacin daily by mouth the bilirubin increase is no longer detectable.	Dietmann and Stork 1976

(Continued on next page)

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TABLE 8

Therapeutic Application of Niacin (*Continued*)

Condition	Number of patients	Treatment	Observations	Reference
Dyslipidemia	65 non-transplant (54 male, 11 female) 17 heart transplant recipients (12 male, 5 female)	Non-transplant: 2.5 +/– 0.9 g oral Niacin daily for 3 years Transplant: 1.3 +/– 0.5 g oral Niacin daily for 3 years	Niacin was well tolerated in the nontransplant group. A reduction of very-low-density lipoprotein cholesterol was observed with the treatment of Niacin which led to an elevation in low-density lipoprotein cholesterol in many patients. In the transplant group, 11 patients discontinued treatment, primarily due to hyperglycemia. Of the 15 patients using sustained-release Niacin, eight cases of hepatitis were reported. No cases of hepatitis were recorded in the 67 patients using regular Niacin.	Henkin et al. 1991
None	4 male	500 mg Niacin tablet three times a day for a total of 13 doses	Marked flushing which reduced during the first two days. Prostaglandin levels were reduced with continued administration.	Stern et al. 1991
Hypercholesteremia	13 female, 8 male children	Niacin treatment in daily doses (500–2250 mg daily)	Flushing (71%), itching (19%), abdominal pain (14%), nausea (14%), vomiting (14%), headache (14%), and constipation (5%) were reported. Six patients had reversible dose-related elevations of serum aspartate aminotransferase.	Colletti et al. 1993
Hyperlipidaemia	treated: 60 male 42 female control: 40 male 48 female	3 g (or more) oral Niacin daily	Dry eyes, eyelid swelling, and cystoid maculopathy were all reported in the treatment group at a higher frequency than controls.	Fraunfelder et al. 1995

Niacinamide is virtually non-toxic to cultured macrophages and it prevented hydrogen peroxide induced necrosis in human pancreatic beta cells in culture.

Effects of metabolic imbalance created by exogenous dosing with Niacinamide and Niacin are consistently reported. While Niacinamide is more toxic than Niacin in acute toxicity studies, both have LD50 values above 1 g/kg in all reports, independent of route of administration. Short-term oral and parenteral toxicity studies did not identify significant irreversible effects. Niacinamide produced no adverse effects in a mouse dermal study using up to 50 mg per treatment, daily for four weeks. Niacinamide was not irritating, but did produce slight liver weight increases in female mice in a dermal study using up to 4.65 M Niacinamide, daily, 5 days a week, for 13 weeks.

Niacinamide was evaluated in an in vitro test to predict ocular irritation and was determined not to present an acute ocular hazard. Animal testing of Niacinamide in rabbits in actual formulations produced mostly non-irritant reactions, with only some marginally irritating responses.

Skin irritation tests of up to 2.5% Niacinamide in rabbits produced only marginal irritation that was significantly less than that produced in the same study with sodium lauryl sulfate at concentrations up to 0.5%. Skin sensitization tests of Niacinamide at 5% during induction and 20% during challenge were negative in guinea pigs.

Neither Niacinamide nor Niacin was mutagenic in Ames tests, with or without metabolic activation. Niacinamide and Niacin at 2 mg/ml were negative in a chromosome aberration test in Chinese hamster ovary cells, but did produce large structural chromosome aberrations at 3 mg/ml. Niacinamide induced sister chromatid exchanges in Chinese hamster ovary cells, but Niacin did not. Under certain circumstances, Niacinamide can cause an increase in unscheduled DNA synthesis in vitro; for example, in human lymphocytes treated with UV or a nitrosoguanidine compound. A rat study in which animals were injected $3\times$ with 500 mg/kg Niacinamide found the rate of DNA synthesis in the kidney unaffected while ornithine decarboxylase activity was increased in the kidney. Niacinamide was found to be radioprotective in several studies in vitro.

Niacinamide has been studied with several known carcinogens. Uniformly, the results of the Niacinamide controls demonstrate no increases in tumor incidence above vehicle control levels. Niacinamide can moderate the induction of tumors by established carcinogens. For example, Niacinamide in combination with streptozotocin (a nitrosourea compound) or with heliotrine (a pyrrolizidine alkaloid), produced pancreatic islet tumors. On the other hand, Niacinamide reduced the renal adenomas produced by streptozotocin; and intestinal and bladder tumors induced by a preparation of bracken fern. Niacinamide plus diethylnitrosamine produced no discernable pattern of effect in one study, while in another study with diethylnitrosamine, Niacinamide appeared to be a kidney tumor promoter. Niacinamide itself was not carcinogenic when administered (1%) in the drinking water of

mice. No data on the carcinogenic effect of Niacin were available.

Niacinamide evaluated in in vitro test systems did affect development; for example, development of embryos in culture was arrested at the two cell stage at a concentration of 69 mM Niacinamide. For comparison purposes, the normal blood level of Niacinamide is 1000 times lower at 0.069 mM. There was a reduction in the amount of amniotic fluid per fetus and in the maternal liver weight, but no significant effects on fetuses when mice were given 61 mg/kg Niacinamide sc in DMSO daily beginning on the 6th day and continuing through the 14th day of pregnancy. Niacinamide was combined with several known reproductive/developmental toxins. Uniformly, Niacinamide alone was not different from the vehicle. Niacinamide reduced the reproductive/developmental toxicity of 2-aminonicotinamide-amino-1,3,4-thiadiazole hydrochloride and urethane. Niacin did not reduce the incidence of urethane-induced malformations. Niacinamide had no effect on sulfadiazine induced reproductive/developmental toxicity.

Clinical testing in 22 adults of a skin lotion with 1% Niacinamide plus 5% lactic acid, 1% Niacinamide alone, or a control resulted in higher irritation but no significant difference in stinging reactions in the Niacinamide plus lactic acid test compared to either the Niacinamide alone or the control, and the latter two groups were similar. Further tests of the stinging sensation at concentrations up to 10% were negative. A skin cream containing 3% Niacinamide tested in 32 adults produced marginally more erythema compared to the control. In simulated use tests, a skin cream with 1% Niacinamide was not significantly more irritating than the control, nor was 5% Niacinamide in distilled water. A use test involving 159 females with a cream containing 2% Niacinamide resulted in no negative reactions. A 21-day cumulative irritation test in 25 subjects at Niacinamide concentrations up to 5% resulted in no irritancy.

Three separate HRIPT tests were conducted to investigate the effects of oil-in-water emulsions containing Niacinamide, 0, 1, 2.5 and 10 mg/cm^2 under semi-occlusive and occlusive patch. The number of volunteers was 100 per study. During the induction phase, no evidence of irritation was found. There was no evidence of the induction or elicitation of delayed type hypersensitivity in any volunteer.

A lipstick containing 2% Niacinamide and a foundation containing 5% Niacinamide were applied under occlusive patches for a day. Half the sites were exposed to UVA radiation. No phototoxic effect was seen. The foundation containing 5% Niacinamide was also applied under occlusive conditions twice weekly for three weeks. A day after each treatment, the site was exposed to 3 MEDs of UV radiation. Challenge at another site two weeks after the last exposure was done and 24 h after the challenge, that site was exposed to 1/2 MED. No photoallergenic response was seen.

Therapeutic use of Niacin alone or in combination with other agents, use of Niacin in healthy individuals, and Niacin as a causitive agent in case reports are each associated with a range

of side effects including blurred vision, cystoid maculopathy, skin flushing, erethematous papules, sensation of warmth, itching, jaundice, hepatitis, abnormal liver function, hypothyroxinemia, leukopenia and thrombocytopenia, nausea, and vomiting. Niacinamide produced no significant side effects in patients treated for necrobiosis lipoidica or acne vulgaris. Niacinamide used by cancer patients was linked to nausea and vomiting; other side effects included flushing, dizziness, sweating, fatigue, and headache. Similar side effects were seen in healthy individuals given Niacinamide.

DISCUSSION

The CIR Expert Panel considered that Niacinamide and Niacin are sufficiently similar from a toxicologic standpoint to combine the available data and reach a conclusion on the safety of both as cosmetic ingredients. Overall, the available information on acute, short-term, and subchronic toxicity of Niacinamide or Niacin suggests these ingredients are non-toxic, at levels considerably higher than would be experienced with Niacinamide or Niacin in cosmetic products. Clinical testing confirms that these ingredients are not significant skin irritants, are not skin sensitizers or photosensitizers, and are not comedogenic.

The Panel noted that certain formulations were marginal to slight ocular irritants. It appears that the formulation in which Niacinamide or Niacin was found played a role. Clearly, it was possible to formulate products with these ingredients such that the products would not be ocular irritants. The industry should be aware of this and formulate products such that ocular irritation is not produced.

While Niacinamide can adversely affect the growth of embryos in culture, animal tests clearly demonstrate that Niacinamide is not a reproductive or developmental toxin, and has been shown to reduce the toxicity of some known reproductive or developmental toxins.

Niacinamide, while not carcinogenic alone, can modulate the induction of tumors by certain established carcinogens. In a two-stage tumor initiator–tumor promoter model, Niacinamide reduced the carcinogenic effect of the initiator in some cases. In other cases, the Niacinamide appeared to promote the development of tumors. These effects were dependent on the tumor type and on the timing of the delivery of Niacinamide. The Panel noted that the doses in these studies are high relative to the low concentrations at which Niacinamide is used in cosmetic formulations. In neither case (tumor protection or tumor promotion) are these findings considered relevant to the use of Niacinamide at its current low concentrations of use in cosmetics; i.e., neither benefits nor adverse effects should be expected.

CONCLUSION

Based on the available data, the CIR Expert Panel concludes that Niacinamide and Niacin are safe in the current practices of use and concentration in cosmetic products.

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