FINAL REPORT ON THE SAFETY ASSESSMENT OF 2-AMINO-6-CHLORO-4-NITROPHENOL AND 2-AMINO-6-CHLORO-4-NITROPHENOL HYDROCHLORIDE[1]

2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt are substituted aromatic compounds that function as colorants in hair dyes. Only the hydrochloride salt is currently reported to be used. 2-Amino-6-Chloro-4-Nitrophenol is poorly absorbed through the skin. Less than 0.25% of 2-Amino-6-Chloro-4-Nitrophenol found in a nonoxidative hair dye was absorbed. Less than 0.2% of 2-Amino-6-Chloro-4-Nitrophenol found in an oxidative hair dye was absorbed. In subchronic oral studies in rats, a no observable adverse effect level of 30 mg/kg/day for 2-Amino-6-Chloro-4-Nitrophenol was determined; at higher doses, increased organ weights were seen. A 2% solution of 2-Amino-6-Chloro-4-Nitrophenol applied under occlusive conditions was found to be nonirritating to rabbits. At 0.1%, 2-Amino-6-Chloro-4-Nitrophenol was nonsensitizing; at 2%, there was sufficient skin coloration from the dye that assessment of sensitization was difficult; but no obviously sensitized areas were reported. An oral teratogenicity study in rats showed no birth defects. In one Ames test, Salmonella *strains TA97, TA98, and TA100 showed an increase in mutations, with and without metabolic activations. Strain TA1535 was negative, however. In another Ames test,* Salmonella *strains TA97 and TA100 showed no increase in mutations upon treatment with 2-Amino-6-Chloro-4-Nitrophenol, diluted in dimethylsulfoxide, with and without metabolic activation. Strain TA98 was negative with metabolic activation but positive without activation. In follow-up testing with strain TA98-NR, there was no increase in mutations in the absence of activation. Mutagenicity assays in mammalian systems were negative. The poor absorption through the skin, lack of any teratogenic effect, and negative mutagenesis data in certain Ames test strains and in mammalian systems suggested that any systemic effects from the use of actual products were unlikely. It was possible to conclude that the highest concentration of 2-Amino-6-Chloro-4-Nitrophenol tested (2%) is safe for use in hair dye formulations.*

INTRODUCTION

2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt are substituted aromatic compounds that function as hair colorants in hair

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International Journal of Toxicology, 16(Suppl. 1):131–143, 1997

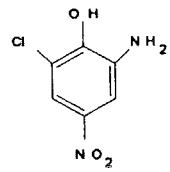
1091-5818/97 $12.00 + .00

dyes and colors that require caution statements and patch test instructions. This report reviews the available safety data on these ingredients.

CHEMISTRY

Definition and Structure

2-Amino-6-Chloro-4-Nitrophenol (CAS No. 6358-09-4) is the substituted aromatic compound that conforms to the following formula:



Another name for this ingredient is Phenol, 2-Amino-6-Chloro-4-Nitro- (Wenninger and McEwen, 1993).

Chemical and Physical Properties

2-Amino-6-Chloro-4-Nitrophenol is described as yellow needles or an orange-yellow, fine-grained powder. The molecular weight of the free base is 188.56, and the monohydrate is 206.60. 2-Amino-6-Chloro-4-Nitrophenol is soluble in water, at a pH greater than 7.0 (COLIPA, 1990).

Ultraviolet Light Absorbance

The absorption maximum for 2-Amino-6-Chloro-4-Nitrophenol is 204.8 nm; additional absorption maxima occurred at 262.3 nm, 229.1 nm, and 316.1 nm, in order of decreasing absorption (COLIPA, 1990).

COSMETIC USE

2-Amino-6-Chloro-4-Nitrophenol Hydrochloride is used as a hair colorant in hair dyes and colors requiring caution statements and patch test instructions and in hair tints (Wenninger and McEwen, 1992; Food and Drug Administration [FDA], 1995). The product formulation data submitted to the FDA in 1995 reported that 2-Amino-6-Chloro-4-Nitrophenol Hydrochloride was used in a total of 15 hair-coloring products (Table 1; FDA, 1995). There was no listing for 2-Amino-6-Chloro-4-Nitrophenol in 1995.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA, 1992); however, product formulation data sub-

Table 1. Cosmetic product formulation data on 2-Amino-6-Chloro-4-Nitrophenol Hydrochloride

Product category	No. of formulations in category	No. containing ingredient
Hair and colors dyes (requiring caution statement and patch test instructions)	1458	11
Hair tints	44	4
1995 Total		***15***

Source. FDA, 1995.

mitted to the FDA in 1984 stated that both 2-Amino-6-Chloro-4-Nitrophenol and 2-Amino-6-Chloro-4-Nitrophenol Hydrochloride were used at concentrations of up to 1% (FDA, 1984).

Hair dyes containing 2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a cautionary statement and patch test instructions for determining whether the product causes skin irritation (FDA, 1979). To qualify for the exemption, the following caution statement must be displayed conspicuously on the labels of coal tar hair dyes:

> *Caution: This product contains ingredients that 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 eyelashes or eyebrows; to do so may cause blindness.*

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

> The CIR 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 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 (North American Contact Dermatitis Group, 1980; Eiermann et al., 1982; Adams et al., 1985). 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 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 (Elder, 1985).

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 hours 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.

In Europe, 2-Amino-6-Chloro-4-Nitrophenol is used in oxidative hair dye formulations and color-setting lotions at a maximum concentration of 3.5% (COLIPA, 1994). COLIPA (1994) calculated a 500-fold margin of safety for this ingredient as used in both oxidative and semipermanent hair dyes (Table 2).

GENERAL BIOLOGY

Absorption and Excretion

The percutaneous absorption of [^{14}C]2-Amino-6-Chloro-4-Nitrophenol (9.99% in water/DMSO) in both oxidative and nonoxidative hair dyes was studied. Groups of three male and three female Sprague-Dawley rats, at an average body weight of 200 g, were topically treated with these solutions for 30 min followed by rinsing of the skin. Radioactivity was measured in the rinsings, applications sites, urine, feces, blood, organs, and carcass. Percutaneous absorption was calculated from the amount of radioactivity eliminated from the body 72 h after treatment and the amount remaining in the carcass. The mean percutaneous absorption was 0.248% for the nonoxidative hair dye, 0.189% for the oxidative hair dye, and 1.213% for the 2-Amino-6-Chloro-4-Nitrophenol solution alone. The majority of the radioactivity was in the urine (89–92%) and feces (8–11%). The application sites of the nonoxidative hair dye, the oxidative

Table 2. Calculation of safety margin for 2-Amino-6-Chloro-4-Nitrophenol as used in oxidative and semipermanent hair dyes

	Oxidative	Semipermanent
Product usage volume	100 mL	35 mL
Maximum concentration of 2-Amino-6-Chloro-4-Nitrophenol in product	3.5%	3.5%
Final maximum concentration after dilution by peroxide (1:1)	1.75%	NA
Amount of 2-Amino-6-Chloro-4-Nitrophenol applied (vol. × conc.)	1750 mg	1225 mg
Maximum absorption (from rat, in vivo data)	0.189%	0.248%
Dermal absorption (amount applied × max. absorption)	3.3075 mg	3.038 mg
Typical body weight	60 kg	60 kg
Systemic exposure dose (dermal absorption/body weight)	0.055125 mg/kg	0.05063 mg/kg
No observed adverse effect level (NOAEL; from rat, in vivo data)	30 mg/kg	30 mg/kg
Margin of safety (NOAEL/systemic exposure dose)	544.2	592.5

Source. COLIPA, 1994.

hair dye, and 2-Amino-6-Chloro-4-Nitrophenol solution retained 0.38%, 0.82%, and 0.59% of the applied radioactivity, respectively. As a point of reference, the investigators reported that following oral administration of 3% 2-Amino-6-Chloro-4-Nitrophenol, 70% of the radioactivity was in the urine and 30% was in the feces (COLIPA, 1990).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Five male and five female Wistar rats were given single oral doses (2000 mg/kg) of 20% 2-Amino-6-Chloro-4-Nitrophenol. No deaths occurred during the 14-day observation period. The only change observed was red to orange urine up to 5 days following administration (COLIPA, 1990).

Subchronic Oral Toxicity

Doses of 10, 30, and 90 mg/kg 2-Amino-6-Chloro-4-Nitrophenol in sodium carboxymethylcellulose were given orally to groups of 15 male

(body weight range of 122–169 g) and 15 female (body weight range of 116–146 g) Wistar derived SPF albino rats for 90 days. An additional 10 male and 10 female rats were given the high-dose regimen and kept an additional 4 weeks without treatment to evaluate recovery. A control group of 25 male and 25 female rats were given the vehicle alone.

No deaths occurred during the study. Discolored (orange) urine was observed in the animals from the mid- and high-dose groups, and some of the animals of the high-dose group had diarrhea. Feed consumption was normal for all the treatment groups, but there was a significant reduction in body weight gains in the males of the high-dose group. During the 4-week recovery period, body weights returned to normal. No significant changes were observed in blood, urine, and clinical chemistry data. Since no histopathologic changes were found in the control or high-dose groups, tissues from the lower-dose animals were not evaluated. No gross lesions were observed, but kidney weights were increased in the female rats of the high-dose group at week 13. During the 4-week recovery period, liver, kidney, lung, and thymus weights were increased. The investigator reported that the no-effect-level was 30 mg/kg 2-Amino-6-Chloro-4-Nitrophenol (COLIPA, 1990).

Dermal Irritation

A 2% solution of 2-Amino-6-Chloro-4-Nitrophenol (0.5 mL) was applied under occlusive patches to intact and abraded skin of six New Zealand rabbits. The trunk of each rabbit was wrapped in a rubber sheet for 4 h. Observations were made 30 min to 60 min after patch removal and at 24 h, 48 h, and 72 h. No evidence of irritation was observed (COLIPA, 1990).

Dermal Sensitization

In a modified maximization test, 20 Pirbright guinea pigs were given intradermal injections (0.5 mL) of 2-Amino-6-Chloro-4-Nitrophenol in 10% propylene glycol and in 10% Freund's complete adjuvant. Injections of Freund's complete adjuvant alone were used as negative controls. Seven days later, 2-Amino-6-Chloro-4-Nitrophenol was applied neat in a 48-h closed patch. Three weeks after the first intradermal injection, 0.1% and 2% 2-Amino-6-Chloro-4-Nitrophenol in propylene glycol were applied to the left flank of each guinea pig for 24 h. Control animals were treated similarly with the vehicle only. Skin reactions were read 24 h and 48 h after the last application. No evidence of sensitization was observed with the 0.1% 2-Amino-6-Chloro-4-Nitrophenol; however, patches of 2% 2-Amino-6-Chloro-4-Nitrophenol colored the skin, so reactions could not be evaluated (COLIPA, 1990).

Ocular Irritation

The left conjunctival sacs of six New Zealand rabbits were instilled with 0.1 mL of a 2% dilution of 2-Amino-6-Chloro-4-Nitrophenol in propylene glycol. The eyes of three rabbits were rinsed after 4 sec. The right eye of each rabbit served as a control. Ocular irritation scores were made at 1 h, 24 h, 48 h, and 72 h after instillation. At the 24-h and 72-h readings, examinations using fluorescein staining were also conducted. Redness was observed in the eye of one rabbit at the 1-h scoring period, but no other signs of ocular irritation were observed with any of the other rabbits or at any other time periods (COLIPA, 1990).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of 20 pregnant SPF albino Wistar rats were given oral doses of 2-Amino-6-Chloro-4-Nitrophenol dissolved in 0.5% sodium carboxymethylcellulose at doses of 10 mg/kg, 30 mg/kg, and 90 mg/kg on days 5 through 15 of gestation. Control rats were given the vehicle only. The rats were killed and necropsied on day 20 of gestation, and all the fetuses were examined for gross malformations. Two thirds of the fetuses were examined for skeletal defects, and the remaining fetuses were evaluated for visceral effects. During dosing, the female rats had orange urine, the intensity of which was dose related. There was a significant reduction in mean maternal body weight and mean feed consumption in the high-dose group, but no gross lesions were found at necropsy. The number and weight of fetuses; number of resorptions, implantations, and corpora lutea; and weight of the placentae and uteri were not significantly different from control values. Five fetuses from the mid-dose group and one fetus of the high-dose group had edema, but the investigators considered these effects to be coincidental. No evidence of teratogenicity was observed. The no-effect-level was 30 mg/kg 2-Amino-6-Chloro-4-Nitrophenol (COLIPA, 1990).

MUTAGENICITY

In Vitro

The mutagenic potential of 2-Amino-6-Chloro-4-Nitrophenol Hydrochloride was evaluated in the Ames test using *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535. Tests were conducted both with and without metabolic activation with S9 fractions from Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamsters. 2-Amino-6-Chloro-4-Nitrophenol Hydrochloride was tested in triplicate at concentrations ranging from 10 μL/plate to 557 μL/plate

for protocols without metabolic activation and from 10 μL/plate to 2000 μL/plate in the protocols using metabolic activation. The solvent was dimethylsulfoxide (DMSO). Solvent and positive controls were run concurrently with each trial. The positive controls used for the protocols without metabolic activation were 9-aminoacridine for TA97, 4-nitro-o-phenylenediamine for TA98, and sodium azide for strains TA100 and TA1535. In the protocols with metabolic activation, 2-aminoanthracene was used as the positive control. This experiment was repeated at least 1 week following the initial trial. 2-Amino-6-Chloro-4-Nitrophenol hydrochloride was positive in tests with strains TA97, TA98, and TA100 both with and without metabolic activation but was negative in tests with TA1535 (Zeiger et al., 1988).

In another Ames test, concentrations of 3000 μg/plate to 6000 μg/plate 2-Amino-6-Chloro-4-Nitrophenol (diluted in anhydrous DMSO) were toxic to the bacteria. At lower concentrations, no evidence of mutagenicity was observed with strains TA97, TA98, or TA100 in the presence of metabolic activation; however, in studies without liver enzymes, a slight mutagenic effect was observed in strain TA98 (the exact concentration of 2-Amino-6-Chloro-4-Nitrophenol was not stated; COLIPA, 1990).

As a follow-up to the positive results observed with strain TA98, the investigators conducted another Ames test using *S. typhimurium* strain TA98-NR, which is capable of detecting false-positive results of substances containing a nitro-group. The bacteria were exposed to 2-Amino-6-Chloro-4-Nitrophenol in DMSO in the absence of S9 mix at doses ranging from 10 μg/plate to 6000 μg/plate. The positive control was 2-nitro-fluorene. No mutagenic response was observed (COLIPA, 1990).

A mouse lymphoma assay was also used to determine the mutagenic potential of 2-Amino-6-Chloro-4-Nitrophenol. Cultures of mouse lymphoma L5178Y cells were incubated with 1.58, 5, 15.8, 50, 158, 500, and 1580 μg/mL 2-Amino-6-Chloro-4-Nitrophenol diluted in DMSO for 2 h both with and without S9 mix. A concentration of 5000 μg/mL with S9 mix and a concentration of 0.5 μg/mL without S9 mix were also tested. The positive controls were 4-nitroquinoline-*N*-oxide for tests without activation and benzo(a)pyrene for tests with activation. One week later, viability and mutation to 6-thioguanine resistance were determined for the 15.8 μg/mL to 500 μg/mL dose groups. There was no statistically significant increase in mutation rate (COLIPA, 1990).

In a chromosome aberration assay, Chinese hamster ovary cells were exposed to 2-Amino-6-Chloro-4-Nitrophenol in DMSO at doses of 5, 16.5, 50, 165, 500, 1650, and 5000 μg/mL diluted in DMSO both in the presence and absence of metabolic activation. The cells were incubated for 2 h, rinsed, and then cultured for 20 h. Mitosis was stopped with

colchicine, and 100 metaphases in the cultures containing 50, 165, and 500 μg/mL 2-Amino-6-Chloro-4-Nitrophenol were analyzed for chromosome aberrations. The positive controls used in this study were methylmethanesulfonate with liver S9 mix and cyclophosphamide without liver enzymes. DMSO was used as the negative control. There was a statistically significant increase in the number of aberrations in the 165 and 500 μg/mL groups without S9 mix, but no effect was observed in the cultures treated with S9 mix. The investigators noted that a reevaluation of the results indicated that the number of aberrations observed did not exceed the control range, so they concluded that there was no real increase in aberrations (COLIPA, 1990).

Because the results of the chromosome aberration assays described earlier were unclear, a second chromosome aberration assay was conducted using human lymphocytes from both male and female donors. Cell cultures of lymphocytes were incubated with 2-Amino-6-Chloro-4-Nitrophenol in DMSO at doses of 39.1, 78.13, 156.25, 312.5, 625, and 1250 μg/mL both with and without metabolic activation. Analyses for chromosome aberrations were conducted for cultures exposed to 312.5, 625, and 1250 μg/mL 2-Amino-6-Chloro-4-Nitrophenol. In the presence of S9 mix, 1250 μg/mL 2-Amino-6-Chloro-4-Nitrophenol induced a statistically significant increase in the number of aberrations, but it was noted that this was a toxic dose to limit of solubility. Lymphocytes from male donors had more aberrations than those from female donors. No evidence of mutagenicity was observed at lower doses tested with S9 mix or in any of the cultures run without S9 mix (COLIPA, 1990).

2-Amino-6-Chloro-4-Nitrophenol was also tested in a sister chromatid exchange (SCE) assay using Chinese hamster ovary CHO-K1-cells. Tests were conducted both with and without rat liver metabolic activating systems. The positive controls were 2-nitro-p-phenylenediamine and 2-acetylamino fluorene. 2-Amino-6-Chloro-4-Nitrophenol, at concentrations ranging from 0.01 mM to 1.0 mM, diluted in DMSO, did not induce SCEs in the presence or absence of S9 mix (COLIPA, ,1990).

In Vivo

In an unscheduled DNA synthesis assay, doses of 15, 50, and 150 mg/kg 2-Amino-6-Chloro-4-Nitrophenol dissolved in DMSO were given orally to groups of six male and six female Wistar rats. The livers were removed from the rats 24 h after dosing and liver preparations were incubated with ^{3}H-thymidine. Microscopic examinations of the cells were conducted and the grains/nucleus counted. No induction of unscheduled DNA synthesis was observed (COLIPA, 1990).

2-Amino-6-Chloro-4-Nitrophenol was also tested in a micronucleus test. Groups of six male and six female NMRI mice were given single

oral doses of 15, 50, or 150 mg/kg 2-Amino-6-Chloro-4-Nitrophenol in DMSO. Positive control mice were given cyclophosphamide and negative control mice were given DMSO alone. Twenty-four hours after dosing, femoral bone marrow cells were extracted and prepared for examnation. Additional femoral preparations were taken at 48 h and 72 h from the high-dose and control groups. One-thousand polychromatic erythrocytes were taken from five male and five female mice in each group and analyzed for micronuclei and the ratio of polychromatic to normochromatic erythrocytes was determined. 2-Amino-6-Chloro-4-Nitrophenol did not induce micronuclei (COLIPA, 1990).

CARCINOGENICITY

No published data on the carcinogenic potential of 2-Amino-6-Chloro-4-Nitrophenol or its hydrochloride salt were found.

CLINICAL ASSESSMENT OF SAFETY

No published clinical studies of 2-Amino-6-Chloro-4-Nitrophenol or its hydrochloride salt were found.

EPIDEMIOLOGY

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years (Cosmetic, Toiletry and Fragrance Association [CTFA], 1993). This estimate is drawn from market research data on hair dye product use, generally from women aged 15 to 60 years.

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber or between cancer and personal use of hair dyes. The World Health Organization's International Agency for Research on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6 to 13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed; to evaluate the results of the epidemiologic and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group concluded that: "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence, "Personal use of hair colourants *cannot be evalu-*

ated as to its carcinogenicity (Group 3)." The IARC Working Group also concluded that "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence, "Occupation as a hairdresser or barber entails exposures that are *probably carcinogenic (Group 2A)*" (IARC, 1993). The Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt are used as colorants in hair dyes and colors. Hair dyes containing 2-Amino-6-Chloro-4-Nitrophenol or its hydrochloride salt as coal tar hair dyes are exempt from the principal adulteration provision from the color additive provisions 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.

The mean percutaneous absorption of 2-Amino-6-Chloro-4-Nitrophenol was 1.213% when applied as a 9.99% solution in water/DMSO. The mean absorption was 0.248% for a nonoxidative hair dye containing 2-Amino-6-Chloro-4-Nitrophenol and was 0.189% for an oxidative hair dye containing this ingredient.

The oral LD_{50} of a 20% solution of 2-Amino-6-Chloro-4-Nitrophenol was greater than 2000 mg/kg for rats. In subchronic oral studies, the no-effect level was 30 mg/kg 2-Amino-6-Chloro-4-Nitrophenol. At higher concentrations, increased organ weights were observed. All treated animals had discolored (orange) urine.

No evidence of irritation was observed when 2% 2-Amino-6-Chloro-4-Nitrophenol was applied under occlusive patches to rabbits. In a modified maximization test using guinea pigs, no sensitization was observed with 0.1% 2-Amino-6-Chloro-4-Nitrophenol.

In a teratogenicity study, pregnant rats orally administered 90 mg/kg 2-Amino-6-Chloro-4-Nitrophenol during gestation had significantly reduced body weight and feed consumption; however, no evidence of teratogenicity was observed.

2-Amino-6-Chloro-4-Nitrophenol produced positive results in Ames tests with *Salmonella typhimurium* strains TA97, TA98, and TA100 but negative results with TA1535 in one laboratory. There was no influence of metabolic activation. In another laboratory, negative results were seen with TA97 and TA100, with and without metabolic activation, but positive results were seen with TA98 without metabolic activation. In a follow-up test with strain TA98-NR without metabolic activation, the results were negative. No evidence of mutagenicity was

observed in a mouse lymphoma assay, chromosomal aberration assays, a sister chromatid exchange assay, an unscheduled DNA synthesis assay, and a micronucleus test.

DISCUSSION

Concentration of use data are no longer reported to the FDA by cosmetic companies. Because it is not known at what concentrations cosmetic companies are using this ingredient, a maximum allowable concentration of use was determined from test data contained in this review.

The Expert Panel noted that, while negative results were seen with some Ames assays, others were positive. Genotoxicity studies using mammalian systems, however, were negative. Additionally, 2-Amino-6-Chloro-4-Nitrophenol is poorly absorbed through guinea pig skin and was not a teratogen in an oral study using rats. These data led the Panel to conclude that systemic adverse effects from use of a product containing these ingredients would be highly unlikely.

The Expert Panel arrived at a maximum safe concentration of use based on animal ocular irritation and dermal irritation and sensitization studies using animals summarized in this review. While acknowledging that evaluations could not be made at the 2% concentration in the skin sensitization study due to skin discoloration, the Panel is of the opinion that if the 2% concentration was significantly sensitizing, the reaction would have been observable. Therefore, the Expert Panel concluded that 2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt are safe for use in hair dye formulations at concentrations of up to 2%.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that 2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt are safe for use in hair dye formulations at concentrations of up to 2.0%.

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