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Final Report on the Safety Assessment of 1-Naphthol

1-Naphthol, a phenol, is used as a coupler in hair dyes and colors at concentrations below 1.0%. Animal studies indicate that this compound at low concentrations is neither an eye nor skin irritant. The human exposure of this ingredient is as a component of hair dyes and not as a pure ingredient. 1-Naphthol has an LD_{50} of 2.3 g/kg. No significant toxicity findings were reported in either subchronic or chronic studies. Hair dyes containing this ingredient when dermally applied were neither teratogenic nor carcinogenic. It is concluded that 1-Naphthol may be safely used in cosmetic products in the present practices of use and concentration.

INTRODUCTION

This safety evaluation is applicable only to the use of 1-Naphthol in cosmetic hair dye formulations. Additional references on the safe use of hair dye formulations are cited in this report.

CHEMISTRY

Definition and Structure

1-Naphthol (CAS No. 90-15-3) is a polycyclic phenol with a formula of $C_{10}H_8O$ and a molecular weight of 114.16. It conforms to the structure[1] in Figure 1.

Synonyms for 1-Naphthol include α-naphthol, 1-hydroxynaphthalene, α-hydroxynaphthalene, and 1-naphthalenol.[2] Numerous trade names for 1-Naphthol are presented elsewhere.[3]

Chemical and Physical Properties

1-Naphthol is a clear, crystalline substance with a disagreeable burning taste and a phenolic odor.[4] 1-Naphthol is soluble in alcohol, ether, acetone, benzene, chloroform, and alkali hydroxide solutions and is slightly soluble in water.[5] It darkens in light and is volatile in steam.[2] 1-Naphthol is combusti-

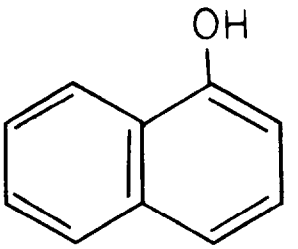

FIG. 1. Structure of 1-Naphthol.

ble[6] (Table 1). 1-Naphthol does not absorb ultraviolet A (UVA) or B (UVB) light.[7]

Method of Manufacture

1-Naphthol can be prepared by fusing sodium 1-naphthalene sulfonate with NaOH. The melt is then decomposed by HCl and distilled.[6] It can also be produced by the oxidation of naphthalene.[3]

Analytical Methods

Analytical methods for the separation and/or determination of 1-Naphthol include infrared spectroscopy,[8] high-performance liquid chromatography,[9] gas chromatography[10] or electron-capture detection,[11] thin-layer chromatography,[12] silica gel column chromatography,[13] high voltage paper electrophoresis,[14] spectrophotometry,[15] fluorimetry,[16] and colorimetry.[17]

Reactions

1-Naphthol is used in permanent (oxidative) hair dyes. A more detailed review of oxidative hair coloring chemistry has been published previously.[18] Chromophores are formed inside the hair fiber from precursors as a result of

TABLE 1. Chemical and Physical Properties of 1-Naphthol

Property	*Value*	*Reference*
Melting point (°C)	96	5
Boiling point (°C)	288 (sublimes)	5
	282.5	19
	278 (sublimes)	6
Density (98.7°/4°)	1.0954	2
(99°/4°)	1.0989	5
(4°)	1.224	6
Vapor pressure (94°)	1 mm	19
Refractive index (99°)	1.6224	5
(98.7°)	1.6206	6

oxidation. Three classes of chemical reactants are required to accomplish the color-forming reactions: primary intermediates, couplers, and oxidants. The primary intermediate (usually *p*-phenylenediamine, *p*-toluenediamine, or *p*-aminophenol) is oxidized by the oxidant (usually hydrogen peroxide) to give a reactive imine. This imine reacts with a coupler to produce an indo dye. 1-Naphthol is used as a coupler; when reacted with the imines produced from the oxidation of either *p*-phenylenediamine or *p*-aminophenol it produces a blue color.[20] The Cosmetic Ingredient Review (CIR) has previously reviewed both *p*-phenylenediamine[18] and *p*-aminophenol.[21]

Impurities

Information concerning impurities in 1-Naphthol is not available.

USE

Cosmetic

1-Naphthol is used as a coupler in hair dyes and colors. Data submitted to the Food and Drug Administration (FDA) in 1987 indicated that 1-Naphthol is used in a total of 236 formulations of hair dyes.[22] Of these listed formulations containing 1-Naphthol, 180 were $\leq 0.1\%$, 55 were in the > 0.1–1% range, and 1 was in the > 1–5% range (Table 2). This represents an increase from the eight formulations containing 1-Naphthol that were reported in 1981 by the FDA.[23]

The FDA cosmetic product formulation computer printout[22,23] is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations, 1982. Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual

TABLE 2. Product Formulation Data[22]

Product category	Total no. of formulations in category	Total no. containing ingredient	*No. of product formulations within each concentration range (%)*		
			> 1–5	*> 0.1–1*	*≤ 0.1*
Hair dyes and colors (all types requiring caution statement and patch test)	915	236	1	55	180
1987 Totals		236	1	55	180

concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2–10-fold error in the assumed ingredient concentration.

The oxidative or permanent hair dyes containing the 1-Naphthol, as "coal tar" hair dye products,[18] are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation.[24] To be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution—this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Patch test instructions call for a 24 h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.[25]

Permanent hair dye preparations are usually packaged in separate containers that are mixed immediately before use. These formulations may come in contact with the hair, skin (especially the scalp), eyes, and nails. Individuals dyeing their hair may use these formulations once every few weeks, whereas hair dressers may come into contact with 1-Naphthol-containing hair dye products several times a day. An estimated 40% of American women are regular users of hair dyes.[25]

1-Naphthol is listed in Annex III in the European Economic Community (EEC) Directive. This Annex contains a list of substances that can only be used in cosmetics subject to the restrictions and conditions laid down in the Annex. 1-Naphthol can be used as a coloring agent for hair dyeing with a maximum authorized concentration of 0.5% in the finished cosmetic product. The warning "contains alpha-naphthol" must be printed on the label of the product.[26]

Noncosmetic

1-Napthol is used in the manufacture of dyes and intermediates and in microscopy.[2] Its use as an antimicrobial agent for treating buildings, building materials, textiles, leather, agricultural products, and foods has been patented.[27]

GENERAL BIOLOGY

Biochemical Effects

Male Swiss-Webster mice, weighing between 17 and 20 g, were fed a diet containing 25 mmol 1-Naphthol/kg of feed to assess the induction of hepatic microsomal drug-metabolizing enzyme activity. After 14 days on the test diet, animals were killed and the livers were immediately removed. There was no significant difference in either body or liver weights between mice fed control diets and mice fed diets containing 1-Naphthol. The rate of in vitro *p*-hydroxylation of aniline and demethylation of benzphetamine was not significantly different from controls in the livers of mice fed 1-Naphthol. 1-Naphthol did not affect the hepatic activities of either cytochrome P-450 or b_5. The concentration of microsomal protein in the liver was not affected by the administration of 1-Naphthol. Mice receiving the diet containing 1-Naphthol slept for the same length of time as mice fed the control diet when injected i.p. with 70 mg/kg pentobarbital.[28]

The effect of 1-Naphthol on arachidonic acid metabolism by cyclooxygenase and lipoxygenase enzymes was studied in intact human platelets. By using intact platelets as a source of the enzymes the investigators were able to evaluate both the drug's effects on the enzymes and the ability of the drug to cross the cell membrane to reach the target enzymes. Fractions of platelets (1 ml) were incubated with 10 μl of 1-Naphthol for 5 min. Arachidonic acid (20 μl) was then added to the suspension and incubated for another 5 min. 1-Naphthol was a selective blocker of cyclooxygenase enzymes but did not affect the activity of lipoxygenase enzymes.[29]

The distribution of glucuronyl transferase and its activity towards 1-Naphthol was investigated in specimens of human intestine and liver. 1-Naphthol at a concentration of 0.5 mmol/l was incubated with homogenates of liver and intestinal tissue for 10 min. Glucuronyl transferase was present in both nuclear and microsomal fractions of the human intestine. A significant rate of 1-Naphthol glucuronidation was found in all specimens of human intestine assayed. The nuclear glucuronyl transferase activity was higher than the microsomal activity in intestinal segments. The activity of glucuronyl transferase towards 1-Naphthol was higher in the microsomal liver fraction than the nuclear liver fraction.[30]

Cytotoxicity

1-Naphthol was tested for its cytotoxicity in four different cell lines. It inhibited the growth of ascites sarcoma BP 8 cells by decreasing the cell growth rate 90–99% when compared with control values. The cells were incubated for 48 h with 1-Naphthol at a concentration of 1 mM. 1-Naphthol, 1 mM, was incubated for 5 min with 10^5 brown fat cells isolated from adult hamsters. Norepinephrine was then added and the cells incubated for another 5 min. 1-Naphthol inhibited the oxidative metabolism of the cells by 90–99%. Human diploid embryonic lung fibroblasts were used to assess the amount of plasma membrane damage caused by 25 mM 1-Naphthol, which caused

70–79% of the maximal release of the labeled cytoplasmic nucleotide marker. The ciliotoxicity of 1-Naphthol was assessed in organ cultures from the tracheas of chicken embryos. A 5 mM concentration of 1-Naphthol caused a 90–99% decrease in the time to ciliostasis. A mean activity index of 8.5 was calculated for 1-Naphthol on a scale of 0–10 with 10 being the value given to the most toxic of the compounds tested.[31]

1-Naphthol had a dosage-dependent toxicity to suspensions of freshly isolated hepatocytes from rats treated with phenobarbital. A dose of 0.35 mM of 1-Naphthol resulted in only 20% of the cells being viable after a 4 h incubation as determined by trypan blue exclusion. The surface morphology of the hepatocytes was also affected by 1-Naphthol; after a 4 h incubation with a dose of 100 μM it caused surface blebbing. The toxicity of 1-Naphthol as well as its early effects on surface morphology were potentiated by the addition of dicoumarol, an inhibitor of DT-diaphorase that catalyses the reduction of quinones to diols without forming reactive semiquinone intermediates. These results suggest that 1-Naphthol was toxic to isolated hepatocytes via the formation of cytotoxic naphthoquinone metabolites. Both oxygen radical formation and covalent binding may be involved in the toxicity.[32]

The effect of 1-Naphthol on the growth of HeLa cells was studied. The cells were grown as a monolayer culture and were incubated for 48 h with 1-Naphthol in the growth medium. At concentrations of 25, 30, 50, and 100 ppm 1-Naphthol caused 33, 49, 61, and 95% inhibition, respectively.[33]

1-Naphthol was tested for its cytotoxic potential in both HeLa cells and human diploid skin fibroblasts. The cells were incubated for 48 h with 1-Naphthol, dissolved in alcohol, in the growth medium. 1-Naphthol, tested in a concentration of 30 ppm, caused a 33 and 37% inhibition in the growth of HeLa cells and human skin fibroblasts, respectively.[34]

Absorption, Distribution, Metabolism, and Excretion

The percutaneous absorption of 1-Naphthol was studied in 3 men, ages 50–52. The subjects were under treatment for skin diseases of their extremities and had normal skin on their trunk. Blood urea and liver function tests were normal for the subjects, who had no clinical evidence of renal or hepatic disease. A circular area, 10 cm in diameter, on the upper back was the selected treatment site. An ointment, 3 g, made up of equal parts soft soap and soft paraffin was applied to the treatment site. The ointment contained approximately 0.3 mg of 1-Naphthol containing ^{14}C on the first carbon. The ointment was left in contact with the skin for 8 h before removal. Urine was collected for 24 h before the application and for three 24 h periods following the application. Radioactive compounds were recovered from the urine and the identity of the radioactive metabolites was determined by paper chromatography. In subject 1, approximately 65% of the dose applied was not recovered from the skin and 88.5, 5.2, and 2.8% of that 65% was found in the urine 1, 2, and 3 days, respectively, after the application. Approximately 40% of the urinary radioactivity recovered from the first 2 days' urine could be extracted after hydrolysis. Of that 40%, approximately 32% was found in the glucuronide fraction and 2.3% was found in the sulfate fraction. In subjects 2 and 3, 23.8%

and 48.1% of the applied dose, respectively, was not recovered from the skin. The amounts not recovered from the skin were assumed to have been absorbed, although no further data were reported regarding the amount of radioactivity found in the urine from these two subjects. Most of the glucuronide fraction of the urine recovered from subjects 1 and 2 after 1 day behaved chromatographically like 1-Naphthol and it was concluded that the major part of the radioactivity recovered consisted of 1-Naphthol. The authors stated "there is a fast and extensive percutaneous absorption of naphthol through the intact skin."[35]

The diffusion of 1-Naphthol through fetal hog and guinea pig skin was investigated in a study to determine an appropriate model for human skin. Whole fetal hog skin was frozen prior to the experiments while the skin from young adult male Hartley guinea pigs was tested both as a fresh sample and after freezing. A 1 inch square of the whole skin was cut and hydrated by floating on water, dermis side down. The skin was then blotted dry and placed epidermal side up on the receiving half of a diffusion cell. The skin was left in the diffusion cell for 24 h and samples of the dermal chamber were taken after several intervals. The results were expressed as the percentage of the applied 0.5M 1-Naphthol, in ethanol, that diffused to the dermal side. 1-Naphthol penetrated the fetal hog skin to a much greater extent than the guinea pig skin. After 4 h in the diffusion cell, an approximate average of 14% had diffused through the fetal hog skin while an approximate average of 3% had diffused through the guinea pig skin. After 24 h approximately 21% had diffused through fetal hog skin while approximately 14% had diffused through the guinea pig skin; these values have been averaged for the three specimens of each species tested.[36]

Nine ICR male mice, weighing between 25 and 30 g, received a single 45 mg/kg oral dose of 1-Naphthol in corn oil with ^{14}C in the 1 carbon position. The mice had free access to water and food and were kept in metabolism cages with urine and feces collected 6, 12, and 24 h after treatment. The mice were killed 72 h after administration of the dose. Of the total administered dose, approximately 68% was eliminated in the urine after 24 h and approximately 13% was eliminated in the feces. The metabolites were mainly 1-naphthyl glucuronide and 1-naphthyl sulfate. The percentage of these two metabolites was approximately equal in the urine, while the feces contained more sulfate. Both the glucuronide and sulfate conjugates were formed rapidly after the administration of 1-Naphthol.[37]

1-Naphthol was injected intraperitoneally in white Sprague-Dawley rats, weighing 160–170 g. A dose of 7.5 μmol/kg 1-Naphthol in 2-methoxyethanol was administered with 1-Naphthol that was ^{14}C labeled in the 1 carbon position. The rats were placed in metabolism cages and the expired CO_2 was collected for 48 h following treatment. Urine was collected every 12 or 24 h and feces were collected after 24 and 48 h. The rats were killed 4 or 48 h after the i.p. injection of 1-Naphthol. After 4 h, approximately 83.5% of the administered radioactivity had been eliminated in the urine while about 16.5% remained in the body tissues. After 48 h, 91.0% of the administered radioactivity had been recovered from the urine and 1.4% from the feces, while 7.6% remained in the body.[38]

The extrahepatic clearance of 1-Naphthol, with ^{14}C on the 1 carbon, was investigated in female Sprague-Dawley rats weighing between 210 and 290 g. The 1-Naphthol, at a dosage of 0.1 mg/kg, was administered to the rats intravenously (i.v.), intra-arterially (i.a.), intraduodenally (i.d.), and via the hepatic portal vein (h.p.v.). The rats were anesthetized and various cannulae were placed to determine the clearance of 1-Naphthol in the various organs investigated. The decline in plasma concentration of 1-Naphthol was polyexponential with time following i.a. administration. The plasma concentrations were considerably reduced due to pulmonary extraction following i.v. administration. Appreciable first pass intestinal extraction was seen when i.d. administration was compared with h.p.v. administration. Both naphthyl glucuronide and naphthyl sulfate conjugates were found. Both the lungs and intestine were more than twice as efficient as the liver in the extraction of 1-Naphthol in vivo.[39]

Male Sprague-Dawley rats, weighing between 335 and 400 g, were used in a study to investigate the renal clearance of 1-Naphthol with ^{14}C at the 1 carbon position. The rats were anesthetized and 1-Naphthol, 1-naphthyl glucuronide, and 1-naphthyl sulfate were each infused at a rate of 1.0 μmol/min/kg through the jugular vein. This infusion resulted in a steady-state plasma concentration of 1.10 nmol/ml of 1-Naphthol. Approximately 77% of the radioactivity was recovered in the urine and 99% of the material excreted was identified as either 1-naphthyl glucuronide (66%) or 1-naphthyl sulfate (33%). The bile, which contained approximately 11% of the infused ^{14}C radioactivity, was an alternative route of excretion.[40]

The metabolism of 1-Naphthol, with ^{14}C on the 1 carbon, was studied in the cat, pig, and rat. The 1-Naphthol was administered at a dosage of 25 mg/kg by i.p. injection. In the cat, after 24 h, an average of 91% of the administered radioactivity had been excreted in the urine; approximately 98% of the excreted material was the sulfate conjugate while 1.4% was the glucuronide conjugate. In the pig, 81% of the administered radioactivity appeared in the urine within 24 h, 66% glucuronide and 32% sulfate. After 24 h, approximately 59% of the administered ^{14}C had been excreted in the urine of the rat. Of the radioactive material that had been excreted, 53% was the sulfate conjugate and 47% was the glucuronide conjugate.[41]

Several different species of primates were used in an experiment to assess the metabolism of 1-Naphthol with ^{14}C on the 1-carbon. The 1-Naphthol was administered in an intramuscular injection at dosages of 0.01, 1, 10, and 25 mg/kg in rhesus, cynomolgus, and capuchin monkeys and at a dosage of 10 mg/kg in patas monkeys and a tamarin, bushbaby, and tree shrew. Of the administered radioactivity, 82–99% was excreted in urine of the rhesus monkeys, 58–97% was excreted in the urine of the cynomolgus monkey, and 19–55% was excreted in the urine of capuchin monkeys after 24 h. In the species receiving only one dose, 40% of the administered radioactivity was excreted in urine of the patas monkeys, 84% in the tamarin, 38% in the bushbaby, and 71% in the tree shrew after 24 h. All of the metabolites found in the urine were identified as either sulfate or glucuronide conjugates.[42]

The transport of 1-Naphthol and its glucuronide and glucoside conjugates in blood was studied both in vitro and in vivo. 1-Naphthol, with ^{14}C at the first

carbon, was incubated with various fractions of human blood for 24 h. The solution was centrifuged and the amount of radioactivity was measured in each fraction of blood. Approximately 97.6% of the 1-Naphthol was bound to the human plasma with 92.8%, 3.6%, and 3.6% in the albumin, heavy lipoprotein, and light lipoprotein fractions, respectively. In the in vivo portion of the study, six or more female Dublin:ICR mice were given i.p. injections of 1-Naphthol at a dosage of 15 mg/kg. The mice were killed after 10 min and blood was removed from the heart. The blood was centrifuged to separate the red blood cell fraction, which contained 20–30% of the radioactivity. The plasma was then divided into three components: albumin, γ-globulins, and lipoproteins; the radioactivity in each component was measured. Approximately 43% of the administered 1-Naphthol was associated with the lipoprotein fraction and 43% was associated with the albumin. The results of both the in vitro and in vivo experiments suggest that albumin plays a significant role in the transport of 1-Naphthol and its conjugates.[43]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

CFY rats, weighing between 90 and 122 g, were used to determine the acute oral toxicity of 1-Naphthol. Groups of five male and five female rats were used in the study and the animals were observed for 14 days following administration of 1-Naphthol. All animals that died were examined macroscopically. 1-Naphthol was tested at a concentration of 40% in a suspension of 0.5% aqueous tragacanth gum containing 0.05% Na_2SO_3 and had a calculated LD_{50} value of 2.3 g/kg.[44]

Inhalation

Four splenectomized dogs were exposed to a mist of 3% 1-Naphthol in deodorized kerosene. The dogs had been splenectomized 4–8 years before this study. Each animal was exposed for four 5 min periods with a 7–10 min pause between the exposures to keep the mist in the air and promote inhalation. The dogs were treated for four consecutive mornings and were observed for a total of 10 days. Blood samples were obtained before the first and fourth exposures and 7 and 10 days after the start of the study. The only effect reported for the dogs exposed to 1-Naphthol was an increase in the number of reticulocytes to at least double the initial number. The increase was noted on days 7 and 10 in one of the four dogs exposed to the treatment.[45]

Subchronic Toxicity

A group of six male and six female New Zealand White rabbits were used in a study to determine the subchronic dermal toxicity of an oxidative hair dye preparation containing 0.5% 1-Naphthol as well as other active ingredients. The formulation was mixed with an equal volume of 6% hydrogen peroxide

and applied in a 1 ml/kg dose to the shaved backs of the rabbits. The applications were made twice weekly for 13 weeks. In three rabbits of each sex the skin of the application site was abraded on the first treatment day of each week. The dye remained in contact with the skin for 1 h, at which time the rabbits were shampooed, rinsed, and dried. Three groups of 12 rabbits that received the same treatment without the dye application were used as controls. At the start of the study and after 3, 7, and 13 weeks, clinical chemistry and hematologic determinations were made and the urine of each animal was examined. All animals surviving the study were killed after 13 weeks and examined for gross abnormalities and selected tissues also were examined microscopically. No evidence of systemic toxicity was seen. The skin of the treated rabbits was slightly thickened, which was attributed to the frequency of the dye application. The mean hemoglobin value was significantly lower in both male and female rabbits treated with the formulation compared with controls. The red blood cell counts of treated females were statistically significantly higher than control values. However, these differences were not considered toxicologically significant. At necropsy, no gross abnormalities were seen and no microscopic lesions were found that were judged related to the administration of the hair dye formulation.[46]

Chronic Toxicity

An oxidative hair dye preparation containing 0.5% 1-Naphthol and other active ingredients was tested for its chronic dermal toxicity to Swiss-Webster mice. The hair dye formulation was mixed with an equal volume of 6% hydrogen peroxide and was applied in a 0.05 ml dose to a clipped interscapular region, approximately 1 cm^2, on each mouse. Groups of 50 female and 50 male mice were used to test the formulation. The animals were treated once weekly for 21 months. Mortality, behavior, and physical appearance were observed daily for the duration of the study. After 7 months of the treatment, 10 male and 10 female mice were killed and necropsied. The survival rate of the mice was not significantly affected by the treatment with the hair dye formulation. There was not any significant difference in ratios of organ to body weight in treated mice as compared to the controls.[47]

Irritation

Dermal

A 2.5% (w/v) preparation of 1-Naphthol in 0.5% aqueous tragacanth gum containing 0.05% Na_2SO_3 was tested for its dermal irritancy potential. The suspension was applied to the intact and abraded skin of three New Zealand White rabbits. None of the rabbits had signs of dermal irritation during the 72 h observation period. 1-Naphthol was given a primary irritation index score of 0.[44]

Three lots of 1-Naphthol were tested for skin irritation on three groups of six guinea pigs. A 0.5 ml test sample prepared as a 3% suspension in a Shultz

vehicle was applied to a shaved area of 1 in^2. The animals were observed at 24, 48, and 72 h. Two lots produced minor irritation at 24 h, but not at 48 and 72 h. One lot, reported to be a purified sample, did not produce any signs of irritation at 24, 48, or 72 h.[48]

When applied to the skin of rabbits for 24 h, 500 mg of 1-Naphthol caused severe irritation. Moderate irritation of the skin was observed when rabbits were treated with 550 mg 1-Naphthol in open patches.[3]

Ocular

1-Naphthol was tested for its ocular irritation potential in New Zealand White rabbits. One group of three rabbits was used to test each concentration of 1-Naphthol in 0.5% aqueous tragacanth gum containing 0.05% Na_2SO_3. The concentrations tested were 0.5, 1.5, 2.0, and 2.5% (w/v). The eyes of all of the rabbits were rinsed with 50 ml of water, 10 s after the instillation of the 1-Naphthol. Mild conjunctival reactions were observed in one of three rabbits tested with the 0.5% concentration and two of three rabbits tested with the 1.5% concentration. In the rabbits tested with the 2.0% concentration, two had corneal opacities persisting for 1–3 days accompanied by well-defined erythema and moderate edema of the conjunctiva in one of the rabbits while the third rabbit had mild conjunctival irritation. Transient corneal opacity lasting for 1–3 days was observed in 1 of 3 rabbits tested with the 2.5% concentration of 1-Naphthol and a 2-day dulling of normal corneal luster was noted in another rabbit, these reactions were accompanied by moderate edema in 1 of the 2 aforementioned rabbits. The third rabbit tested with 2.5% 1-Naphthol had mild conjunctival irritation. 1-Naphthol was an eye irritant at concentrations of 2.0 and 2.5% and its minimum irritant level for the rabbit eye was between 1.5 and 2.0%.[44]

When applied to the surface of rabbit eyes, 1-Naphthol caused damage to the corneal epithelium at a grade of 9 on a scale of 1–10.[49] 1-Naphthol, 1 mg, when instilled in to the eyes of rabbits, caused severe irritation.[3]

Sensitization

A guinea pig maximization test was conducted using 19 test animals plus 8 controls. Each test animal received three intradermal injections of 0.1 ml of Complete Freund's Adjuvant, 0.1 ml of 0.1% 1-Naphthol in water at pH 7.0, or 0.05 ml of 1-Naphthol emulsified with 0.05 ml adjuvant. One week after the intradermal injection a saturated filter paper containing the test substance was applied to each area under an elastic adhesive for 48 h. After 7 days, a challenge patch containing either 0.05% or 0.1% 1-Naphthol, as for the topical induction, was applied and the test site was observed after 24 and 48 h. No evidence of sensitization was observed in any of the test or control animals.[50]

Nineteen guinea pigs were tested with 3.0% 1-Naphthol using an open epicutaneous test procedure. The test material was applied daily (except Sundays) for 3 weeks to the clipped flank of each animal. After a 2 week nontreatment period the animals were challenged by a single administration of the test compound; no sensitization was observed.[51]

Teratogenicity

An oxidative hair dye formulation containing 0.5% 1-Naphthol as well as other active ingredients was tested for its teratogenic potential in rats. The formulation was mixed with an equal volume of 6% hydrogen peroxide prior to application to the shaved backs of 20 pregnant Charles River CD rats. The 2 ml/kg dose was applied on days 1, 4, 7, 10, 13, 16, and 19 of gestation. The study included three negative control groups in which the only treatment was the shaving of the back and a positive control group, which received an oral dose of 250 mg/kg acetylsalicylic acid on days 6–16 of gestation. The rats were killed on day 20 of gestation and the uteri were examined, the corpora lutea were counted, and the number, distribution, and location of live, dead, and resorbed fetuses were recorded. The fetuses from each liter were examined for visceral and skeletal anomalies. No signs of toxicity were observed throughout the study. The only changes in appearance were in the color of the skin and hair at the site of the application of the hair dye formulation. No significant differences were seen between the group treated with the hair dye and the three negative control groups for any of the parameters measured. The authors concluded that the administration of the hair dye formulation "every third day of the gestation period produces no embryotoxic or teratogenic effects."[46]

In a large-scale screening study, 1-Naphthol had no teratogenic activity in mice.[52] The full report on the preceding study noted that 1-Naphthol given subcutaneously produced a slight increase in fetal mortality but the incidence of anomalies was within the normal range. The authors concluded that 1-Naphthol was not teratogenic under the test conditions, but may affect growth.[53]

Mutagenicity

1-Naphthol was tested for its mutagenic potential by the Ames test.[54] It was nonmutagenic when tested using five strains of *Salmonella typhimurium*: TA1535, TA1537, TA1538, TA98, and TA100, both with and without metabolic activation by rat liver microsomes. 1-Naphthol was also tested in a DNA-repair test, which used a procedure similar to the rec-assay system, in three strains of *Escherichia coli*, WP2, WP67, and CM871, and was nonmutagenic when tested both with and without metabolic activation[55] (Table 3).

The incidence of sister chromatid exchanges and chromosomal aberrations was studied in lymphocytes from subjects who had their hair repeatedly dyed. A test group of 6 women and 4 men had their hair dyed every 3–6 weeks for a total of 11 months and 12 total applications of the dyes. 1-Naphthol was in the dye applied to 4 of 10 subjects tested along with 7 other active ingredients. The concentrations of the active ingredients were not specified, although the amount of active ingredients applied to the subjects varied between 0.5 and 4.0 g/subject. The dye was mixed with an equal volume of 3–6% hydrogen peroxide solution and was applied to the hair for 30 min and then washed out. A control group was made up of 10 subjects who did not have their hair dyed. Blood was collected from the test subjects 24 h following each dye applica-

TABLE 3. Mutagenicity of 1-Naphthol

Study type, organism	*Method notes*[a]	*Results, comments*	*Reference*
Ames test, *S. typhimurium*	Strains: TA1535, TA1537, TA1538, TA98, and TA100. +S9 and −S9	Negative	55
Ames test, *S. typhimurium*	Strains: TA1535, TA1537, TA1538, TA98, and TA100. +S9 and −S9. Dosage ≤ 1142 μg/plate	Negative	59
Ames test, *S. typhimurium* and *E. coli*	Strains: TA1535, TA1537, TA1538, TA1000, TA98, G46, C3076, and D3052; WP2 and WP2uvrA$^-$. +S9 and −S9	Negative	60
Ames test, *S. typhimurium*	Strains: TA1535, TA1537, TA1538, TA98, and TA100. +S9 and −S9. Dosage ≤ 3600 μg/plate	Negative	61
Ames test, *S. typhimurium*	Strains: TA1535, TA1537, TA98, and TA100. +S9 and −S9. Dosage = 342 μg/plate	Negative	62
Ames test, *S. typhimurium*	Strains: TA98 and TA100. +S9 and −S9	Negative	63
Ames test, *S. typhimurium*	Strains: TA1535, TA1538, TA98, and TA100. +S9. Dosage ≤ 2500 μg/plate	Positive in TA1538, maximum effect at 500 μg/plate; negative in other three strains	64
Ames test, *S. typhimurium*	5 strains. −S9	Positive	65
Ames test, *S. typhimurium*	Strains: TA98 and TA100	Negative	66
Ames test, *S. typhimurium*	Strain: TA98. +S9 and −S9	Negative	67
Ames test, *S. typhimurium*	Strains: TA1535, TA1537, TA98, and TA100. +S9. Dosage ≤ 100 μg/plate	Negative	68
Rec assay, DNA repair, *E. coli*	Strains: WP2, WP67, and CM871. +S9 and −S9	Negative	55
Rec assay, *B. subtilis*	+S9 and −S9	Positive with −S9 Negative with +S9	63
Micronucleus assay, mice bone marrow	Dosages: 144 or 288 mg/kg	Negative	61
Micronucleus assay, rat bone marrow	Strain: Sprague-Dawley CFY. Dosage: 600 mg/kg	Negative	69
Chromosomal aberrations	Human subjects: hair dyed every 3–6 weeks for 11 months	Negative	57
Chromosomal aberrations	Rat bone marrow cells	Negative	63
Sister chromatid exchange	Human subjects: hair dyed every 3–6 weeks for 11 months	Negative	56
Rodent cell line	L5178Y TK$^+$/$^-$ cells. +S9	Negative	58
Unscheduled DNA synthesis, rat hepatocytes		Negative	60
Basc test, *Drosophila*	Dosage: 12.5 mM, three generations	Negative	61
Silk worm mutations		Negative	63

[a] +S9 = with metabolic activation, −S9 = without metabolic activation, other information listed regarding specific study methods.

tion. The repeated hair dyeing did not have significant effects on either the frequency of sister chromatid exchange[56] or the rate of chromosomal abberation in the lymphocytes of test subjects[57] (Table 3).

A rodent cell line was incubated in the presence of metabolic activation with 1-Naphthol to determine its mutagenic potential. The L5178Y TK$^+$/$^-$ cells were incubated for 3 h with the concentration of 1-Naphthol ranging from 0 to 11.4 μg/ml. The number of mutants in the surviving cells was not increased by 1-Naphthol and it was nonmutagenic[58] (Table 3).

An Ames test with 1-Naphthol was used in five strains of *S. typhimurium*, TA1535, TA1537, TA1538, TA98, and TA100, both with and without metabolic activation. When tested at concentrations of up to 1142 μg/plate, 1-Naphthol was nonmutagenic[59] (Table 3).

1-Naphthol was nonmutagenic when tested both with and without metabolic activation in an Ames test using eight strains of *S. typhimurium*, TA1535, TA1537, TA1538, TA1000, TA98, G46, C3076, and D3052, and two strains of *E. coli*, WP2 and WP2uvrA$^-$. 1-Naphthol was also nonmutagenic in an assay based on chemically induced unscheduled DNA synthesis in rat hepatocyte cultures[60] (Table 3).

In the Ames test, when tested at dosages up to 3600 μg/plate in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, 1-Naphthol was nonmutagenic when tested both with and without metabolic activation. The Basc test in *Drosophila* was also performed using 1-Naphthol. The flies were given a dose of 12.5 mM by the adult feeding method and approximately 1200 X chromosomes were tested in each of three successive generations. 1-Naphthol was determined to be nonmutagenic according to the results of the Basc test. Groups of eight mice were given two doses of either 144 or 288 mg/kg by i.p. injection 24 h apart. Bone marrow smears were made after 30 h and 1000 polychromatic erythrocytes were scored for each mouse. 1-Naphthol was nonmutagenic according to the micronucleus assay[61] (Table 3).

1-Naphthol was nonmutagenic when tested at a dosage of 342 μg/plate in *S. typhimurium*. It was tested both with and without metabolic activation using four strains: TA1535, TA1537, TA98 and TA100 according to the procedure of Ames[62] (Table 3).

Several additional assays were done to assess the mutagenicity of 1-Naphthol. Negative results were obtained for tests performed in the TA100 and TA98 strains of *S. typhimurium*, both with and without metabolic activation. In a rec-assay test with *Bacillus subtilis*, negative results were reported with metabolic activation and positive results were found without metabolic activation. Negative results were reported for both an in vivo test of chromosomal aberrations in rat bone marrow cells and a test for mutations in silk worms[63] (Table 3).

1-Naphthol was tested in an assay using the procedure of Ames in four strains of *S. typhimurium*: TA1535, TA1538, TA98, and TA100. The test was conducted with a range of dosages from 4 to 2500 μg/plate and metabolic activation. A positive result was reported only in the TA1538 strain with a fivefold increase in the number of revertants above the negative control values; this effect was maximized at the dosage of 500 μg/plate. The results were negative in the other three strains[64] (Table 3).

Sprague-Dawley CFY rats, weighing 130–160 g, were used in a micronucleus test to determine the mutagenicity of 1-Naphthol. The groups of five male and five female rats were given a dose of 6000 mg/kg 1-Naphthol suspended in 0.5% tragacanth gum containing 0.05% sodium sulfite. The dose was given by gastric intubation in two equal parts 24 h apart. One rat died during the testing. The rats were killed 6 h after the second dose and bone-marrow smears were prepared; 2000 polychromatic erythrocytes were

examined per rat. The mean number of micronucleated cells was not significantly different from that of controls and 1-Naphthol was nonmutagenic[69] (Table 3).

1-Naphthol was tested for its mutagenic potential in five strains of *S. typhimurium* without activation according to the procedure of Ames. Pronounced mutagenic responses were found with 1-Naphthol[65] (Table 3).

An Ames test was conducted to determine 1-Naphthol's mutagenic potential. 1-Naphthol was nonmutagenic when tested in the TA98 and TA100 strains of *S. typhimurium* both with and without metabolic activation[66] (Table 3).

1-Naphthol was nonmutagenic when tested in the TA98 strain of *S. typhimurium*. It was tested both with and without metabolic activation at doses of up to 150 μg/plate[67] (Table 3).

1-Naphthol was tested in an Ames test in the TA1535, TA1537, TA98, and TA100 strains of *S. typhimurium* with metabolic activation. When tested at concentrations of up to 100 μg/plate, 1-Naphthol was found to be nonmutagenic[68] (Table 3).

Carcinogenicity

In the skin painting study described previously in the chronic toxicity section of this report, mice were treated for 21 months with a hair dye formulation containing 0.5% 1-Naphthol. No unusual tumor types developed in either treated or control groups. The authors concluded that no carcinogenic effects were produced by the hair dye formulations as tested in the skin painting study.[47]

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization: Occupational Exposure

A survey of 250 workers in the tie dye industry in India was made to investigate occupational skin diseases. Of the 250 workers, 49 had dermatitis. Patch tests were performed on 26 of the workers with dermatitis and also on 25 healthy volunteer subjects and 25 healthy workers in the tie dye industry as controls. The patch tests were performed for a variety of chemicals used in the industry, including 1-Naphthol. A 0.2% solution of 1-Naphthol was applied on a 1 cm^2 piece of gauze to the skin on the upper backs of the subjects. The reactions were scored after 48 and 72 h. One of the 26 workers tested had a positive reaction to 1-Naphthol. None of the control subjects had any reactions to any of the chemicals tested.[70]

Epidemiology

The association between occupational exposure to and use of hair dyes and the risk of cancer has been assessed in several epidemiologic studies presented in previous CIR reports on *p*-phenylenediamine[18] and *p*-, *m*-, and

o-aminophenols.[21] Occupational exposure to hair dyes was thought to increase the risk of bladder cancer[71-74] and lung cancer.[75,76] The use of hair dyes was thought to increase the risk of bladder cancer[77] and breast cancer.[78-83] There has been insufficient evidence of any carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined.[84] Many of these studies do not specify which hair dye ingredients were components of the hair dyes tested or whether any adjustment was made for subjects who smoked. It should be noted that with two exceptions all of these epidemiologic studies were performed prior to 1981, when the frequency of use data listed with the FDA showed that 1-Naphthol was used in only 8 hair dye formulations while by 1983 that frequency of use had risen to 214 hair dye formulations. Since most of these epidemiologic studies were conducted when there were relatively few (8 of 811) formulations containing 1-Naphthol, it is logical to assume that many of the formulations tested did not contain 1-Naphthol.

SUMMARY

1-Naphthol, a phenol, is used as a coupler in hair dyes and colors at concentrations normally below 1.0%. The ingredient is applied to the hair as a component of the hair dye formulation, and not as the pure ingredient.

Results of ingestion and skin painting studies have established that in the free form the ingredient is absorbed and excreted in the urine, predominantly as the sulfate and glucuronide conjugates.

The oral LD_{50} of 1-Naphthol was 2.3 g/kg. The results obtained from a subchronic dermal study of a hair dye containing 0.5% 1-Naphthol were unremarkable. No significant toxicity findings were reported from a chronic study of the same hair dye formulation.

1-Naphthol in tragacanth gum at 2.5% was not a rabbit skin irritant, but was slightly irritating at 3% when applied in a Shultz vehicle. 1-Naphthol when tested at 100% concentration was a severe skin irritant. Three separate guinea pig sensitization tests indicated that 1-Naphthol was not an irritant in two studies, but was in the third. This cosmetic ingredient is an eye irritant at concentrations of 2.5% and above, but only minimally at concentrations between 1.5 and 2.0%.

Hair dye formulations containing 0.5% 1-Naphthol applied dermally were neither teratogenic nor carcinogenic. Numerous mutagenic assays of 1-Naphthol have been reported. With but 3 exceptions in the 22 reported studies, the results were negative.

Human skin studies of 1-Naphthol were not available. However as a coal tar derivative used in hair dyes, this ingredient is exempt from the color additive provisions of the Food, Drug and Cosmetic Act when cautionary statements and patch test instructions are displayed conspicuously on the labels.

DISCUSSION

This report reviews the data from animal studies indicating that 1-Naphthol is neither significantly toxic at low doses nor an eye or skin irritant at concentrations of use. Although the animal sensitization data are conflicting, it is most probable that 1-Naphthol is not a sensitization agent in the guinea pig.

The Expert Panel recognizes that clinical data for 1-Naphthol are not available. Hair dyes containing 1-Naphthol are exempt from the principal adulteration provision and from the color additive provisions in the Federal Food, Drug and Cosmetic Act of 1938 when cautionary statements and instructions for patch testing are displayed conspicuously on the labels; therefore, the Expert Panel is not requiring clinical testing.

CONCLUSION

On the basis of the available animal data presented in this report, the CIR Expert Panel concludes that 1-Naphthol is safe as a cosmetic ingredient in the present practices of use and concentrations.

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