Final Safety Assessment for PCA and Sodium PCA[1]

PCA is the cosmetic ingredient term used for the cyclic organic compound known commonly as pyroglutamic acid. Sodium PCA is the sodium salt of PCA. Both are used as hair and skin conditioning agents. These ingredients are recommended to be used in a concentration range of 0.2–4%. One optical isomer of PCA (the L form) is a naturally occurring component of mammalian tissue. PCA applied to the skin is absorbed to a limited extent. Absorption is in addition to PCA already present in the skin. In short-term and subchronic studies in several animal species, findings were unremarkable except for neurotoxicity in mice when injected interstriatally. No such findings were seen in similar studies using rats or with oral administration using mice. In animal studies, Sodium PCA was nonirritating to the eye and skin at concentrations up to 50%. No evidence of phototoxicity, sensitization, or comedogenicity was found. These ingredients were not genotoxic. In a range of clinical tests, PCA and Sodium PCA were found to be nonirritating and nonsensitizing (with and without UV exposure). Based on the low actual skin penetration of dermally applied PCA and in recognition of the endogenous levels found in the skin, it was considered that reproductive and developmental toxicity data were not critical to completion of the safety assessment. Based on the available data, it was concluded that PCA and Sodium PCA are safe as presently used in cosmetic formulations. These ingredients, however, should not be used in cosmetic products containing nitrosating agents.

PCA is a cyclic organic compound, more commonly known as pyroglutamic acid; Sodium PCA is the sodium salt of PCA. Both compounds are used as hair and skin conditioning agents in cosmetic formulations. This report reviews the available safety data on these ingredients.

CHEMISTRY

Definition and Structure

PCA (CAS No. 98-79-3) is the cyclic organic compound that conforms to the formula shown in Figure 1 (Wenninger and McEwen 1997). It is an internal amide of L-glutamic acid found in vegetables, fruits, grasses, and molasses (Budavari 1989). Other technical names for this ingredient include: 5-Oxo-L-Proline; L-Proline, 5-Oxo-; L-Pyroglutamic Acid; 2-Pyrrolidone-5-Carboxylic Acid (Wenninger and McEwen 1997); 5-Oxo-2-Pyrrolidinecarboxylic Acid; Glutimic Acid; Glutiminic Acid; α-Aminoglutaric Acid Lactam; and Glutamic Acid Lactam (Budavari 1989). Trade names for PCA are Ajidew A-100 and Pidolidone, and trade names of mixtures containing PCA are Ajidew SP-100 and Hydro-Diffuser Microreservoir (Wenninger and McEwen 1997).

Sodium PCA (CAS No. 28874-51-3) is the sodium salt of PCA (q.v.) that conforms to the formula shown in Figure 1 (Wenninger and McEwen 1997). Other technical names for this ingredient are: 5-Oxo-DL-Proline, Monosodium Salt; PCA Soda; DL-Proline, 5-Oxo-, Monosodium Salt; Sodium Pyroglutamate; and Sodium DL-2-Pyrrolidone-5-Carboxylate. Trade names for Sodium PCA include: Ajidew N-50, Dermidrol, and Nalidone. This ingredient is also found in mixtures with the following trade names: Ajidew SP-100, Aquaderm, Endomine NMF, Hydrolyzed NMF, Lactil, Moisturizing Liposomes, Phyto NMF, Prodew 100, Prodew 200, Prodew 300, and Ritaderm (Wenninger and McEwen 1997).

Chemical and Physical Properties

PCA is an orthorhombic bisphenoidal crystal with a molecular weight of 129.11. The melting point of PCA is 162–163°C and it is soluble in water, alcohol, and acetone (Budavari 1989). PCA is nonhygroscopic, but its sodium salt is extremely hygroscopic (Ajinomoto 1994a). The ultraviolet (UV) absorption spectrum of PCA indicates very weak absorption from 320–240 nm and strong absorption from 240 nm to shorter wavelength (Lin, Shieh, and Tung 1971).

Method of Manufacture

PCA is prepared from L-glutamic acid by autoclaving with an equal weight of water at 135–140°C (Budavari 1989).

Impurities

No by-products are reported in the production of PCA and Sodium PCA from glutamic acid and sodium glutamate, respectively (Ajinomoto USA, Inc. 1995). It could be expected that some dimer or polymer of glutamic acid would be found, but none was detected with carnet analytical methods. However, glutamic acid and sodium glutamate are possible impurities.

Analytical Methods

Analytical methods for determining and/or isolating PCA include: capillary isotachophoresis (Stehle and Fürst 1987); high-performance liquid chromatography (Shih 1985); reverse phase high-performance liquid chromatography (Bousquet et al. 1983);

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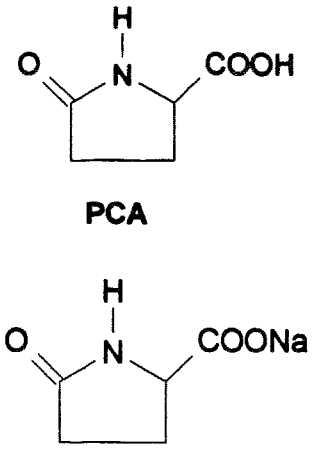

FIGURE 1
Chemical formulas for PCA and Sodium PCA (Wenninger and McEwen 1997).

enantiomeric separation (Zukowski, Pawlowska, and Armstrong 1992); and mass and gas spectrometry (Tham, Nyström, and Holmstedt 1968).

Nitrosation

Yamada, Yamamoto, and Tanimura (1981) investigated the nitrosation of PCA under conditions simulating those in the stomach following a meal. PCA was reacted with sodium nitrite at pH 2.5 and 37°C, and sulfamic acid was added to the mixture. The initial rate of reaction was very slow and the rate constant was 1.23×10^{-3} M^{-1} min^{-1}. The investigators noted that this rate value was 1.7% of that observed with hydanoic acid and 0.03% of that of nitrosomethylurea formation.

COSMETIC USE

PCA and Sodium PCA are used as a skin and hair conditioning agents (humectants) in cosmetic formulations (Wenninger and McEwen 1997). The product formulation data submitted to the Food and Drug Administration (FDA) in 1996 reported that PCA was used in a total of 25 cosmetic formulations and that Sodium PCA was used in a total of 437 products (Table 1) (FDA 1996).

One supplier of PCA and Sodium PCA recommends that these ingredients be used in a concentration range of 0.2–4%, and that the use concentration be <5% because of the possibility of inducing a reddening of the skin at that concentration (similar to alcohol blush) (Ajinomoto USA, Inc. 1995). Because concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992), the supplier has no way of knowing at what concentration its customers are using PCA and Sodium PCA; however, they suspect it is in the range of 0.1–4%. Data have been submitted to the Cosmetic Ingredient Review (CIR) by the Cosmetic, Toiletry, and Fragrance Association (CTFA) that give some use concentrations (CTFA 1995). These data are summarized in Table 2.

In 1984, product formulation data submitted to the FDA stated that PCA was used at concentrations up to 1% and that Sodium PCA was used at up to 10% (FDA 1984).

International

PCA and Sodium PCA are listed in the *Comprehensive Licensing Standards of Cosmetics by Category (CLS)* and must conform to the standards of the *Japanese Standards of Cosmetic Ingredients* (Yakuji Nippo, Ltd. 1994). They can be used in all *CLS* categories without restriction.

BIOLOGICAL PROPERTIES

Natural Occurrence

PCA is a naturally occurring component of mammalian tissue. Tabachnick and LaBadie (1970) reported that 270 μmol/g wet weight was found using ion exchange chromatography in epidermal scrapings taken from albino guinea pigs. In further studies with epidermal samples from guinea pigs, humans, dogs, rats, and mice, the total amount of free PCA was 186.0, 44.9, 30.9, 21.3, and 19.0 μmol/g wet weight, respectively. Optical rotatory dispersion studies of PCA isolated from guinea pig skin indicated that the epidermal PCA was the L isomer (Wolfersberger et al. 1973).

The concentration of PCA in other tissues of guinea pigs was much lower than that found in the epidermis. Wolfersberger et al. (1973) examined the liver, spleen, pancreas, kidneys, intestine, brain, and blood cells of guinea pigs and found that the highest concentration of PCA was in the brain (4.06 μmol/g wet weight). PCA content was much lower in the other tissues. Whole blood and plasma had concentrations of 53.1 and 31.2 μmol/100 ml, respectively.

L-PCA is also present in the human epidermis at approximately 16.5 mg/g fresh tissue (Marstein, Jellum, and Eldjarn 1973) and in normal human plasma at approximately 21.6 μmol/100 ml plasma (Wolfersberger and Tabachnick 1973). Free PCA is also found in the cerebrospinal fluid as the L-isomer and in the urine as both the L- and D-isomers (Wilk and Orlowski 1973).

PCA (and Sodium Lactate) constitute the most hygroscopic fraction of the stratum corneum (Middleton 1978).

Biosynthesis

The major pathway by which L-PCA is formed involves the catalysis of γ-glutamyl amino acids by γ-glutamyl cyclotransferase (van der Werf and Meister 1975).

TABLE 1
Cosmetic product formulation data on PCA and SodiumPCA (FDA 1996)

Product category	Total no. formulations in category	Total no. containing ingredient	Product category	Total no. formulations in category	Total no. containing ingredient
PCA			**Sodium PCA (cont.)**		
Other eye makeup preparations	136	1	Tonics, dressings, and other hair grooming aids	604	13
Hair conditioners	715	8	Wave sets	95	1
Permanent waves	434	2	Other hair preparations	395	29
Other hair coloring preparations	71	1	Blushers (all types)	277	2
Blushers (all types)	277	2	Foundations	355	2
Cleansing	820	1	Lipstick	997	1
Face and neck (excluding shaving)	300	2	Other makeup preparations	157	1
Moisturizing	942	5	Bath soaps and detergents	372	5
Night	226	1	Douches	19	1
Paste masks (mud packs)	300	1	Other personal cleanliness products	339	1
Other skin care preparations	810	1	Aftershave lotion	268	6
1996 total		**25**	Beard softeners	4	1
Sodium PCA			Cleansing	820	23
Baby lotions, oils, powders, and creams	64	1	Face and neck (excluding shaving)	300	11
Eyeliner	533	1	Body and hand (excluding shaving)	1012	35
Eye lotion	22	2	Moisturizing	942	74
Eye makeup remover	95	1	Night	226	11
Other eye makeup preparations	136	4	Paste masks (mud packs)	300	11
Other fragrance preparations	195	1	Skin fresheners	244	16
Hair conditioners	715	31	Other skin care preparations	810	31
Hair sprays (aerosol fixatives)	334	24	Suntan gels, creams, and liquids	196	8
Permanent waves	434	14	Indoor tanning preparations	67	3
Rinses (noncoloring)	60	2	Other suntan preparations	68	3
Shampoos (noncoloring)	972	30	Listing under trade names		11
			Listing under mixture trade names		26
			1996 total		**437**

Enzymatic synthesis in situ was considered the mechanism responsible for the high concentration of PCA in the guinea pig epidermis. The specific activities of PCA-forming enzymes was determined using homogenates of epidermis. The specific activities for γ-glutamyl cyclotransferase, glutamine cyclotransferase, glutamic acid cyclotransferase, and pyrrolidone carboxyl peptidase were 3.05, 0.99, 0.58, and 0.03 μmol/h/mg protein, respectively. It was concluded that if the specific activities of the three cyclotransferases reflect their activity in situ, any of the three could account for the formation of the high concentration of PCA in the epidermis (Wolfersberger and Tabachnick 1974).

In another study, DeLapp and Dieckman (1977) investigated the biosynthesis of PCA in the epidermis of the hairless mouse by administrating a single subcutaneous injection of [^{3}H]-glutamic acid to HRS/J mice and observing the specific activity of PCA in the skin. The specific activity of PCA increased slowly in the epidermis and reached a peak 3–4 days after injection. Ninety-seven percent of the PCA content of the skin was in the stratum corneum. The investigators noted that topical application of cycloheximide inhibited the incorporation of [^{3}H]-glutamic acid into the epidermal PCA. This inhibitory effect was greater prior to injection of [^{3}H]-glutamic acid than after injection. The researchers concluded that protein synthesis was involved in the formation of PCA from glutamic acid rather than a direct conversion of the amino acid. The high concentration of PCA in the epidermis is probably due to its accumulation in the stratum corneum and its relatively slow rate of turnover in comparison to other tissues.

Absorption, Distribution, Metabolism, and Excretion

The in vitro percutaneous absorption of 5, 10, and 20% *w/v* [^{14}C]-Sodium PCA was determined using fresh dermatomed human cadaver thigh skin (Surge Laboratory, 1995). The amount of radioactivity recovered in the receptor fluid over 24 hours was 1.23, 1.67, and 1.08% for applied doses of 5, 10, and 20% Sodium PCA, respectively. The amount recovered in the

TABLE 2
Concentration of use data for PCA and Sodium PCA (CTFA 1995)

Product in which used	Concentration
PCA	
Skin care preparations	<0.1%
PCA and Sodium PCA	
Liquid soap	0.05%
Moisturizer	2.5%
Cleanser	1.2%
Facial mask	1%
Sodium PCA	
Body lotions	0.1%
Foundations	2%
Other makeup preparations	0.225%
Bath soaps and detergents	0.001%
Cleansing (cold creams, etc.)	1%
Face and neck product	1.04%
Moisturizing skin care preparations	1%
Night skin care preparations	2%
Paste masks (mud packs)	0.05%
Other skin care preparations	1%
Sodium PCA 50%	
Other bath preparations	0.001%
Hair conditioner	1%
Hair spray	0.001%
Shampoos	0.50–1%
Tonics, dressings, and other hair grooming aids	0.5%
Other hair preparations	0.001%

epidermis was 2.47, 2.48, and 2.05% and in the dermis was 2.27, 2.63, and 2.76% with 5, 10, and 20% Sodium PCA, respectively. Because receptor fluid accumulations increased with time and the greater receptor fluid accumulations were at the latter part of the study, it was assumed that absorption was an ongoing process at 24 hours and therefore the skin content should be considered part of the absorption process. Accordingly, the percutaneous absorption of 5, 10, and 20% Sodium PCA through human skin was 5.97, 6.78, and 5.89%, respectively. The calculated flux for 5, 10, and 20% [^{14}C]Sodium PCA was 12.9, 29.1, and 50.4 μg/cm^2/h; flux increased almost linearly with dose.

Chanal et al. (1988) investigated the penetration of Sodium PCA into the brain and plasma of Wistar rats. Seven male rats were given orally 500 mg/kg [^{3}H]-Sodium PCA (20 μCi/rat) and one rat was killed for blood and brain analyses at 15, 30, 60, 90, 120, 240, and 480 minutes following administration. A 30-fold increase in the plasma concentration of [^{3}H]-PCA was observed and maximum values were achieved between 90 and 120 min. Brain concentrations increased by 100% after 120 minutes. Over 60% of the cerebral radioactivity was identified as [^{3}H]-PCA, which remained unchanged for at least 2–4 hours.

A fasted rabbit was fed 300 μl ^{14}C-PCA (20 μCi of activity) in a 3-g carrier solution of PCA. Blood samples were taken by cardiac puncture at 0.5, 1, 2, 3, 4, 6, 18, and 24 hours for determination of serum concentrations of radioactivity and for identification of certain amino acids. Radioactivity was identified in the serum as 42% glutamic acid, 42% γ-aminobutyric acid, and 16% PCA. The absorption rate was not determined (Lange and Carey 1966).

The metabolism of PCA was also investigated in mice. Three mice were given orally 1 ml of a PCA carrier solution containing 1 μCi ^{14}C-PCA. After 3 hours, the mice were killed and the blood, brain, and kidneys were pooled for radioactivity evaluation. Radioactivity was found in the serum as PCA, in the brain as glutamic acid, and in the kidneys as γ-aminobutyric acid. The absorption rate was not determined (Lange and Carey 1966).

Greenberg and Schmidt (1936) reported that oral administration of PCA to dogs resulted in approximately 70% of the administered dose being absorbed. Thirty percent of the absorbed dose was excreted unchanged in the urine and the remainder was converted to urea. Consistent with these findings, Pederson and Lewis (1944) reported that when PCA was administered via stomach tube to rabbits, some of the administered PCA nitrogen was excreted as urinary urea nitrogen.

In a study with bile fistula rats, bile was not a major route for the excretion of PCA when given either orally or subcutaneously (Greenberg and Schmidt 1936).

Ramakrishna, Krishnaswamy, and Rajagopal (1970) injected a male albino rat intraperitoneally with [U-^{14}C]PCA (134 μCi; specific radioactivity 55 mCi/mmol). The rat was housed in a metabolic cage for 8 hours, and the distribution of radioactivity in the excretory products and tissues was determined. A total of 64% of the administered radioactivity was recovered from the various fractions. Approximately 87% of the radioactivity was found in expired CO_2, of which 80% was exhaled within the 1st hour. Radioactivity was also found in fractions of various tissues, but at much lower concentrations.

Additionally, the investigators used isolated slices of the liver, kidneys, and brain incubated with radioactive PCA to determine whether different tissues could metabolize PCA. They found 4–5% of the total radioactivity in the free amino acid pool of the liver and kidneys, 50% of which was identified as glutamic acid. Additional studies indicated that metabolism of PCA by these tissues could be inhibited with electron transport inhibitors. No oxidation of PCA by the tissues of the brain was observed.

In another study, groups of two to three NCS mice were injected subcutaneously (s.c.) with 2 μmol to 2 mmol [U-^{14}C]PCA (1.2×10^6 cpm of ^{14}C). The mice were housed in separate metabolic cages and $^{14}CO_2$ was collected for up to 6 hours. At concentrations up to 80 μmol, the percent recovery of $^{14}CO_2$ was 58–70% of the dose after 3 hours and 65–72% after 6 hours. However, at the two higher concentrations tested, 0.2 and 2 mmol, the recovery of $^{14}CO_2$ was much lower. The investigators noted that a relatively large amount of PCA was metabolized during this period. After a dose of 2 mmol PCA, approximately

240 μmol of PCA was metabolized within 6 hours. It was concluded that the mouse has a substantial capacity for utilization of 5-oxoprolinase (Hsu and Meister 1985).

These researchers then investigated the effects of 5-oxoprolinase inhibitors on $^{14}CO_2$ formation. Mice were injected s.c. with one of three inhibitors, L-2-imidazolidone-4-carboxylate, L-2-oxothiazolidine-4-carboxylate, and 3-methyl-5-oxoproline, followed by 10 μmol PCA (1.2×10^6 cpm) 10 minutes later. As a control, the effects of these inhibitors on the metabolism of glutamate were also determined. The three inhibitors significantly reduced the rate of $^{14}CO_2$ formation from [U-^{14}C]PCA, but had little effect on the rate of $^{14}CO_2$ formation from [U-^{14}C]glutamate (Hsu and Meister 1985).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

The oral LD_{50} of Sodium PCA was 10.4 g/kg for male mice (Ajinomoto Co., Inc. 1994c). In another study, the LD_{50} for 50% Sodium PCA was >2.0 g/kg for rats (Centre International de Toxicologie 1990).

Short-Term Oral Toxicity

Five male and five female Sprague-Dawley rats were fed 1.5% PCA in their diet for 12 days and body weight gain was monitored. A control group of rats was fed untreated feed. No significant effect on growth was observed (Lin, Shieh, and Tung 1971).

Subchronic Oral Toxicity

The effect of PCA on body weight gain was studied using Sprague-Dawley rats. Six male rats were fed 1% PCA in their diet for 70 days and a control group of six rats was fed untreated feed. No significant effect on growth was observed during the first 10 days of the experiment. However, at the end of the study, the net gain in body weight was 238.4 g for the rats fed 1% PCA and 214.4 g for the controls. The investigators concluded that the difference between these two groups was of borderline significance (Lin, Shieh, and Tung 1971).

In another study, male and female rats were fed diets containing 2, 4, or 8% Sodium PCA for 13 and 26 weeks. A separate group of rats was fed a diet containing sodium propionate to serve as a sodium ion control. No adverse effects were observed in hematologic parameters, blood chemistry, urinalysis, macroscopic appearance or microscopic findings of the major organs. Diarrhea or softened feces was observed in the high dose group as well as in the control group for up to 9 weeks, by which time the intestinal tract probably adapted to the dietary treatment. The kidneys of these rats were also enlarged, an indication that renal function could have physiologically adapted to compensate for the prolonged sodium ion loading (Ishii et al. 1992).

Neurotoxicity

Caccia et al. (1983) investigated the kinetics and neurotoxicity of PCA using CD-1 (ICR) mice. In the kinetic study, groups of adult male mice and 10-day old male and female mice were given orally 0.5 g/kg PCA. The mice were killed at different time intervals ranging from 5–480 minutes after administration, and blood samples and brains were collected for analysis. In adults, the concentration of PCA in the plasma increased by a factor of 55.8 at 30 minutes, whereas the concentration in the brain reached a maximum by a factor of 4.5 at 60 minutes. A 1.3-fold increase was observed in the concentration of glutamic acid in the plasma, but no changes in concentration were observed in the brain. In 10-day-old mice, PCA increased 69-fold in the plasma and 5.7-fold in the brain at 30 minutes after dosing. In the neurotoxicity study, groups of 12 10-day-old mice were given 2 and 4 g/kg 10% aqueous PCA by gastric intubation. Six hours after administration the mice were killed and the brains were removed. Control mice that were not treated with PCA were also examined. The number of necrotic neurons in the arcuate nuclei was not significantly different between the experimental and control group of mice.

PCA has also been studied for neurotoxicity using the intrastriatal route of administration. In behavioral and neurology studies with mice, Rieke, Scarfe, and Hunter (1984) observed dose-related changes in behavior and dose-dependent increases in the number of lesion of the neuropil following 1-μl injections of 0.02–100 μmol PCA. However, no neurotoxicity was observed in a study of rats stereotaxically injected in the striatum with 250 nM PCA (McGeer and Singh 1984).

Dermal Irritation

The primary skin irritation potential of 2, 4, 8, 16, 32, and 50% aqueous Sodium PCA was tested using groups of 10 white female Hartley guinea pigs. Sodium PCA was applied to the shaved trunk of each animal once a day for 14 days. Observations were made every day and for an additional 2 weeks following the last application. Distilled water was used as the control. No irritation was observed at any of the concentrations tested (Ajinomoto Co., Inc. 1994b).

In another study, 5% aqueous Sodium PCA was applied to the abraded skin of 30 white female Hartley guinea pigs. Applications were made once a day for 3 days. A control group of guinea pigs was treated similarly with distilled water. Sodium PCA was nonirritating to the skin (Ajinomoto Co., Inc. 1994b).

Sodium PCA (50% aq.) was also tested for primary skin irritation using rabbits. Applications were made to both intact and abraded sites for 24 hours, and the sites were scored 24 and 72 hours after application. Sodium PCA was classified as a nonirritant (Usines Chimiques d'Ivry-la-Bataille [UCIB] 1989).

Sensitization

The 10 guinea pigs used in the primary skin irritation study (described earlier) were also used in a sensitization study. Two

weeks after being treated with 2, 4, 8, 16, 32, and 50% aqueous Sodium PCA for 14 consecutive days, 5% aqueous Sodium PCA was applied to the right mammary region of each guinea pig. These sites were scored after 48 and 72 hours. No signs of sensitization were observed (Ajinomoto Co., Inc. 1994b).

Phototoxicity

In a phototoxicity study, 1% aqueous Sodium PCA was applied to the shaved backs of 10 female white Hartley guinea pigs on days 1, 4, and 7. Each of the guinea pigs was irradiated with UV light (wavelengths not reported) for 10 minutes a day from days 1 to 10. The sites of application were scored on days 4 and 10. No evidence of phototoxicity was observed (Ajinomoto Co., Inc. 1994b).

Comedogenicity

A 50% solution of Sodium PCA (0.1 ml) was applied to the right ears of six male New Zealand albino rabbits 5 days a week for 2 weeks. The left ears were left untreated and served as controls. The rabbits were killed 6–8 hours after the last application and the epithelial tissue of the ears was removed for examination. No significant difference in the number of pilosebaceous units was found between the treated and control epithelial samples (UCIB 1987).

Ocular Irritation

A Draize test of 50% aqueous Sodium PCA was conducted using six male white rabbits. The right conjunctival sac of each rabbit was instilled with 0.1 ml 50% aqueous Sodium PCA. The eyes of three rabbits were rinsed 2–4 seconds after instillation, while the eyes of the remaining rabbits were left unrinsed. A control group of rabbits was treated with 0.9% NaCl. Each of the eyes was scored after 24, 48, 72, 96, and 168 hours. Very slight inflammation was observed in one rabbit of both the treated and control groups, but these signs subsided by the 72- or 96-hour readings (Ajinomoto Co., Inc. 1994b).

In a similar study, a 50% solution of Sodium PCA was also classified as nonirritating. Mild conjunctivitis was observed during the first hour following instillation but cleared by the 24-hour reading (UCIB 1990).

Mutagenicity

A *Salmonella* mutagenicity assay was performed with 780–25,000 μg/ml PCA and Sodium PCA using *Salmonella typhimurium* TA100 and TA98 with and without metabolic activation (Ajinomoto Co., Inc. 1992). PCA and Sodium PCA were not mutagenic.

The ability of PCA to induce chromosome damage was studied in vitro using cultured human peripheral lymphocytes (Huntingdon Life Sciences 1996). The vehicle, sterile water, was used as a negative control and chlorambucil and cyclophosphamide were used as the positive controls without and with metabolic activation, respectively. A preliminary toxicity test with 20- and 44-hour sampling times was first performed using doses of 80.625, 161.25, 322.5, 645, and 1290 μg/ml PCA without and with metabolic activation; the highest concentration tested was 10 mM. The first cytogenetic test used concentrations of 322.5, 645, and 1290 μg/ml PCA for the 20-hour sampling time with and without metabolic activation and for the 44-hour sampling time without metabolic activation. For the 44-hour sampling time with metabolic activation, concentrations of 322.5, 645, 860, 1075, and 1290 μg/ml were used. In the second cytogenetic test, which had only a 20-hour sampling time, concentrations of 322.5, 645, and 1290 μg/ml were used with and without metabolic activation.

In the first cytogenetic test, reductions in mean mitotic indices of 18 and 25% were observed with 322.5 and 645 μg/ml PCA, respectively, with metabolic activation and a 26% reduction was observed with 1290 μg/ml without metabolic activation at the 20-hour sampling time. Without and with metabolic activation, PCA did not result in biologically or statistically significant increases in the frequency of metaphases with aberrant chromosomes. In the second test, 1290 μg/ml PCA without metabolic activation produced a reduction in the mean mitotic index of 24%. This dose without metabolic activation also resulted in a small but statistically significant increase in the number of cells with aberrant chromosomes, including gaps. However, the investigators did not consider this of biological significance. No marked toxicity was apparent in either test. The number of cells with polyploidy was within the normal range in both tests. The investigators concluded that "PCA, under the conditions of test, did not show any evidence of clastogenic activity."

CLINICAL STUDIES

Dermal Irritation

Immediate contact reactions to Sodium PCA were investigated by Larmi, Lahti, and Hannuksela (1989). A dose of 10 μl of 6.25, 12.5, 25, and 50% Sodium PCA in distilled water was applied using open patch test methods to the forehead, cheek, neck, and upper back (1 × 1 cm area) of 13 male volunteers. The sites were examined at 5-minute intervals for up to 40 minutes and cutaneous blood flow at the test sites and control sites was measured with a laser-Doppler flowmetry (LDF) device. Three of the subjects developed erythema on their upper backs with concentrations of 12.5% Sodium PCA and greater and two subjects reacted to 6.25%. Irritant reactions observed within the first 5 minutes disappeared by 30 minutes. No irritancy was observed on the skin of the forehead, cheek, or neck, and no significant changes in LDF measurements were observed between the different test areas.

A skin fatigue test of 30% Sodium PCA using open patch test methods was performed using 46 male volunteers. Sodium PCA was applied to the upper left arm of each subject once a day for 14 days, and irritancy was scored on days 6 and 14. Water was used as the control agent. No signs of irritation were observed throughout the test period (Ajinomoto Co., Inc. 1972).

In another study, 4, 8, 16, and 32% aqueous Sodium PCA was applied under occlusive patches to the left side of the backs of 46 male volunteers for 24 hours. The sites were scored 3 hours after patch removal. Negative control sites were treated with distilled water, 5% polyethylene glycol, and 5% glycerine; one site was untreated. Overall, the average irritation scores were not significantly different between the different concentration groups or between those of the test and control groups. One of the subjects had diffuse erythema from all of the concentrations tested, and another had this type of reaction to 4 and 8% Sodium PCA but only partial erythema when tested with 16 and 32% Sodium PCA. It was noted that reactions of equal or lesser intensity were observed at the control sites of these individuals, even the site that received no applications (Ajinomoto Co., Inc. 1972).

A primary irritancy test of 0.2% Sodium PCA was also conducted using patients with eczematous dermatitis. Sodium PCA was applied under occlusive patches for 48 hours to two sites on the backs of 47 patients. Water was applied in a similar fashion and served as the control. The sites of application were scored 24 and 48 hours after patch removal. No significant evidence of irritation was observed (Ajinomoto Co., Inc. 1972).

A formulation containing 2.0% Sodium PCA was negative in a 4-day mini-cumulative irritancy test using occlusive patches and 18 volunteers (CTFA 1990).

Sensitization and Photosensitization

A maximization test with a formulation containing 2.0% Sodium PCA was conducted using 25 volunteers. Sodium lauryl sulfate (1.0% aqueous) was applied to the upper outer arm of each subject using an occlusive patch. After 24 hours, the patch was removed and 0.1 g of the formulation was applied to the same site under occlusive patches for 48 hours during the week and 72 hours during the weekend. This procedure was repeated for a total of five induction exposures. After a 10-day nontreatment period, the subjects were challenged on the opposite arm with 0.1 ml of a 10.0% aqueous solution of sodium lauryl sulfate for 1 hour, followed by application of the formulation using occlusive patches for 48 hours. Sites were scored 1 hour after patch removal and 24 hours later. No irritation was observed during the induction phase of the study, and no evidence of sensitization was observed (Ivy Laboratories 1991).

In the dermal irritancy study using occlusive patch tests (described earlier in this report), 39 subjects tested with 4, 8, 16, and 32% aqueous Sodium PCA were subsequently tested 3 months later with the same concentrations of Sodium PCA. The series of applications were made under occlusive patches to three sites on the back, two on the left and one on the right. One series was scored at 24 hours and the remaining two series at 48 hours. Two controls, distilled water and no treatment, were included in each series. No significant evidence of sensitization was observed. Of the two subjects who had significant reactions to the first exposure to Sodium PCA, the one that had the strongest reaction also had strong positive results after a second exposure. It was noted that this individual also had positive reactions at negative control sites. The other subject did not react to the second exposure to Sodium PCA (Ajinomoto Co., Inc. 1972).

The volunteers of this study were further tested in a photosensitization test. Following the last observation period of the sensitization test, the site of the patch series challenged for 24 hours was irradiated 24 hours later with UV light for 50 seconds (Patch Test 1). The site of one series challenged for 48 hours was irradiated similarly immediately after patch removal (Patch Test 2). The light source used was a Toshiba Fluorescent Lamp FL-20SEX, 2 pulse FL-20BLB X2, Total 80W (no wavelengths were reported). The remaining 48-hour challenged series received no radiation (Patch Test 3) and served as a control for Patch Test 2. A separate group of 18 male volunteers was treated in a similar manner except that they had never been sensitized with Sodium PCA (Patch Test 4). All of the sites were scored at 24-hour intervals after irradiation.

In Patch Tests 1 and 2, no increase was observed in irritancy after UV exposure as compared to the values obtained before UV exposure, rather the trend was dissipating. No significant differences between the test and control values were observed for either of these tests. In Patch Test 4, in which the subjects had not been sensitized to Sodium PCA, the results were similar to that observed in Patch Test 3. The investigators concluded that Sodium PCA was neither a phototoxic nor photosensitizing agent (Ajinomoto Co., Inc. 1972).

Skin Effects

A 2^2 factorial design was used to examine the effect of PCA and urea on transepidermal water loss (TEWL) (McCallion and Li Wan Po 1995). Two and 5% *w/w* PCA solutions in propylene glycol were used. TEWL was measured three times at five sites on four Caucasian female subjects. Baseline values were established. Increasing the PCA concentration from 2% to 5% *w/w* in the presence of both 10 and 20% urea resulted in a statistically significant increase in TEWL. Increasing the urea concentration from 10% to 20% *w/w* in the presence of 2% *w/w* PCA increased TEWL, but there was no additional influence on the effect of 5% PCA. The researchers stated that "the magnitude of effects of altering the concentration of urea depends on the concentration of PCA present and vice versa," indicating that PCA and urea were interactive.

SUMMARY

PCA and Sodium PCA are used as hair and skin conditioning agents in cosmetic formulations. Collectively, these compounds were used in 462 product formulations in 1996. One supplier recommends using these ingredients at a concentration range of 0.2–4%, not exceeding 5%. PCA and Sodium PCA are used in a variety of skin products at concentrations ranging from 0.001–2.5% and Sodium PCA 50% is used in bath preparations at 0.001% and in hair care products at concentrations ranging from 0.001–1%.

No by-products are reported in the production of PCA and Sodium PCA from glutamic acid and sodium glutamate, respectively. However, glutamic acid and sodium glutamate are possible impurities.

PCA is a naturally occurring component of mammalian tissue. PCA (and Sodium Lactate) constitute the most hygroscopic fraction of the stratum corneum. In general, the major biochemical pathway by which it is formed involves the catalysis of γ-glutamyl amino acids by γ-glutamyl cyclotransferase.

The percutaneous absorption of 5, 10, and 20% Sodium PCA through fresh human cadaver skin in a 24-hour period was 5.97, 6.78, and 5.89%, respectively. PCA was present in the plasma and brain of rats following oral administration. In studies with rabbits and mice, it was reported that PCA was metabolized into glutamic acid and γ-aminobutyric acid. A study using dogs reported that of the 70% of the oral dose absorbed, 30% was eliminated unchanged in the urine and the remainder was converted to urea. PCA given subcutaneously was also rapidly metabolized in mice.

The oral LD_{50} of Sodium PCA was 10.4 g/kg for male mice and >2.0 g/kg for a 50% solution in a study with rats. No adverse effects were observed in either a short-term study using rats fed 1.5% PCA or in subchronic studies with rats fed diets containing up to 8% PCA. In a study using mice, PCA was neurotoxic when injected intrastriatally. However, no effects were observed in a similar study with rats or after oral administration to mice.

No phototoxic effects were observed in guinea pigs treated topically with 1% aqueous Sodium PCA.

Sodium PCA was nonirritating when applied to the skin of guinea pigs and rabbits at concentrations up to 50%. No evidence of dermal sensitization was observed when guinea pigs were induced with 2–50% aqueous Sodium PCA and challenged with 5% aq. Sodium PCA. Sodium PCA was noncomedogenic in rabbits.

No ocular irritation was observed when 50% aqueous solutions of Sodium PCA was instilled into the conjunctival sac of the eye of rabbits.

PCA and Sodium PCA were not mutagenic in a *Salmonella* mutagenicity assay with or without metabolic activation, and PCA was not considered clastogenic in a chromosome damage assay.

In a clinical study of dermal irritation using open patch test methods on various sites of the body, 2 of 13 volunteers had reactions to 6.25% Sodium PCA applied to their backs and 3 volunteers developed erythema when concentrations of 12.5% Sodium PCA and greater were applied. These reactions disappeared within 30 minutes. No reactions were observed when Sodium PCA was applied to the skin of the forehead, cheek, or neck.

In another study, no significant irritation was observed when 46 volunteers were treated with 30% Sodium PCA using open patch test methods. Negative results were also obtained when 46 volunteers were tested with concentrations up to 32% Sodium PCA using occlusive patches. Provocative tests of 0.2% Sodium PCA using occlusive patches were also negative.

A formulation containing 2.0% Sodium PCA was negative in a minicumulative irritation test.

Clinical studies using 39 subjects indicated that 32% aqueous Sodium PCA is neither a sensitizer nor a photosensitizer. A maximization test of a cosmetic formulation containing 2.0% Sodium PCA was also negative.

In a 2^2 factorial design study, TEWL was significantly increased when PCA concentrations were increased from 2 to 5% in the presence of 10 and 20% urea. Increasing the concentration of urea in the presence of 2% also increased TEWL; the presence of 5% PCA did not have an additional influence. PCA and urea were interactive.

DISCUSSION

Upon review of the data included in this report, the CIR Expert Panel was concerned that developmental toxicity data were absent. A safety assessment could be completed, however, if PCA was found not to significantly penetrate the skin. Data were made available indicating that the amount of exogenously applied PCA absorbed through the skin was on the order of 1% of the applied dose, but that up to 5% was distributed between the dermis and epidermis.

Concern was expressed over the potential that the PCA adsorbed in the dermis and epidermis would eventually move across the skin and result in a cumulative penetration that could be significant. This concern was mitigated by the low actual penetration through the skin over a 24-hour period and the recognition that PCA is naturally resident in the skin. Additionally, it was noted that adverse effects were absent in a 26-week oral study. With these factors considered, the Expert Panel concluded that the extent of penetration was not significant and that developmental toxicity data were not critical to completion of the safety assessment.

The Expert Panel also recognized that although Sodium PCA was reported to be used in aerosol products, there was a lack of inhalation toxicity data. The Expert Panel noted that PCA is structurally similar to 4-hydroxy-L-proline, a major component of mammalian collagen. Also important was the minimal, transient ocular irritation produced by 50% Sodium PCA, which is used in hair sprays. The Expert Panel considered that such a compound, then, is unlikely to elicit a serious toxicological effect if inhaled as a result of an exposure to hair spray. Based on the structure of the ingredient and the existing data included in this report, the Expert Panel did not envision that Sodium PCA would be a respiratory irritant and therefore did not require inhalation toxicity data to make a determination of safety.

CONCLUSION

On the basis of the animal and clinical data presented in this report, the CIR Expert Panel concludes that PCA and Sodium PCA are safe as presently used in cosmetic formulations. These ingredients should not be used in cosmetic products containing nitrosating agents.

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