FINAL REPORT OF THE SAFETY ASSESSMENT FOR 2-BROMO-2-NITROPROPANE-1,3-DIOL

2-Bromo-2-Nitropropane-1,3-Diol (BNPD) is used in cosmetics as an antibacterial agent. Data presented indicate that BNPD produces minimal contact allergy and/or contact irritation in both animals and humans at concentrations below 0.1%. Unformulated BNPD in concentrations of 1% or greater has been shown to be a considerable irritant. BNPD is moderately toxic orally in a variety of laboratory mammals, the LD50 varying with the test circumstances.

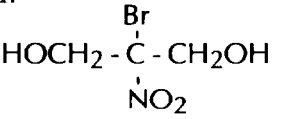
BNPD had no effect on reproduction, was not a teratogen, and had no embryotoxic effects. It was not a mutagen by the standard mouse dominant lethal test nor by the bacterial reverse mutant system.

The evidence at hand indicates that BNPD to be safe as a cosmetic ingredient at concentrations up to and including 0.1% except under circumstances where its action with amines or amides can result in the formation of nitrosamines or nitrosamides.

CHEMICAL AND PHYSICAL PROPERTIES

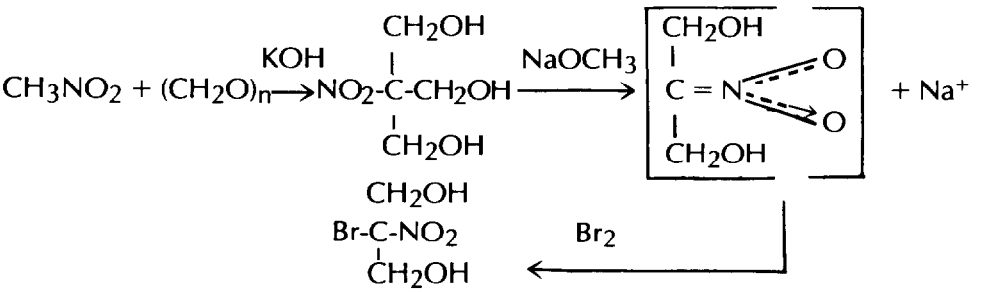
Structure

2-Bromo-2-Nitropropane-1,3-Diol (BNPD) is a substituted aliphatic diol that conforms to the formula:



BNPD is one of a family of halo-nitro compounds which have been found to inhibit the growth of bacteria, fungi, and yeasts. In a large group of aliphatic nitro compounds tested for antimicrobial properties, the most active appear to be alcohols containing a -$CBrNO_2$- group (Bowman and Stretton, 1972; Clark *et al.*, 1974; Clausen, 1973; Croshaw *et al.*, 1964).

The published method of manufacture is as follows (CTFA, 1972a):



Physical Properties

BNPD is a colorless-to-pale, brownish yellow, odorless crystalline solid which is soluble in water, alcohol, tetrahydrofuran and propylene glycol. It is slightly soluble in mineral oil and vegetable oils. The distribution coefficient of water: chloroform is 14.7:1 at 22-29°C. Its melting point is approximately 130°C (CTFA, 1972a; Marzulli and Maibach, 1973; Fan *et al.*, 1978).

Reactivity

In solid form BNPD is stable for at least one year at temperatures up to 45°C and at relative humidities up to 90% with no observable photodecomposition. Also, BNPD does not decompose during storage at room temperature in darkness up to two years. Freshly prepared aqueous solutions of BNPD are weakly acidic (pH 5.1-5.5) and upon storage and heating become more acidic with the liberation of formaldehyde. The decomposition of BNPD is accelerated with increasing pH and with increasing temperature of the solutions. Half lives of 0.2% w/v solutions of BNPD were determined to be >5 years at pH 4, 1.5 years at pH 6, and two months at pH 8. Major decomposition products were formaldehyde, 2-hydroxymethyl-2-nitro-1,3-propanediol and bromonitroethanol (Marzulli and Maibach, 1973; Sheppard and Wilson, 1974; Bryce *et al.*, 1978).

A solution of BNPD in tetrahydrofuran with morpholine produces N-nitroso-morpholine. In aqueous solution and in the presence of diethanolamine and triethanolamine, BNPD serves as a nitrosating agent leading to formation of N-nitrosodiethanolamine. In a 5mM aqueous solution, the pH of diethanolamine plus BNPD is initially 11.5-12.0. After one hour, 0.06% of diethanolamine is N-nitrosated; after six hours, 1% has reacted; after 24 hours, 2.2% has reacted; and after 72 hours, 10.3% is N-nitrosated. During this reaction time, the pH decreases to 8.1, 6.0, and 5.2, respectively. With decreasing pH the N-nitrosating activity of BNPD decreases: in solution at pH 4, virtually no N-nitrosation of diethanolamine occurs even after 190 hours. In a solution of equimolar amounts of BNPD and triethanolamine, 0.05% of the tertiary amine is nitrosated to N-nitrosodiethanolamine (NDELA) after 24 hours (Fan *et al.*, 1978; Schmeltz and Wenger, In press).

It has been suggested that BNPD oxidizes sensitive thiol groups of enzymes (Bowman and Stretton, 1972; Clark *et al.*, 1974; Stretton and Manson, 1973).

Analytical Methods

The recent advances in analytical chemistry, which now provide sensitive and specific methods for BNPD and its decomposition products, have been reviewed elsewhere and are only briefly summarized here. BNPD, as a raw material or as extracted from a formulation, can be specifically determined by gas-liquid chromatography of trimethylsilylated or acetylated material with detection by electron capture or flame-ionization. Sensitivity is 5 ppm or more in aqueous formulations. Polarography may also be applied to aqueous

systems containing BNPD, but calibration curves must be prepared for each system. This detects the alkyl nitro group and is thus subject to interference by degradation products. Its precision is approximately ± 2%. Thin layer chromatography is more specific but its relative errors may be as high as 15%. Microbiological assay in agar diffusion plates may be sensitive to as little as 0.005% but is non-specific (Bryce *et al.*, 1978).

Analyses of BNPD decomposition products, bromide ion, formaldehyde, and nitrate/nitrite are useful. Bromide is titrated potentiometrically in an acidic solution with silver nitrate. Formaldehyde reacts with chromotropic acid in strong sulfuric acid and the absorbance of the product may be measured at 570 nm. Nitrate and nitrite may be dtermined by reaction with 2,6-xylenol. High pressure liquid chromatography has recently been reported as having a limit of detection for BNPD of 3 μg per μl injected. The standard methods of the cosmetic industry for BNPD analysis are based on gas-liquid chromatography of the acetylated sample and titration of bromide (CTFA, 1972a; Bryce *et al.*, 1978; Schmeltz and Wenger, In press).

USE

Purpose and Extent of Use in Cosmetics

BNPD as a preservative is used in cosmetics and in pharmaceutical preparations because of its antibacterial and antifungal properties. It is effective against both gram-negative and gram-positive organisms (Bowman and Stretton, 1972; Clark *et al.*, 1974; Clausen, 1973; Croshaw *et al.*, 1964).

In the United States, BNPD is used as a preservative for a wide variety of cosmetics, especially shampoos, creams, lotions, rinses, and eye makeup. The number of product formulations containing BNPD and the concentrations of BNPD used in each of the several cosmetic categories are listed in Table 1.

Potential Interactions With Other Ingredients

It has been suggested (FDA, 1978a, b; Boots Co., 1978a, 1979; WHO: IARC, 1978) that BNPD might be a source of nitrosating agents which could react with amines or amides in cosmetics (Fan *et al.*, 1978; Bryce *et al.*, 1978; Schmeltz and Wenger, In press; FDA, 1978a; Boots Co., 1978a). An on-going study by FDA has provided initial and incomplete information on 191 off-the-shelf cosmetic formulations regarding their content of N-Nitrosodiethanolamine (NDELA), BNPD, and triethanolamine or its salts (TEA). Table 2 displays data on NDELA obtained by analyses at FDA.

These results give the following comparison details which are shown below:

1. Seventy-seven of the 191 samples analyzed contained NDELA. Of the 77 samples containing NDELA, 19 contained both BNPD and TEA, 1 contained BNPD but no TEA, 47 contained TEA but no BNPD, 5 had neither BNPD nor TEA, and 5 had incomplete or no ingredient information. Of these groups of 77 cosmetic product samples, 17 were found to contain

TABLE 1. Product Formulation Data (FDA, 1976)

Ingredient	Cosmetic Product Type	Concentration (%)	Number of Product formulations
2-Bromo-2-Nitropropane-1, 3-Diol	Bath oils, tablets, and salts	≤ 0.1	1
	Bubble baths	≤ 0.1	4
	Other bath preparations	≤ 0.1	5
	Eyebrow pencil	≤ 0.1	14
	Eyeliner	≤ 0.1	11
	Eye shadow	≤ 0.1	3
	Mascara	≤ 0.1	6
	Other makeup preparations	≤ 0.1	2
	Other fragrance preparations	> 0.1 to 1	2
	Hair conditioners	> 0.1 to 1	2
		≤ 0.1	20
	Rinses (noncoloring)	> 0.1 to 1	3
		≤ 0.1	3
	Shampoos (noncoloring)	≤ 0.1	9
	Tonics, dressings, and other hair grooming aids	> 0.1 to 1	1
		≤ 0.1	2
	Wave sets	≤ 0.1	1
	Other hair preparations	≤ 0.1	1
	Hair dyes and colors (all types requiring caution statement and patch test)	> 0.1 to 1	3
	Hair shampoos (coloring)	≤ 0.1	6
	Blushers (all types)	≤0.1	20
	Foundations	≤ 0.1	6
	Leg and body paints	≤ 0.1	2
	Makeup bases	≤ 0.1	3
	Makeup fixatives	≤ 0.1	134
	Other makeup preparations	≤ 0.1	1
	Bath soaps and detergents	≤ 0.1	1
	Deodorants (underarm)	≤ 0.1	2
	Aftershave lotions	≤ 0.1	1
	Cleansing (cold creams, cleansing lotions, liquids, and pads)	≤ 0.1	17
	Face, body, and hand (excluding shaving preparations)	>0.1 to 1	3
	Moisturizing	≤ 0.1	9
	Night	≤ 0.1	3
	Paste masks (mud packs)	≤ 0.1	8
	Skin fresheners	≤ 0.1	3
	Other skin care preparations	≤ 0.1	6
	Suntan gels, creams, and liquids	> 0.1 to 1	2
		≤ 0.1	1
	Indoor tanning preparations	≤ 0.1	1
	Other suntan preparations	≤ 0.1	1

NDELA at levels above 2000 ppb, 43 were found to contain NDELA at levels between 30 ppb and 2000 ppb, and 17 samples were found to contain NDELA at trace levels (10 to 30 ppb).

2. One hundred fourteen of the 191 samples analyzed contained no NDELA. Of these 114 samples, 4 contained both BNPD and TEA, 2 contained BNPD but not TEA, 81 contained TEA but not BNPD, 16 had neither BNPD nor TEA, and 11 had incomplete or no ingredient information.

FDA reported a change in sensitivity number during the analytical program between 10 ppb and 30 ppb. Values in this range are considered trace values. All negative values are below 10 ppb.

These findings suggest the possibility that the presence of BNPD and/or TEA in some cosmetics may lead to the formation of NDELA but not necessarily in all formulations. Further investigation is needed to clarify what relationship, if any, these ingredients have to the presence of NDELA in some but not all cosmetics containing them.

FDA studies have demonstrated that NDELA is absorbed through excised human skin with a permeability constant of 0.50×10^{-5} cm/hr (FDA, 1978b).

NDELA and other nitrosamines and nitrosamides are known to have varying degrees of potency as carcinogens in animals. Up to the present time, neither group of compounds has been shown to cause cancer in humans (WHO: IARC, 1978; Magee *et al.*, 1976).

TABLE 2. Association of NDELA With Certain Ingredients in Cosmetics Analyzed by FDA (1978a).

Cosmetic Product Samples Reported to Contain	NDELA	No NDELA Detected
BNPD + TEA	19	4
BNPD	1	2
TEA	47	81
Samples Containing neither BNPD or TEA	5	16
Samples with incomplete or no ingredient information	5	11
Total results reported by FDA	77	114

Surfaces To Which Commonly Applied

As implied in Table 1, BNPD is used in formulations applied to all areas of the human integument and is in contact with many, or in close proximity to all, body orifices. Some are used near sensitive and absorptive tissues (eyelids or ocular mucosa) and in proximity to mucous membranes. Formulations containing BNPD may be applied several times a day and may remain in contact with the skin for hours, e.g., in makeup (FDA, 1976).

BIOLOGICAL PROPERTIES

General Effects

BNPD is used as a preservative in a variety of cosmetic products which are applied to the skin. It has been suggested this is due to its oxidation of sulfhydryl groups in critical enzymes of the micro-organisms. In concentrations of 1% or more it is an irritant, and human test results show that BNPD has a significant potential for sensitization (Bowman and Stretton, 1972; Clark *et al.*, 1974; Marzulli and Maibach, 1973; Stretton and Manson, 1973).

Absorption, Metabolism, and Excretion

Percutaneous absorption is generally low (11% in 24 hours) for aqueous solutions of 4 mg/ml applied to the skin of rats and rabbits. The rate of absorption remains low even when the material is applied beneath an occlusive dressing. Absorption can be enhanced by using acetone rather than water as a vehicle. Absorption appears to occur by way of hair follicles. Absorption, metabolism and excretion of the compound have been studied using BNPD 2-^{14}C administered topically and orally, and BNPD-1,3 ^{14}C intravenously. Elimination in the urine of 60-80% of the dose given to rabbits intravenously occurs within 24 hours. Rats excrete 80.9% of an oral dose in the urine within 24 hours. Approximately 8.4% of the ^{14}C is eliminated in the expired air. Plasma concentrations after oral doses peaked at 2.5 to 9.0% of the total dose in two species within about two hours (in tests using small numbers of animals) (Moore *et al.*, 1976a, b; Naito *et al.*, 1974).

Distribution (as seen by whole body autoradiography) is fairly even among body organs with somewhat higher concentrations in the kidney and lower concentrations in fatty tissues (Moore *et al.*, 1976b).

Metabolic breakdown includes reductive dehalogenation resulting in 2-nitropropane-1,3diol. This in turn, may be further metabolized to glycerol and eventually CO_2 (Moore *et al.*, 1976b).

Animal Toxicology

General Studies

Acute Toxicity

Oral BNPD administered orally to rats and mice in varying doses caused gastrointestinal lesions and indicated the following LD50 values (Bryce *et al.*, 1978):

	Mouse	Rat
Male	374 mg/kg	307 mg/kg
Female	327 mg/kg	342 mg/kg

Another study established the oral LD50 of BNPD to be 180 mg/kg in rats, 270 mg/kg in mice, and 250 mg/kg in dogs (Frear, Ed., 1969); sex of the animals was not reported.

Administered orally as a solution containing 30 mg/ml in distilled water at five doses ranging from 150-525 mg/kg to ten rats at each dose, BNPD had an LD50 of 292 ± 31.9 mg/kg, while another sample of BNPD had an LD50 of 320 ± 26.3 mg/kg (CTFA, 1972b).

For aqueous solutions, the oral LD50 for mice was reported to be 350 mg/kg and for rats 400 mg/kg (Croshaw *et al.*, 1964).

An additional study found the oral LD50 to be 193 mg/kg in rats given oral doses of 182-205 mg/kg. Symptoms observed at four hours included decreased motor activity and respiratory rates (CTFA, ET42B).

Single doses of 40 or 100 mg/kg of BNPD in dogs caused transient gastric irritation. No methemoglobinemia occurred in cats given single oral doses of 25 mg/kg of BNPD, although 20 mg/kg acetanilide produced a marked increase in the percentage of methemoglobin present (Bryce *et al.*, 1978).

Intraperitoneal Intraperitoneal administration of BNPD in single doses allowed calculations of LD50s as follows:

	Mouse	Rat
Male	34.7 mg/kg	22.0 mg/kg
Female	32.8 mg/kg	30.2 mg/kg

Some of the injections led to peritonitis (Bryce *et al.*, 1978).

For aqueous solutions, the intraperitoneal LD50 in mice was reported to be 20 mg/kg (Croshaw *et al.*, 1964).

Subcutaneous injections of BNPD in rats produced hemorrhage at the injection sites, lesions in the stomach, edema and congestion of the lungs. The subcutaneous LD50 was approximately 200 mg/kg (Bryce *et al.*, 1978).

Skin Irritation Holding 0.5 g of dry BNPD in contact with the moistened, abraded and unabraded skin of rabbits for 24 hours resulted in a primary irritation score of 0.75 out of a maximum possible score of 8. Erythema occurred only on abraded skin. In the Federal Hazardous Substance Act procedure, scores of less than 5 indicate that the test material is not a primary irritant. A dose of 0.4 ml of a 20% aqueous solution of BNPD applied to abraded and non-abraded skin of rabbits gave a score of 6.75/8.0 and should be considered moderately to severely irritating (CTFA, 1973a; BS147B)

BNPD in 0.5 and 2% emulsions and solutions was tested on rabbit skin. At 2%, irritation was produced from one application; whereas, no irritation was produced from four daily applications of 0.5% concentrations (Croshaw *et al.*, 1964).

When applied to non-abraded, shaved skin of rabbits in a variety of solvents, BNPD's level of irritancy depended on the vehicle. Acetone solutions were nonirritating on single occluded application at 1%, while repeated application of 0.5% was highly irritating when not occluded. BNPD at 0.5% in aqueous methylcellulose gave similar results. In Polyethylene Glycol 300, a 5% concentration of BNPD was nonirritating on single occluded application. A single application of a 2% emulsion caused skin irritation but a 0.5% emulsion applied on four successive days did not (Bryce *et al.*, 1978).

Eye Irritation BNPD in amounts of 106 mg (apparently as the crystalline material) in the eyes of rabbits caused immediate irritation of the conjuctiva and delayed effects on the cornea and iris. These later effects were noted on the fourth day and remained on the last day of observation (seventh day). Scores, according to the Draize scale, on the seventh day were maximum in all but two of six unwashed eyes. Washing with water five minutes after the compound was allowed to contact the eye for five minutes did not modify the damage produced (CTFA, 1973b).

A 0.1 ml dose of a 10 or 20% aqueous solution of BNPD placed in the conjunctival sac of a rabbit's eye produced severe ocular damage. Washing four seconds after the application of the 20% solution reduced the reaction somewhat. Complete clearing of the damage required as many as 35 days in the unwashed eye and as many as 14 in the washed eye. When 3 mg of the solid compound was placed in the eye, damage was severe and clearing again required 35 days. Washing reduced recovery time to 14 days in one test and 21 in another (CTFA, BS147B, ET26B).

Two percent BNPD in solution and in emulsion was reported to be irritating to the rabbit eye. However, four daily applications of 0.5% solution and emulsion reportedly was not irritating (Croshaw *et al.*, 1964).

BNPD was also tested as a 0.5% solution in 1N saline and was found to be nonirritating when applied daily for four successive days to the eyes of rabbits. A solution of 5% in Polyethylene Glycol 400 was irritating on single application, but 2 percent under the same conditions was not (Bryce *et al.*, 1978).

Inhalation The approximate four-hour LC50 of BNPD was 0.18 mg/1 when administered by inhalation to 10 male and 10 female rats per exposure concentration. Survivors were described as having "rather severe" irritation of the ears and paws. This could have been increased redness resulting from increased blood flow and may have been an indication of a systemic effect rather than of skin irritation of exogenous origin. Survivors showed reduced body weight gain in the two weeks following exposure to 0.17 mg/l or greater, indicating some systemic effect (CTFA, BS147B).

Percutaneous Dermal applications of an acetone solution of BNPD to rats caused death at 160 mg/kg or more (Bryce *et al.*, 1978).

Subchronic Toxicity

Oral Studies of BNPD administered by oral intubation to rats showed that daily doses of 20 mg/kg for 90 days were tolerated well. At 80 and 160 mg/kg, respiratory distress, gastrointestinal lesions, and some deaths occurred. Rats given BNPD in drinking water for six weeks had reduced water intake and slightly enlarged kidneys at 160 mg/kg/day. Some deaths occurred when the dose level was 300 mg/kg/day. Dogs given 20 mg/kg/day by oral intubation for 90 days showed no significant toxic reaction, except for some vomiting (Bryce *et al.*, 1978; Boots Co., 1978a).

Male and female albino rats, 5-6 weeks of age, were fed 100 and 1000 ppm in the diet for 12 weeks without apparent effect on growth, food consumption, blood, liver, and kidney weight or histopathologic changes in the major organs (Croshaw *et al.*, 1964).

Skin Irritation BNPD as a 0.2 or 0.5% solution in aqueous 2.5% methylcellulose was applied to rabbits once daily in doses of 1 ml/kg for three weeks. It was applied to the intact and abraded clipped skin of the back. The abrasions penetrated the stratum corneum but did not disturb the derma. The 0.5% solution produced moderate edema, erythema, and eschar formation, while the 0.2% solution produced local erythema. The vehicle alone produced an effect similar to the 0.2% BNPD (Bryce *et al.*, 1978).

Skin Sensitization A guinea pig sensitization test was conducted using a combination of intradermal injections and topical applications following the Magnusson and Kligman procedure in which two intradermal injections of 0.02% BNPD in normal saline were given in the shoulder region. This was followed by two injections of 0.02% in 50:50 Complete Freunds Adjuvant (CFA): normal saline after which another two injections of 50:50 CFA:saline were given. Seven days later a booster application was given on the same site by an occluded patch of 1.5% BNPD in water which was left in place for 48 hours. Fourteen days later an occluded challenge patch of 0.4% in water was applied to the flank for 24 hours. Skin reactions at the challenge sites were observed at 24 and 48 hours after removal of the flank patches. The challenges and observations were repeated for a total of four applications. Two of the ten guinea pigs became sensitized after three challenges. A comment in the report stated, "In the Magnusson and Kligman test, sensitization is normally assessed after one challenge. At this stage in the present test there is no sensitization." It was concluded that BNPD was a weak sensitizer by this method of testing. Formaldehyde, a decomposition product of BNPD, which was also applied at 0.2% during the fourth challenge, was found not to be responsible for the sensitization in guinea pigs (Boots Co., 1978b).

Intradermal injections of a 0.05% aqueous solution of BNPD were given to guinea pigs on alternate days for a total of 10 injections. The first dose was 0.1 ml and the others were 0.05 ml. The challenge dose, 0.05 ml of 0.05%, given two weeks later produced no evidence of skin sensitization (Croshaw *et al.*, 1964). In another test using a 1% solution in acetone, BNPD failed to sensitize guinea pigs by the ear-flank method of Stevens (Bryce *et al.*, 1978).

Special Studies

Reproduction and Teratogenicity Studies

Rats given 10, 30, or 100 mg/kg daily by oral intubation during days 1 to 20 of pregnancy showed no embryotoxic or teratogenic effects. Some dams had a dose-related retardation in weight gain, and some died from pulmonary and gastric lesions. At the highest dose level, a slight delay in the calcification of the fetal skeleton was noted. Doses of 1, 3.3, and 10 mg/kg administered orally to rabbits from day 8 to 16 of pregnancy did not induce embryotoxic or teratogenic effects; however, the 10 mg/kg dose suppressed the weight gain of the does (Bryce *et al.*, 1978).

There was no effect on parturition, litter size, postnatal survival or development of the young in rats given 20 or 40 mg/kg of BNPD orally from day 15 of gestation throughout lactation. Reproductivity of male rats was not

impaired by daily doses of 20 or 40 mg/kg for 63 days before mating. Likewise, similar doses given to females from 14 days before mating to day 12 of pregnancy or until litters were weaned had no effect on reproduction. The males receiving 40 mg/kg daily had slightly reduced weight gain (Bryce *et al.*, 1978).

Application of 1 ml/kg of 0.5 or 2% BNPD in 2.5% aqueous methylcellulose to the dorsal skin of rats daily from day 6 to 15 of pregnancy produced local skin reaction at the site of application, but had no other adverse effects on the dams or the fetuses (Bryce *et al.*, 1978).

Rats given oral doses in 2% gum acacia of 0.3, 3, and 8 mg/kg BNPD on days 6 through 15 of pregnancy, when compared with control rats given a 2% suspension of gum acacia, showed no teratogenic effects (CTFA, 1972c).

Mutagenesis Male mice in five groups of 20 were given BNPD at a maximum tolerated dose, a calculated exposure dose and intermediate dose. (Actual values were not reported.) Doses were given daily for five days. One other group was given vehicle and the fifth group was untreated. Results of repeated matings of test animals with fresh females each week throughout spermatogenic cycle showed no effect from the compound. Therefore, BNPD was not considered a mutagen (CTFA, ET40B).

The mutagenic potential of BNPD was tested in a reverse mutation system using auxotrophic mutants of *Salmonella typhimurium* with and without Ames S-9 rat liver microsomes for bioactivation. The following strains of *S. typhimurium* were used:

With microsomes: TA1535, TA1536, TA1537, TA1538.
Without microsomes: G46, TA1535, TA1536, TA1537, TA1538.

There was no evidence of mutagenic activity. Maximum dose levels were not stated (Bryce *et al.*, 1978; Boots Co., 1979).

Carcinogenesis BNPD in concentrations of 0.2 and 0.5% in aqueous acetone applied to the skin of mice three times a week for 80 weeks did not affect the tumor incidence (Bryce *et al.*, 1978; Boots Co.,1978a). Data on this study are displayed in Table 3.

Oral administration of BNPD to rats in drinking water at doses as high as 160 mg/kg/day for two years did not reveal an effect on tumor incidence (Bryce *et al.*, 1978; Boots Co., 1978a). Data on this study are displayed in Table 4.

The manufacturer of BNPD reports no known cases of cancer among its workers who have been exposed during production for the last 7 to 8 years. It was also pointed out, quite correctly, that the number of workers exposed and the years of their exposure are too small for any meaningful conclusion at this time.

In view of the indications discussed earlier that N-nitrosodiethanolamine (NDELA) has been found in some cosmetics, it is important to note the report of the International Agency for Research on Cancer which states that NDELA is carcinogenic in two species of animals by different routes of administration. The Agency also notes, "Although no epidemiological data were available, N-nitro-sodiethanolamine should be regarded for practical purposes as if it were carcinogenic to humans" (WHO:IARC, 1978).

TABLE 3. Tumor Incidence in Mice Exposed Topically to BNPD (Boots Co., 1978a).

Tumor Site	Number of Mice with Tumors					
	Males			Females		
	Control	0.2[1]	0.5	Control	0.2	0.5
Lymphoreticular system	6	4	11	7	8	10
Liver	1	1	0	2	0	0
Heart	1	0	0	0	0	0
Lungs	13	13	13	10	9	11
Endocrine glands	3	3	0	3	3	1
Mesentery	0	1	0	0	0	0
Subcutaneous tissues	1	0	1	3	0	0
Cutaneous tissues	1	0	1	1	1	3
Kidney	0	0	0	1	0	0
Harderian gland	0	0	1	-	-	-
Testes	3	0	2	-	-	-
Ovary	-	-	-	1	1	0
Uterus/Vagina	-	-	-	3	0	0
Number Examined	50	50	50	51	50	49

[1]Percent BNPD

Clinical Assessment of Safety

Dermatologic Evaluation Ten volunteers were tested for skin irritation with closed patches of BNPD at 0.0, 0.5, 1.0, and 2.0% in soft paraffin and 0.0, 0.05, 0.1, and 0.25% in aqueous buffer at pH 5.5. The paraffin patches produced slight erythema in two volunteers at 1% BNPD, and moderate erythema in four volunteers at 2% BNPD. The aqueous patches produced slight erythema in one of the volunteers at 0.25% BNPD. It was concluded that BNPD is "slightly irritant to human skin at 1% in soft paraffin and at 0.25% in aqueous buffer at pH 5.5" (Bryce *et al.*, 1978).

Marzulli and Maibach (1974) and Maibach (1977) studied the potential contact sensitization to a number of biocides and concluded that BNPD at 2.5% in soft paraffin was a potential sensitizer but a nonirritant in that concentration. Their subsequent tests showed BNPD to be an irritant to human skin at concentrations greater than 1% (Marzulli and Maibach, 1973). However, Maibach later was unable to demonstrate contact sensitization in a study of 93 normal subjects on whose skin 5% BNPD in yellow paraffin was applied 10 times in three weeks followed by a two-week rest period prior to challenge with 0.25% BNPD in paraffin (Maibach, 1977).

Occupation Exposure In the industrial experience of 50 workers from 1970 to date, it was found that a documented 23 of 50 workers had reported rashes and/or superficial burns secondary to exposure to saturated aqueous solutions or powder of BNPD on at least one occasion. Of these 23, there were

TABLE 4. Tumor Incidence in Rats Exposed Orally to BNPD (Boots Co., 1978a)

Tumor Site	Number of Rats with Tumors											
	Males (main group)				Males (satellite group)				Females			
	Control	10[1]	40	160	Control	10	40	160	Control	10	40	160
Lymphoreticular tissue	1	2	2	1	0	0	0	0	1	1	1	0
Mediastinum	1	0	0	0	0	0	0	0	0	0	0	0
Liver	1	0	0	0	1	0	0	0	0	0	0	0
Endocrine glands	21	22	12	2	3	2	1	1	30	34	33	22
Pancreas	0	1	1	0	0	0	0	0	0	0	0	0
Kidney	0	2	0	0	0	0	0	0	0	2	1	0
Stomach	0	1	0	2	0	0	0	1	0	0	0	1
Duodenum	0	1	0	0	0	0	0	0	0	0	0	0
Skin	6	6	6	5	0	0	1	0	0	0	0	0
Subcutaneous tissue	10	7	8	2	3	0	1	0	38	46	49	33
Abdominal cavity	1	1	0	0	0	0	0	0	0	0	0	0
Bone	0	0	0	0	0	1	0	0	0	1	0	0
Testes	1	0	0	0	0	0	0	0	-	-	-	-
Scrotum	0	0	1	0	0	0	0	0	-	-	-	-
Ovary	-	-	-	-	-	-	-	-	1	0	0	0
Uterus	-	-	-	-	-	-	-	-	0	0	1	5
Number Examined	43	43	42	41	6	4	6	13	52	53	49	51

[1]mg/kg/day BNPD

8 who reported a second occurence, 6 a third, and 3 a fourth. These reactions were described as apparently the result of a breakdown of protective measures and appeared to be irritant reactions rather than contact allergy (Boots, 1978a). The records indicate that no individual involved was required to terminate employment as a consequence of these injuries.

Clinical Experiences Patients attending a dermatitis clinic were subjected to a battery of closed patch tests for diagnosis which included BNPD at 0.25% in soft paraffin. Three of the 149 patients showed a slight transient erythema. There was no evidence of sensitization or of cross-sensitization with formalin (Bryce *et al.*, 1978).

Data reported for 1975-76 by the North American Contact Dermatitis Group gives the incidence of contact dermatitis among dermatology patients. The following data were presented (Rudner, 1977):

Test Material	No. of Patients	% Incidence
1% BNPD (aqueous)	190	13.2
2% Formaldehyde (aqueous)	900-2000	3.8

No information has been made available on studies of phototoxicity or photosensitization.

SUMMARY

BNPD has been shown to possess a wide spectrum of antibacterial activity with effective activity against gram-positive and gram-negative organisms, particularly *Pseudomonas aeruginosa*. Its effectiveness is enhanced by the addition of other antibacterials or biocides such as the parabens.

BNPD is most stable under acid conditions, although it demonstrates high bacterial activity over a wide pH range. Its mode of decomposition has revealed several decomposition products including formaldehyde. Decomposition of BNPD *in vitro* produces an N-nitrosating agent. This may be expected to occur also *in vivo*.

Contact allergy and contact irritant reactions in animals and humans are reported as minimal. The spectrum of these cutaneous reactions appears to be dose dependent at 0.25% and above. Cosmetic preparations containing BNPD at levels of 0.01 to 0.1% are considered to produce minimal contact irritation. However, unformulated BNPD in concentrations of 1% or greater has been shown to be a considerable irritant.

BNPD is moderately toxic orally in a variety of laboratory mammals, the LD50 varying with the test circumstances. Intraperitoneally, it is highly toxic to rats and mice. On skin contact the dry powder produced only slight irritation, while a 20% aqueous solution caused moderately severe irritation in rabbits. Results with BNPD dissolved organic solvents and applied under occlusive dressings varied from practically nonirritating to being highly irritating for humans as well as animals.

Contact with the rabbit eye caused immediate irritation which was not relieved by irrigation. Inhalation of concentrated vapor produced an approximate 4-hour LC50 of 180 mg/1.

Repeated dosing by intubation or feeding in the diet was tolerated well while repeated skin application produced no effects different from those produced by the vehicle. It did not have a carcinogenic effect in studies of limited numbers of mice by skin painting and rats by ingestion in drinking water. In the guinea pig it appeared to be a weak sensitizer.

BNPD had no effect on reproduction, was not a teratogen, and had no embryotoxic effects. It was not a mutagen by the standard mouse dominant lethal test nor by the bacterial reverse mutant system.

CONCLUSIONS

The evidence at hand indicates 2-Bromo-2-Nitropropane-1,3-Diol to be safe as a cosmetic ingredient at concentrations up to and including 0.1% except under circumstances where its action with amines or amides can result in the formation of nitrosamines or nitrosamides.

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