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Final Report on the Safety Assessment of 4-Nitro-*m*-Phenylenediamine

ABSTRACT

4-Nitro-*m*-Phenylenediamine is a coal tar cosmetic used at concentrations of ≤0.1%. In an acute oral toxicity study involving mice, the LD_{50} was 0.5 g/kg. 4-Nitro-*m*-Phenylenediamine was mutagenic in the chromosome aberrations assay and in the preincubation assay, using strains TA97, TA98, and TA 1537 of *Salmonella typhimurium*. The safety of use of 4-Nitro-*m*-Phenylenediamine in cosmetic products has not been documented and substantiated. The needed safety test data include: methods of production, ultraviolet absorption spectrum, impurities, skin absorption data, adequately performed skin irritation and sensitization studies, and dermal toxicity. It cannot be concluded that this ingredient is safe for use in cosmetic products until the cited safety data have been obtained and evaluated.

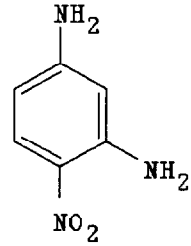
INTRODUCTION

DATA CONCERNING THE CHEMISTRY, use in cosmetic products, acute oral toxicity, and mutagenic potential of 4-Nitro-*m*-Phenylenediamine are included in this report. No other safety test data on this compound were available.

CHEMISTRY

Chemical and Physical Properties

4-Nitro-*m*-Phenylenediamine (CAS No. 5131-58-8) is the substituted aromatic amine that conforms to the formula (Estrin et al., 1982):



It has a molecular weight of 153.16 (RTECS, 1990). Other names for this chemical are: 1,3-benzenediamine, 4 nitro; 4-nitro-1,3-diaminobenzene; 2,4-diaminonitrobenzene, and 4-nitro-1,3-phenylenediamine (Estrin et al., 1982; RTECS, 1990).

USE

Purpose in Cosmetics

4-Nitro-*m*-Phenylenediamine is used as a hair colorant in cosmetic products (Nikitakis, 1988).

Scope and Extent of Use in Cosmetics

The FDA cosmetic product formulation computer printout (FDA, 1989) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations (1984). 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 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 the same as one entered at the highest end of that range, thus introducing the possibility of a two- to tenfold error in the assumed ingredient concentration. 4-Nitro-*m*-Phenylenediamine has been used in as many as 12 cosmetic products at concentrations of ≤0.1% (Table 1) (FDA, 1989). Current product formulation data indicate that 4-Nitro-*m*-Phenylenediamine is not being used (FDA, 1991).

Hair coloring formulations containing 4-Nitro-*m*-Phenylenediamine are applied to or may come in contact with hair, skin (particularly the scalp), eyes, and nails. These formulations may be used as often as once per week.

The oxidative or permanent hair dyes containing 4-Nitro-*m*-Phenylenediamine, as "coal tar" hair dye products (Elder, 1985a), 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 (Federal Register, 1979). In order 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 direc-

Table 1. Product Formulation Data on 4-Nitro-*m*-Phenylenediamine

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%) ≤0.1*
Hair dyes and colors	1073	12	12
1989 Totals		12	12

Source: FDA, 1989.

tions 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 (Corbett and Menkart, 1973).

At its February 11, 1992 meeting, the CIR Expert Panel issued the following policy statement on coal tar hair dye product labeling:

> The Cosmetic Ingredient Review Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.
>
> Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.
>
> There is a general consensus among dermatologists that screening of patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group.[1,2,3] Basically these procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch and evaluated for sensitization at 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients.[4]
>
> During the August 26–27, 1991 public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 to 48 hours after application of the test material.
>
> The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel this policy statement was adopted.

TOXICOLOGY

Acute Oral Toxicity

In an acute oral toxicity study involving mice (weights and strain not stated), the LD_{50} was 500 mg/kg (RTECS, 1990).

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[1]North American Contact Dermatitis Group. 1980. Patch testing in allergic contact dermatitis. American Academy of Dermatology.

[2]Fiermann et al. 1982. Prospective study of cosmetic reactions. J. Am. Acad. Dermatol. **6**:909–917.

[3]Adams et al. 1985. A five-year study of cosmetic reactions. J. Am. Acad. Dermatol. **12**:1062–1069.

[4]Elder 1985. Final report on the safety assessment of *p*-Phenylenediamine. J. Am. Coll. Toxicol. **4**(3):203–266.

Mutagenicity

The mutagenicity of 4-Nitro-*m*-Phenylenediamine in DMSO in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 was evaluated with and without metabolic activation according to a modification of the preincubation assay by Haworth et al. (1983). The positive controls in the absence of metabolic activation were as follows: sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). In the presence of metabolic activation, 2-aminoanthracene was the positive control for all strains. DMSO served as the solvent control. The test substance (0.0–10,000 μg/plate) was incubated with each *Salmonella* culture for 20 min (37°C) prior to plating. Two different S9 mixes (rat and hamster liver) were used. For strain TA97, S9 concentrations in metabolic activation cultures were 5.0, 10.0, and 30.0%. In the remaining strains, the S9 concentration was 10.0%. Histidine-independent colonies arising on the plates were counted after two days of incubation at 37°C. A chemical was classified as mutagenic if it produced a reproducible dose-related response over the solvent control in replicate trials. 4-Nitro-*m*-Phenylenediamine was mutagenic in strain TA98 both with and without metabolic activation, but was not mutagenic in strains TA100 and TA1535. In strain 1537, positive (S9 mix, hamster liver) and negative (S-9 mix, rat liver) results were obtained with metabolic activation, and, negative results, without metabolic activation. In strain TA97, 4-Nitro-*m*-Phenylenediamine was mutagenic only in the presence of metabolic activation (10.0% S9, hamster liver). Based upon the available data, the authors concluded that 4-Nitro-*m*-Phenylenediamine was mutagenic (Zeiger et al., 1988).

4-Nitro-*m*-Phenylenediamine was mutagenic in the chromosome aberrations assay (*in vitro*), and its mutagenic potential was questionable in an *in vitro* assay for the detection of sister chromosome exchanges. The mutagenic potential of a chemical was judged questionable for any of the following reasons: if the results of individual trials were not reproducible, if increases in his^+ revertants did not meet the criteria for a weak mutagenic response, or if only single doses produced increases in his^+ revertants in repeat trials. Both assays were conducted with and without metabolic activation (National Toxicology Program, 1989).

Epidemiology

Approximately 40% of American women dye their hair, often at monthly intervals over a period of years (Corbett and Menkart, 1973). The U.S. EPA reported that [approximately] 15 million people are potentially exposed to hair dye ingredients as a result of personal use or in the application of hair dyes to other people (47 FR 979).

A variety of published studies have assessed the association between occupational exposure to and use of hair dyes and the risk of cancer. These studies do not note which specific hair dye ingredients were involved in the human exposure. A summary of reports of how occupational exposure to hair dyes affects the risk of bladder cancer (Cole et al., 1972; Anthony and Thomas, 1970; Dunham et al., 1968; Wynder et al., 1963) and lung cancer (Garfinkel et al., 1977; Menck et al., 1977), or use of hair dyes affects the risk of bladder cancer in men or women (Jain et al., 1977) and breast cancer in women (Wynder and Goodman, 1983; Hennekens et al., 1979; Shore et al., 1979; Nasca et al., 1979; Kinlen et al., 1977; Shafer and Shafer, 1976) has been published in previous Cosmetic Ingredient Review reports on *p*-Phenylenediamine, 2-Nitro-*p*-Phenylenediamine, and 4-Nitro-*o*-Phenylenediamine (Elder, 1985a,b). In the small case-controlled study by Shore et al. (1979), a positive correlation between hair dye use

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and breast cancer was reported. When their study was extended to include 398 breast cancer cases, the same investigators could not implicate hair dye use as an important cause of human breast cancer (Koenig et al., 1991). The latter study indicated that beauticians who work for five or more years in this occupation have an increased breast cancer risk. However, the increased risk was not a strong finding, and "if beauticians are at increased breast cancer risk, exposures other than hair dyes may be responsible."

An epidemiology study involving 118,404 U.S. women concluded that the use of permanent hair dyes appears unlikely to cause any important increase in the risk of breast cancer (Green et al., 1987).

Evidence of any carcinogenic effect from hair dyes investigated among the occupations and users examined is insufficient (Clemmesen, 1981). Clemmesen discussed the difficulties implicit in epidemiologic studies and reviewed many of the papers that investigated the relationship of the risk of cancer to occupational exposure to or use of hair dyes. He concluded that most researchers used samples that were too small to allow conclusions and that analyses of duration and intensity of exposure, lag time, and the influence of lifestyle factors, such as tobacco use, were deficient in many cases.

SUMMARY

4-Nitro-*m*-Phenylenediamine has been used as a hair colorant (concentrations of $\leq$0.1%) in as many as 12 cosmetic products. Current product formulation data (November 18, 1991) submitted to FDA indicate no reported uses for this ingredient.

The oxidative or permanent hair dyes containing 4-Nitro-*m*-Phenylenediamine, as "coal tar" hair dye products, 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 appropriate "patch test" instructions for determining whether the product causes skin irritation. The patch test, in which the intermediates and hydrogen peroxide are mixed in the same manner as in use, is to be performed prior to each and every application of the hair dye.

In an acute oral toxicity study involving mice, the LD_{50} was 500 mg/kg.

4-Nitro-*m*-Phenylenediamine was mutagenic in the preincubation assay, using strains TA97, TA98, and TA1537 of *Salmonella typhimurium*. It was also mutagenic in the chromosome aberrations assay, and its mutagenic potential was questionable in an assay for the detection of sister chromosome exchanges.

DISCUSSION

Section 1 paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on 4-Nitro-*m*-Phenylenediamine were not sufficient for determining whether the ingredient, under relevant conditions of use, was either safe or not safe. The Panel released a Notice of Insufficient Data on September 13, 1991, outlining the data needed to assess the safety of 4-Nitro-*m*-Phenylenediamine.

The data required included: (1) Methods of production; (2) impurities; (3) UV absorption spectrum; (4) Skin irritation and sensitization (animals); (5) 28-day dermal toxicity (animals).

No comments regarding the data requested were received during the 90-day public comment period specified in the Notice of Insufficient Data on 4-Nitro-*m*-Phenylenediamine.

The CIR Expert Panel also advises any users or potential users of 4-Nitro-*m*-Phenylenediamine that data on absorption should be obtained and evaluated in order to determine if other types of safety test data are needed.

CONCLUSION

The safety of 4-Nitro-*m*-Phenylenediamine has not been documented and substantiated. The CIR Expert Panel cannot conclude that this ingredient is safe for use in cosmetic products until the appropriate safety data have been obtained and evaluated.

ACKNOWLEDGMENT

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