International Journal of Toxicology
28(Suppl 3) 217S-251S

DOI: 10.1177/1091581809354651
http://ijt.sagepub.com
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Final Report on the Safety Assessment of Amino Nitrophenols as Used in Hair Dyes

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Abstract

2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-3-nitrophenol, 4-amino-2-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are substituted aromatic compounds used as semipermanent (nonoxidative) hair colorants and as toners in permanent (oxidative) hair dye products. All ingredients in this group except 2-amino-4-nitrophenol sulfate, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol have reported uses in cosmetics at use concentrations from 2% to 9%. The available toxicity studies for these amino nitrophenol hair dyes did not suggest safety concerns except for the potential carcinogenicity and mutagenicity of 4-amino-2-nitrophenol. 2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-4-nitrophenol sulfate, 2-amino-5-nitrophenol, 4-amino-3-nitrophenol, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment, but the data are insufficient to make a safety determination for 4-amino-2-nitrophenol.

Keywords

amino nitrophenols, cosmetics, hair dye, safety

This report addresses the safety of 2-amino-3-nitrophenol (CAS No. 603-85-0), 2-amino-4-nitrophenol (CAS No. 99-57-0), 2-amino-5-nitrophenol (CAS No. 121-88-0), 4-amino-3-nitrophenol (CAS No. 610-81-1), 4-amino-2-nitrophenol (CAS No. 119-34-6), 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol (CAS No. 65235-31-6), and 4-hydroxypropylamino-3-nitrophenol (CAS No. 92952-81-3). These ingredients are used as semipermanent hair colorants in cosmetic products.

Chemistry

Definition and Structure

2-Amino-3-nitrophenol, 2-amino-5-nitrophenol, 4-amino-2-nitrophenol, 4-amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are the substituted aromatic compounds that conform to the structures shown in Figure 1. Synonyms and trade names can be found in Table 1. The chemical and physical properties are given in Table 2.

Method of Manufacture

Farris[1] described a method of manufacture for 2-amino-4-nitrophenol by the partial reduction of 2,4-dinitrophenol by hydrosulfide, hydrazine, or copper. Electrolytic reduction may be done using vanadium.

The preparation of 2-amino-5-nitrophenol involves catalytic reduction of 2-nitrophenol with Raney Ni, ring closure with acetic anhydride to give 2-methylbenzoxazole, nitration in mixed HNO_3-H_2SO_4, and subsequent hydrolysis with HCl.

4-Amino-2-nitrophenol can be produced by 1 of 4 methods: (1) reduction of 4′-hydroxy-3′-nitroazobenzene-4-sulfonic acid with iodine and sulfurous acid, (2) reduction of 4-chloro-3′-nitro-4′-hydroxyazobenzene-3-sulfonic acid with HI and red phosphorus, (3) saponification of 2-nitro-4-acetaminophenol with sulfuric acid, and (4) heating with 3-nitrophenylhydroxylamine with sulfuric acid.[1]

No information was available on the method of manufacture of 2-amino-3-nitrophenol, 4-amino-3-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, or 4-hydroxypropylamino-3-nitrophenol.

Analytical Methods

Kottemann[2] used 2-dimensional thin layer chromatography to identify 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and

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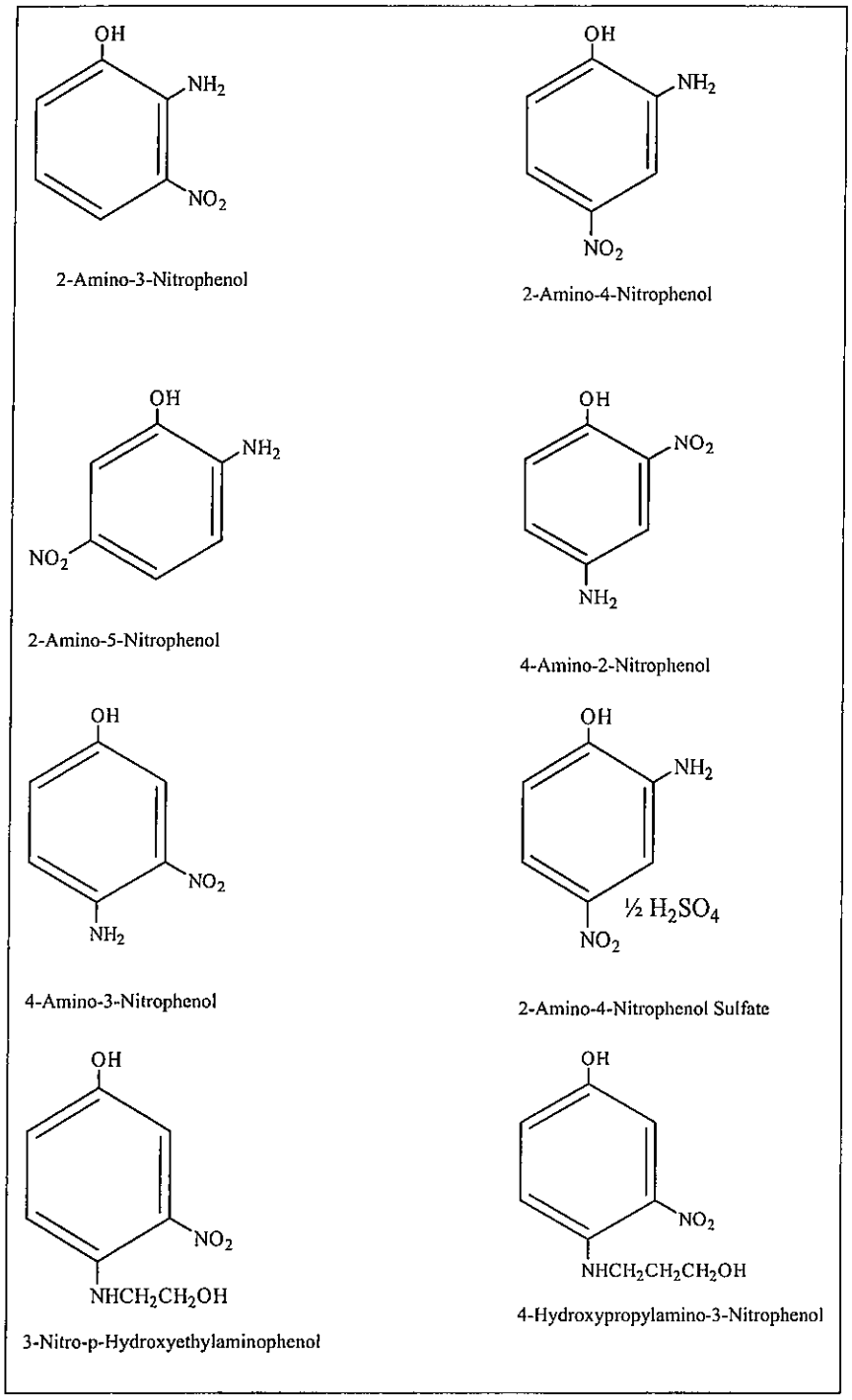

Figure 1. Structures of amino-nitrophenols[9]).

4-amino-2-nitrophenol in various commercial hair dye products.

Polystyrene-based anion exchangers with aqueous eluents have been used to separate 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol from solutions.[3]

Analytical techniques to detect 4-amino-3-nitrophenol are similar to those for 3-nitro-p-hydroxyethylaminophenol.[4]

3-Nitro-p-hydroxyethylaminophenol can be detected by the following analytical techniques: infrared and UV-visible spectrophotometry, mass and nuclear magnetic resonance spectrometry, titer by potentiometry, liquid chromatography/mass spectroscopy (impurity detection and identification), high-performance liquid chromatography (impurity content), Karl Fischer method (water content), and gas chromatography (residual solvents).[5]

Table 1. Synonyms and Trade Name[9]

Ingredient	Synonyms	Trade Name
2-amino-3-nitrophenol	1-hydroxy-2-amino-3-nitrobenzene 2-hydroxy-6-nitroaniline 3-nitro-2-aminophenol Phenol, 2-amino-3-nitro-	Imexine FO
2-amino-4-nitrophenol	1-hydroxy-2-amino-4-nitrobenzene 4-nitro-2-amino-1-hydroxybenzene p-nitro-o-aminophenol 5-nitro-2-hydroxyaniline	Rodol 42
2-amino-5-nitrophenol	3-hydroxy-4-aminonitrobenzene 2-hydroxy-4-nitroaniline 5-nitro-2-aminophenol Phenol, 2-amino-5-nitro- CI 76535	Rodol YBA
4-amino-2-nitrophenol	None given	None given
4-amino-3-nitrophenol	2-amino-5-hydroxynitrobenzene 3-nitro-4-aminophenol 2-nitro-4-hydroxyaniline Phenol, 4-amino-3-nitro-	4-amino-3-nitrophenol Covariane orange W 2121 Imexine FN Colorex 4A3NP JAROCOL 4A3NP
2-amino-4-nitrophenol sulfate	None given	Rodol 42S
3-nitro-p-hydroxylaminophenol	Phenol, 4-[(2-hydroxyethyl)amino]-3-nitro- 4-[(2-hydroxyethyl)amino]-3-nitrophenol	Colorex red 54 Imexine FH Jarocol NHEAP Rodol HENP Velsol red 54
4-hydroxypropylamino-3-nitrophenol	4-(3-hydroxypropylamino)-3-nitrophenol Phenol, 4-[(3-hydroxypropyl)amino]-3-nitro- 4-hydroxypropylamino-2-nitrophenol	Colorex RBN Covariane rouge W 3127 Velsol red BN

Table 2. Chemical and Physical Properties

	2-Amino-3-Nitrophenol	2-Amino-4-Nitrophenol	2-Amino-5-Nitrophenol	4-Amino-2-Nitrophenol	4-Amino-3-Nitrophenol	3-Nitro-p-Hydroxyethyl aminophenol	4-Hydroxypropylamino-3-Nitrophenol
Chemical description		Yellow-brown to orange prisms	Olive-brown, brown, to orange crystalline solid	Dark red plates or needles	Dark red prisms	Reddish-brown powder	Reddish-brown crystals or powder
Molecular weight	154.12	154.12	154.12	154.14	154.12	198.16	212.3
Empirical formula	$C_6H_6N_2O_3$	$C_6H_6N_2O_3$	$C_6H_6N_2O_3$	$C_6H_6N_2O_3$	$C_6H_6N_2O_3$	$C_8H_{10}N_2O_4$	$C_9H_{12}N_2O_4$
Solubility		Soluble in ethanol, acetone, acetic acid, diethyl ether; slightly soluble in water	Soluble in ethanol, acetone, benzene; slightly soluble in water	Soluble in hot water, ethanol, ether	Soluble in isopropyl alcohol; slightly soluble in water	In water: <1; in ethanol: 1<S<10; DMSO: >20	In water 1.8%, in ethanol 5.0%
Melting point, °C	212-213	Anhydrous 143-145; hydrated 80-90	Decomposes at 200	131	154	133-140	103 minimum
Spectroscopy				Absorption maximum at 234 nm in methanol	231.6 nm—0.740 abs units in ethanol 453 nm—0.646 abs units in ethanol	237.2 nm—0.561 abs units) in ethanol 477.0 nm—0.589 abs units) in ethanol	

ABS, absorbance; DMSO, dimethyl sulfoxide.

Impurities

2-Amino-3-Nitrophenol. A certificate of analysis reported by Centre International de Toxicologie (CIT) indicates that 99.6% pure 2-amino-3-nitrophenol contains 0.24% 2-amino-5-nitrophenol and less than 0.1% of an unknown impurity that had an absorption maximum of 220 nm. Residual solvents were less than 100 μg/g.

4-Amino-3-Nitrophenol. Colipa[4] performed analytical studies on 5 batches of 4-amino-3-nitrophenol that were 97.5% to 99.5% pure.[4] Impurities for 4-amino-3-nitrophenol may be intermediates: p-aminophenol, acetic acid 4-acetylamino-phenyl-ester, acetic acid 4-acetylamino-3-nitro-phenyl-ester, and N-(4-hydroxy-2-nitrophenyl) acetamide. Residual solvents may include isopropanol and methanol (<100 μg/g, each). Heavy metals that may be present include As and Sb (<5 mg/kg each), Pb (<20 mg/kg), Cd (<10 mg/kg), and Hg (<5 mg/kg).

3-Nitro-p-Hydroxyethylaminophenol. Maximum levels of iron and lead in 3-nitro-p-hydroxyethylaminophenol are reported as 50 ppm and 5 ppm, respectively.[6]

Analytical studies performed by Colipa[4] on 2 batches of 3-nitro-p-hydroxyethylaminophenol found the purity to be 98.3% to 98.7%. Impurities may include 4-amino-3-nitrophenol (0.14 g/100 g), 2-[[4-(2-hydroxyethoxy)-2-nitrophenyl]amino] ethanol (0.52 g/100 g), and 4-[[2-(2-hydroxyethoxy)ethyl]-amino]-3-nitrophenol (0.11 g/100 g). Residual solvents may include isopropanol and methanol (<100 μg/g each). The following heavy metals are present: As, Cd, Pb, Sb (each <1 mg/kg), and Hg (<0.1 mg/kg).

4-Hydroxypropylamino-3-Nitrophenol. Iron content for 4-hydroxypropylamino-3-nitrophenol is reported to be 200 ppm maximum.[6]

Reactivity and Stability

Centre International de Toxicologie (CIT)[7] reported that the stability of 3-nitro-p-hydroxyethylaminophenol was satisfactory after 2, 4, and 6 hours of storage at room temperature and for 4 to 9 days at 4°C at quantities of 1 and 200 mg/mL in 0.5% carboxymethylcellulose under inert gas and with no exposure to light. The material was also found to be stable after 2 and 4 hours of storage at room temperature under inert gas and no exposure to light for the quantities 0.1 and 500 mg/mL in dimethyl sulfoxide (DMSO) and 1, 10, and 500 mg/mL in dimethylformamide. 3-Nitro-p-hydroxyethylaminophenol maintained homogeneity in 0.5% carboxymethylcellulose after 9 days of storage at 4°C with the above conditions.

CIT[8] also determined the homogeneity and stability of 4-amino-3-nitrophenol. Homogeneity was reported to be satisfactory in 0.5% carboxymethylcellulose and in acetone/olive oil (0.5, 10, and 250 mg/mL) under inert gas and protection from light. Stability was satisfactory after 2, 4, and 6 hours of storage at room temperature and 4 and 9 days of storage at 4°C at 1 and 100 mg/mL in 0.5% carboxymethylcellulose, all under inert gas and protection from light. The material was also stable after 2 and 4 hours of storage at room temperature at 0.1 and 500 mg/mL DMSO and 0.5, 10, and 250 mg/mL acetone/olive oil, also under inert gas and light protection.

Use

Cosmetic

2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-2-nitrophenol, 4-amino-3-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol all function as hair colorants in cosmetic products.[9] Ordinarily, permanent hair dyes will penetrate the hair shaft, and, if an oxidizing agent is added, the dyes become irreversibly bound within the hair shaft. These ingredients, however, act as toners in permanent hair dyes and do not interact with an oxidizing agent.

As reported by industry to the US Food and Drug Administration (FDA) in 2006 in the voluntary cosmetic registration program (VCRP), only 2-amino-3-nitrophenol, 2-amino-4-nitrophenol, 4-amino-3-nitrophenol, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are currently used in hair dyes and colors, tints, and coloring shampoos (see Table 3). 2-Amino-5-nitrophenol, 4-amino-2-nitrophenol, and 2-amino-4-nitrophenol sulfate were not reported to be used.[10]

Also given in Table 3 are the current data from the VCRP on the total number of products in each product category, allowing the reader to determine how frequently these ingredients are used in a particular product category. For example, in Table 3, 18 of the 1600 hair coloring products reported to be on the market contain 2-amino-4-nitrophenol.

Concentration of use information in Table 3 was based on an industry survey of current practice.[11] Although uses were voluntarily reported to the FDA in 2006, in some cases no use concentrations were reported in the industry survey. For example, in Table 3, 18 uses of 2-amino-4-nitrophenol were voluntarily reported to the FDA, but no use concentrations were reported in the industry survey.

According to the International Agency for Research on Cancer, 2-amino-4-nitrophenol and 2-amino-5-nitrophenol are found in both semipermanent and permanent hair dyes.[12]

According to Cosmetic, Toiletry, and Fragrance Association (CTFA),[13] semipermanent hair dyes remain on the hair through several washings and do not require the use of an oxidizing agent.

Hair coloring formulations are applied to or may come in contact with hair, skin (particularly at the scalp), and nails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing these ingredients several times a day. Under normal conditions of use, skin contact with hair dye is restricted to 30 minutes.[11]

2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-2-nitrophenol, 4-amino-3-nitrophenol,

Table 3. Ingredient Uses and Concentrations as a Function of Product Category

Product Category	Ingredient Uses in Each Product Category[10] (Total No. of Products in Each Category)[123]	Use Concentrations, %[11]
2-amino-3-nitrophenol		
Hair dyes and colors	3 (1600)	2
2-amino-4-nitrophenol		
Hair dyes and colors	18 (1600)	—[a]
4-amino-3-nitrophenol		
Hair dyes and colors	21 (1600)	9
3-nitro-p-hydroxyethylaminophenol		
Hair dyes and colors	32 (1600)	10 (5 after dilution)
Hair tints	2 (56)	—[a]
4-hydroxypropylamino-3-nitrophenol		
Hair dyes and colors	1 (1600)	2.6 in semipermanent and permanent hair colors[b]
Hair shampoos (coloring)	3 (27)	—[a]

[a] Although use of this chemical was voluntarily reported to the US Food and Drug Administration (FDA) in 2006, no use concentrations were reported in a survey of industry.
[b] Colipa.[23]

2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests to be exempt from certain adulteration and color additive provisions of the of the Federal Food, Drug, and Cosmetic Act. To be exempt, a coal tar hair dye product must display the following caution statement:

> 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.

Product labels shall also bear a caution statement and patch test instructions for determining whether the product causes skin irritation. The Cosmetic Ingredient Review Expert Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 hours after application of the test material and prior to the use of a hair dye formulation.

2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-2-nitrophenol, 4-amino-3-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are not included on the list of ingredients that must not be used in cosmetic products that are marketed in Japan.[14]

However, in Japan, hair dyes are regulated as quasi-drugs, and all ingredients, both active and inactive, must be specifically approved. Quasi-drugs are defined as "having a mild effect on the body, but are intended for neither the diagnosis, prevention, nor treatment of disease, nor to affect the structure or function of the body." 2-Amino-4-nitrophenol and 2-amino-5-nitrophenol are approved hair dye actives.[15]

In the European Union, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol have been prohibited from use in all cosmetics, apparently based on concerns about carcinogenesis.[16] 2-Amino-3-nitrophenol, 4-amino-3-nitrophenol, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol have been provisionally listed in Annex III (part 2) with concentrations limits apparently related to sensitization. The first 2 ingredients are limited to 3% in semipermanent hair dyes and 1.5% with hydrogen peroxide in permanent dyes. 3-Nitro-p-hydroxyethylaminophenol has a maximum authorized concentration in the finished product (as oxidizing and nonoxidizing coloring agent in hair dye) of 6%, whereas in combination with hydrogen peroxide the maximum use concentration upon application is 3.0%. 4-Hydroxypropylamino-3-nitrophenol has a maximum authorized concentration in the finished product of 5.2% and 2.6% as oxidizing and nonoxidizing coloring agents in hair dye, respectively, whereas in combination with hydrogen peroxide the maximum use concentration upon application is 2.6%.

Noncosmetic

2-Amino-4-nitrophenol has been used as a catalyst in the manufacture of hexadiene and as an antioxidant and light stabilizer in butyl rubber.[1]

In addition to their use as hair dye ingredients, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol are used as intermediates in the production of dyes. 2-Amino-4-nitrophenol is used to color leather, nylon, silk, wool, and fur. 2-Amino-5-nitrophenol is used to color synthetic resins, lacquers, inks, and wood stains. and 4-amino-2-nitrophenol is used to dye furs.[12,17]

General Biology

Absorption, Distribution, Metabolism, Excretion

Bronaugh and Congdon[18] measured octanol/water partition coefficients and determined percutaneous absorption using excised human cadaver skin.

The octanol/water partition coefficient for 2-amino-4-nitrophenol was 13.5, and the permeability constant was less than 3.0×10^{-5} cm/h using borate buffer with a pH of 7.9 as vehicle and 6.6×10^{-4} cm/h using water as vehicle to prevent ionization.

The octanol/water partition coefficient for 4-amino-2-nitrophenol was 9.1 and the permeability constant was less than 8.6×10^{-5} cm/h using borate buffer, pH 7.9, as vehicle and 2.8×10^{-3} cm/h using water as vehicle to prevent ionization.[18]

Bronaugh and Maibach[19] reported a study in which monkeys (species and number not mentioned) were dosed on shaved abdominal skin with 4 μg/cm^2 of ^{14}C-labeled 4-amino-2-nitrophenol in acetone to measure percutaneous absorption. 4-Amino-2-nitrophenol was left on the skin for 24 hours. The mean percentage of 4-amino-2-nitrophenol percutaneous absorption was 64.0% (from 6 determinations). The urinary excretion of a parenteral dose to correct for excretion by other routes was 68.3%. The maximum absorption of 4-amino-2-nitrophenol occurred within the first 2 hours of application of 4-amino-2-nitrophenol; absorption decreased rapidly in subsequent samples.

In addition to conducting the in vivo study, these authors[19] reported in vitro studies with excised human and monkey abdominal skin. A 4 μg/cm^2 sample of ^{14}C-labeled 4-amino-2-nitrophenol was applied to the excised skin. The human skin absorbed 45.1% of the applied dose whereas the monkey skin absorbed 48.2% of the applied dose.

An in vitro human skin penetration study of a formulation containing 1.03% 2-amino-3-nitrophenol resulted in a cumulative penetration of 0.26% in skin samples with hair and 0.46% without hair 24 hours after contact.[20]

Inveresk[21] reported that human skin samples were used to study percutaneous absorption of radiolabeled 3-nitro-p-hydroxyethylaminophenol in both oxidative and semipermanent formulations of the hair dye. The concentration of the oxidative preparation was approximately 3% (wt/wt) after mixing with developer (1:1 wt/wt). The concentration of the semipermanent preparation was about 1.85% (wt/wt). The different preparations were applied to human split-thickness skin membranes mounted in flow-through diffusion cells.

In the oxidative preparation, most of the applied dose was removed at 30 minutes post application (96.92%). Another 0.17% was removed 24 hours post application. The total dislodgeable dose was 97.09% and the total absorbed was 0.12%. The dislodgeable dose refers to the amount of the dose that is removed from skin. In the semipermanent preparation, 97.82% was removed at 30 minutes post application. At 24 hours post application, another 0.30% was removed giving the total dislodgeable dose of 98.11%. The total semipermanent preparation absorbed was 0.03%. For 3-nitro-p-hydroxyethylaminophenol, the dermal delivery of the oxidative preparation was 2.50 μg Eq/cm^2 and 0.45 μg Eq/cm^2 for the semipermanent preparation.[21]

In another study, Inveresk[22] determined percutaneous absorption of radiolabeled 4-amino-3-nitrophenol using human skin samples. The rate and extent of absorption from topical application were tested using both oxidative and semipermanent formulations. The oxidative formulation was incorporated at about 3% (wt/wt) before mixing with a developer (1:1, wt/wt) for a final concentration of about 1.5% (wt/wt), whereas the semipermanent formulation was incorporated at about 1% (wt/wt). The [^{14}C]-4-amino-3-nitrophenol preparations were applied and absorption was measured using the protocol that was described above in the 3-nitro-p-hydroxyethylaminophenol absorption study.

In the oxidative preparation, most of the applied dose was removed at 30 minutes post application (96.15%). At 24 hours post application, another 0.74% was removed. The total dislodgeable dose was 96.89% and the total absorbed was 0.59% (the absorbed dose was the mass of 4-amino-3-nitrophenol in the receptor fluid and receptor rinse). In the semipermanent preparation, 95.41% was removed at 30 minutes post application. At 24 hours post application, another 1.06% was removed giving the total dislodgeable dose of 96.47%. The total semipermanent preparation absorbed was 0.23%. For 4-amino-3-nitrophenol, the dermal delivery of the oxidative preparation was 3.00 μg Eq/cm^2 and 0.59 μg Eq/cm^2 for the semipermanent preparation.[22]

Colipa[23] reported that the clipped dorsal skin of male and female Sprague-Dawley rats was treated with approximately 0.99 g of a formulation containing 1.5% ^{14}C-ring-labeled 4-hydroxypropylamino-3-nitrophenol for a period of 30 minutes.

Percutaneous absorption, calculated from the amount of ^{14}C eliminated from the body in urine and feces within 72 hours plus the amount still present in the carcass, was determined to be 0.27% of the administered ^{14}C. Eighty-two percent of the absorbed amount was rapidly excreted within the first 24 hours, mainly via urine (76% of the eliminated ^{14}C) and feces (24% of the eliminated ^{14}C).

Retention of 4-hydroxypropylamino-3-nitrophenol was not observed in any organ, and the mean ^{14}C concentration in blood and organs was below or near detection limits after 24 hours. Before the end of this time period, the highest concentration of 4-hydroxypropylamino-3-nitrophenol was detected in kidneys, thyroids, and ovaries. Approximately 2.8% of the ^{14}C administered was recovered from the application site at the end of the study.

In the same study, different groups of male and female Sprague-Dawley rats were orally given 1.02 g of 1.5% solution of 4-hydroxypropylamino-3-nitrophenol in DMSO/water. The oral administration resulted in a similar elimination pattern with 99% of administered ^{14}C being eliminated in first 24 hours.

Most of the radiolabel was excreted via urine (70% of total excretion) followed by fecal excretion (30% of the total excretion). After oral administration, the highest concentrations of 4-hydroxypropylamino-3-nitrophenol were found in kidneys, liver, and skin, and the lowest concentrations were in brain, muscles, and fat.[23]

Animal Toxicology

Acute Oral Toxicity

Yellow, orange, or red colorations of rat and mice organs, tissues, skin, fur, and urine were observed in most oral studies of

2-amino-4-nitrophenol, 4-amino-3-nitrophenol, and 3-nitro-p-hydroxyethylaminophenol. The colorations were observed at most dose levels and were dose dependent. Specific mention of this effect is not included in the discussion of each study unless it influenced data collection.

2-Amino-4-Nitrophenol, 2-Amino-5-Nitrophenol, and 4-Amino-2-Nitrophenol. 2-Amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol were orally administered to groups of 5 Charles River CD rats of each sex in an oil-in-water emulsion. The median lethal dose (LD_{50}) values for 2-amino-4-nitrophenol and 2-amino-5-nitrophenol were greater than 4000 mg/kg, and the LD_{50} for 4-amino-2-nitrophenol was 3300 mg/kg.[24]

2-Amino-3-Nitrophenol. CIT[25] conducted an oral toxicity study of 2-amino-3-nitrophenol using 3 groups of 10 Sprague-Dawley rats (5 males and 5 females in each group; rats were approximately 6 weeks old at the start of the study). The test material was administered by gavage at 1000, 2000, and 3000 mg/kg in a suspension of 1,2-propanediol at a volume of 10 mL/kg. Mortality, general behavior, and body weight gain were monitored for 14 days after the single administration, and all rats were killed and necropsied at study end.

Clinical signs of toxicity observed were sedation, dyspnea, tonic–clonic convulsions, ataxia, and hypersalivation, which appeared 15 to 30 minutes post dosing. Survivors completely recovered between days 3 and 5. Death rates of 10%, 30%, and 20% occurred in dose groups 1000, 2000, or 3000 mg/kg, respectively, and death occurred 1 to 2 hours post treatment. There were no remarkable differences between the sexes with the exception of slightly reduced body weight gains in males from days 1 to 5, but gains were comparable to controls thereafter. There were no macroscopic abnormalities in the animals killed at the end of the study. The LD_{50} value of 2-amino-3-nitrophenol was greater than 2000 mg/kg. The noneffect dose was determined to be less than 1000 mg/kg.[25]

4-Amino-3-Nitrophenol. In all dose groups, sedation, dyspnea, and tonic–clonic convulsions occurred 15 to 30 minutes after treatment. Ataxia and hypersalivation were noted in the 1000- and 1500-mg/kg dose groups. Deaths occurred at 1 to 2 hours post treatment at rates of 10%, 70%, and 70% in the 500-, 1000-, and 1500-mg/kg dose groups, respectively. Surviving animal recovered between days 2 and 3. Macroscopic examination found no abnormalities in the survivors. From this study, the LD_{50} was determined to be greater than 500 mg/kg but less than 1000 mg/kg. [26]

3-Nitro-p-Hydroxyethylaminophenol. CIT[27] evaluated the acute toxicity of 3-nitro-p-hydroxyethylaminophenol using Sprague-Dawley rats. Two groups of 5 fasted females were administered a single dose of 1000 or 2000 mg/kg via oral gavage (1 rat from the 2000-mg/kg group was used in a preliminary test). Clinical signs and mortality were monitored for 14 days following the treatment. Body weight gain was also recorded. The rats were necropsied at the end of the observation period.

In the 2000-mg/kg dose group, 2 of the 4 animals died within 4 hours of treatment. Hypoactivity, piloerection, lateral recumbency, and dyspnea were noted prior to death and in the surviving animals on day 1. Surviving animals were completely recovered on day 2. No mortality was observed in the 1000-mg/kg dose group; however, piloerection was observed in all animals on day 1 and orange fur was noted from days 1 to 15. Body weight was not affected at either dose level, and no abnormalities were observed at necropsy. The maximum nonlethal dose was 1000 mg/kg and the minimum lethal dose was 2000 mg/kg.[27]

An acute oral toxicity study of 3-nitro-p-hydroxyethylaminophenol performed using albino Wistar rats and Swiss mice (5 males and 5 females per dose group per species) resulted in a 10% mortality in rats and 0% mortality in mice at 3 g/kg (dose groups not reported).[28]

4-Hydroxypropylamino-3-Nitrophenol. A 20% solution of 4-hydroxypropylamino-3-nitrophenol was administered once orally to 5 males and 5 female Wistar Crl:(WI)BR rats at a dose of 10 mL/kg. No mortalities or adverse effects on weight gain were observed, and necropsy 14 days after administration showed no abnormal results. 4-Hydroxypropylamino-3-nitrophenol caused red-stained urine up to 4 days after the administration.[23]

Acute Intraperitoneal Toxicity

2-Amino-4-Nitrophenol, 2-Amino-5-Nitrophenol, and 4-Amino-2-Nitrophenol. Male Charles River CD rats (10 per group) received intraperitoneally either 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, or 4-amino-2-nitrophenol in a 10% aqueous DMSO solution. The LD_{50} for 2-amino-4-nitrophenol was reported to be 246 mg/kg, the LD_{50} for 2-amino-5-nitrophenol was reported to be greater than 800 mg/kg, and the LD_{50} for 4-amino-2-nitrophenol was reported to be 302 mg/kg.[24]

Acute Ocular Toxicity

2-Amino-3-Nitrophenol. Centre de Recherches Biologiques[29] reported that 2-amino-3-nitrophenol was tested for ocular irritation using 3 female New Zealand albino rabbits.[29] The left eyes of the rabbits were instilled with 0.1 g of the test material, whereas the right eyes were left untreated and served as control. Observations for lesions in the conjunctiva, iris, and cornea were made at 1, 24, 48, and 72 hours after instillation.

At 1 hour after treatment, slight redness in the conjunctiva was noted in all animals and was noted to intensify slightly in 2 of the animals at 24 hours post instillation. Slight redness was present in the same 2 rabbits at 48 hours post instillation. The iris was folded more than the controls in 1 animal an hour after treatment, but this animal recovered by the 24-hour observation period. The cornea had translucent zones in 2 animals

1 hour post treatment and in 1 animal 24 hours post treatment, but the cornea recovered completely by 48 hours post instillation. The researchers determined 2-amino-3-nitrophenol to be a nonirritant.[29]

4-Amino-3-Nitrophenol. Laboratoire de Recherche et D'Experimentation[30] tested undiluted (99.3% pure) 4-amino-3-nitrophenol (as Imexine FN) using 3 male New Zealand albino rabbits. A dose of 0.1 mL of paste was instilled into 1 eye of each rabbit whereas the other eye was left untreated and served as a control. The eyes were observed 1, 24, 48, and 72 hours after treatment.

Irritation of the palpebral and bulbar conjunctivae occurred in all the rabbits, although the orange staining of the material made observations difficult. Twenty-four hours after treatment, redness, slight discharge, and chemosis occurred in all the animals. Iris congestion and partial corneal opacity were also noted and were totally reversible less than 72 hours after treatment. The reactions involving the conjunctivae disappeared within a week. It was concluded that 4-amino-3-nitrophenol is irritating to rabbit eyes. [30]

An acute eye irritation study was performed by CIT.[31] A single dose of 0.1 mL of 6% 4-amino-3-nitrophenol (as Imexine FN) in 1,2-propanediol (99%) vehicle was instilled into the conjunctival sac of the left eyes of 3 male New Zealand white rabbits. The right eyes were left untreated and served as controls. The eyes were not rinsed after instillation. Reactions were observed 1, 24, 48, and 72 hours after treatment. No ocular reactions were observed at any time after treatment, and the researchers concluded that 4-amino-3-nitrophenol was nonirritating to rabbits in ocular exposure.

3-Nitro-p-Hydroxyethylaminophenol. CIT[32] tested the eye irritation potential of 6% 3-nitro-p-hydroxyethylaminophenol using male New Zealand white rabbits. Three animals had the single dose of 0.1 mL of test material instilled into the left conjunctival sac. The right eye received no treatment and served as the control. After instillation, the eyes were not rinsed. The eyes were observed 1, 24, 48, and 72 hours after administration and then daily until any reactions reversed. Very slight chemosis and very slight redness of the conjuntiva were observed in all animals on day 1 and lasted until day 4. It was concluded that 3-nitro-p-hydroxyethylaminophenol was a slight irritant to rabbit eyes.

Institut Français de Recherches et Essais Biologiques (IFREB)[33] performed an ocular irritation study of 4% 3-nitro-p-hydroxyethylaminophenol using 6 male New Zealand white rabbits. The conjunctival sac of the right eye of each rabbit was instilled with 0.1 mL of the test material. The left eyes of the rabbits served as controls. Observations for effects were made at 1, 24, 48, 72 and 168 hours after treatment. At 1 hour after administration, the rabbits experienced chemosis, slight discharge, and slight conjunctival enanthema. These effects were gone by the 48-hour observation. The rabbit irises were folded at the 1-hour observation, but they recovered by the 24-hour observation. The researchers determined that 4% 3-nitro-p-hydroxyethylaminophenol was a slight irritant after 1 hour.

4-Hydroxypropylamino-3-Nitrophenol. The left eyes of 6 New Zealand white rabbits were treated with 0.1 mL of 3% solution of 4-hydroxypropylamino-3-nitrophenol. Eyes of 3 rabbits were rinsed off after 4 seconds using lukewarm water. Potential lesions were checked by instillation of 1 drop of 1% fluorescein solution per eye after 24 and 72 hours of application. After 1, 24, 48, and 72 hours, minimal redness of the conjunctiva was present in the 3 animals whose eyes were not rinsed. This effect was reversible within 24 hours. No other adverse reactions were observed in any test animals.[23]

Short-Term Oral Toxicity

2-Amino-3-Nitrophenol. In a 28-day oral toxicity study of 2-amino-3-nitrophenol performed by CIT,[34] 3 groups of 10 male and 10 female Sprague-Dawley rats (approximately 6 weeks in age) received daily doses of the test material at 100, 300, or 1000 mg/kg/d by gavage (volume received was 5 mL/kg/d). An additional group of 10 male and 10 female rats received 0.5% carboxymethylcellulose vehicle as a control. Clinical signs and mortality were checked twice daily, and body weight and food consumption were measured once a week.

No clinical signs of toxicity were observed in the animals receiving 100 mg/kg/d.

In the 300-mg/kg/d dose group, 1 female died during week 1 of unknown causes, but the death was not considered treatment related.

Body weight gain in the 1000-mg/kg/d dose group was slightly decreased in males during the last 2 weeks of treatment, and a relationship to the test material could not be excluded. In both sexes, slight to moderate increases in the absolute and relative weight of liver and spleen were recorded. In the 1000-mg/kg/d group, a blackish color of the spleen was observed in 50% of the males and 80% of the females. In the 1000-mg/kg/d group, hemosiderin-laden macrophages were observed in the spleen of 50% of the males and 70% of the females, which was associated with the increase in spleen weight and splenic congestion. The researchers of this experiment concluded that the no observable adverse effect level (NOAEL) was 300 mg/kg/d. [34]

2-Amino-4-Nitrophenol. The National Toxicology Program (NTP)[35] conducted oral toxicity studies of 2-amino-4-nitrophenol in rats and mice.

Rats. Male and female F344/N rats were divided into groups of 5 rats per sex per group and were orally administered either 2-amino-4-nitrophenol or vehicle control (corn oil). The rats were dosed with 0, 313, 625, 1250, 2500, or 5000 mg/kg 2-amino-4-nitrophenol for 11 or 12 doses over 15 days. Observations were performed twice daily, body weights were recorded weekly, and at study termination all animals underwent necropsy.

Male and female rats receiving 2500 or 5000 mg/kg 2-amino-4-nitrophenol died within 3 days of the beginning of dose administration. In the 1250-mg/kg group, only 1 male survived whereas none of the females survived to study termination. Diarrhea related to the administration of 2-amino-4-nitrophenol was observed in all groups except 313 mg/kg. No gross lesions were observed at necropsy.

Mice. Male and female $B6C3F_1$ mice were divided into 6 groups of 5 mice per sex per group and were orally administered either 2-amino-4-nitrophenol or vehicle control (corn oil). The mice were dosed with 0, 313, 625, 1250, 2500, or 5000 mg/kg for 11 or 12 doses over 15 days. As with the rat study, observations were performed twice daily, body weights were recorded weekly, and at study termination all animals underwent a necropsy.

During the first 5 days of the study, all the mice in the 2500- and 5000-mg/kg groups died; in addition, 2 males and all the females in the 1250-mg/kg group died. All treated mice that survived to study end had body weights comparable to controls.

2-Amino-5-Nitrophenol

Rats. NTP[36] conducted a study in which F344/N rats were used to test various doses of 2-amino-5-nitrophenol in corn oil by oral gavage. Sixty rats were divided into 6 groups containing 5 rats per sex per group. The rats received 12 doses of 2-amino-5-nitrophenol or corn oil (control material) at 1 of the following doses: 0, 156, 313, 625, 1250, or 2500 mg/kg over a 16-day period. Clinical observations were performed twice daily, body weights were recorded weekly, and a necropsy was performed at study termination.

Lesions found at necropsy in exposed rats were the same type as those found in vehicle controls. Four rats died prior to study end; 1 male in the 2500-mg/kg group, 1 female in the 1250-mg/kg group, and 2 females in the 313-mg/kg group. Body weights of the 2500- and 1250-mg/kg groups (males and females) were depressed compared with the controls. Male body weights in the 2500-mg/kg group were 30% less than controls; female body weights were 13% less. Male body weights in the 1250-mg/kg group were 11% less than the controls; female body weights were 9% less than controls. The only clinical signs recorded were loose stools in the 3 highest dose groups. No differences were seen in gross lesions between the treated and control rats.[36]

Mice. This NTP[36] study also examined 60 $B6C3F_1$ mice that were divided into 6 groups containing 5 mice per sex per group. The mice received 12 doses of either 2-amino-5-nitrophenol or corn oil (control material) at 1 of the following doses: 0, 313, 625, 1250, 2500, or 5000 mg/kg over a 16-day period. Clinical observations were performed twice daily, body weights were recorded weekly, and animals were necropsied.

Nineteen mice died prior to the end of the study. Two males and 5 females died in the 5000-mg/kg group, 3 males and 3 females died in the 2500-mg/kg group, 3 females died in the 1250-mg/kg group, 1 female died in the 625-mg/kg group, and 2 vehicle control males died. No significant differences in body weights occurred between treated and control animals; clinical observations were limited to loose stools in the males in the 5000-mg/kg group, and 2 males were prostrate for the first week of the study in the 2500-mg/kg group.[36]

4-Amino-2-Nitrophenol. In a study by the National Cancer Institute (NCI),[37] Fischer 344 rats and $B6C3F_1$ mice (both sexes) were used to estimate the maximum tolerated dose of 4-amino-2-nitrophenol, which would be used in a later chronic study. Groups of 5 males and 5 females were tested at 1 of the following concentrations: rats were given diet with either 147, 215, 316, 464, 681, 1000, 1470, 2150, 3160, or 4640 ppm; mice received either 100, 147, 215, 316, 464, 681, 1000, 1470, 2150, 3160, or 4640 ppm in their diet. Control groups received feed only.

After 6 weeks of administration, neither the rats nor the mice had any compound-related deaths, body weights were comparable to controls, and no gross lesions were noted. The lack of findings led the authors to set the dose levels for a chronic study (described in the Chronic Oral Toxicity section) at 1250 and 2500 ppm.[37]

4-Hydroxypropylamino-3-Nitrophenol. In a 28-day oral gavage study, male and female COBS CD rats received 20, 100, 500, or 1000 mg/kg/d of 4-hydroxypropylamino-3-nitrophenol.[23] No animals died during the study. Dose-related staining of fur, paws, tails, and bedding was observed. At 1000 mg/kg, dark red staining of fecal pellets was observed. A slight decrease in white blood cell count and a slight increase in blood urea nitrogen were observed in males receiving 500 mg/kg. There was no effect on organ weights; however, increased pallor and friability of the liver and pallor of the kidneys were observed in test animals at the 1000-mg/kg dose.

Subchronic Oral Toxicity

2-Amino-3-Nitrophenol. In a 13-week toxicity study of 2-amino-3-nitrophenol conducted by CIT using Sprague-Dawley rats, 3 groups of 10 males and 10 females received test material at 50, 200, or 800 mg/kg/d through oral gavage. An additional group of 10 males and 10 females received the 0.5% carboxymethylcellulose vehicle as the control. The animals were checked daily for clinical signs and mortality.

In the 800-mg/kg/d dose group, ptyalism was observed in almost all animals (starting week 4 in males), and loud breathing was observed in a few animals during weeks 2 and 4.

The authors stated that slightly lower glucose levels and slightly higher cholesterol levels occurred in females and slightly increased protein levels and lower albumin/globulin ratio were measured in males. Males in this dose group also had moderately high urinary volume. Slightly greater absolute and relative kidney weights were noted in males, as was enlargement or a gray-green coloration of the kidneys. Moderate tubular nephrosis occurred in some animals (both sexes), and moderate accumulation of acidophilic globules in the cortical

tubular epithelium of the kidneys was observed in almost all the males. Moderately high total bilirubin levels (in serum) and slight regenerative anemia were noted in both sexes. Both sexes also had greater relative and absolute liver and spleen weights and blackish coloration of the spleen. The splenic congestion and increased liver function produced slight to moderate centrilobular hepatocyte hypertrophy.

Slightly lower glucose, slightly higher cholesterol, and moderately higher total bilirubin levels were noted in females in the 200-mg/kg/d dose group. Males had slightly higher protein levels and lower A/G ratios.

2-Amino-3-nitrophenol was considered to be tolerated well by the 50-mg/kg/d dose group. The NOAEL in rats was reported to be 50 mg/kg/d.[38]

2-Amino-4-Nitrophenol. The NTP[35] conducted a study in which male and female F344/N rats and $B6C3F_1$ mice were randomized into 6 groups of 10 animals per sex per group per species. The animals orally received either 2-amino-4-nitrophenol or corn oil for 5 days a week for 13 weeks. Doses of 2-amino-4-nitrophenol were 0, 62.5, 125, 250, 500, and 1000 mg/kg. Rats and mice were observed twice daily, body weights were recorded weekly, and the animals were necropsied at study termination.

All rats in the 1000-mg/kg group and 2 males and 2 females in the 500-mg/kg group died during the first week of the study. Mortalities for the mouse study in the 500-mg/kg group (3 females) occurred at weeks 1, 8, and 11. In the 1000-mg/kg group, the 5 male deaths occurred at weeks 8 (3 mice), 9, and 10 and the female deaths occurred at weeks 4, 5, 9 (4 mice), and 11 (3 mice). In the 500- and 1000-mg/kg groups, the rats were observed to have diarrhea and lethargy; however, no compound-related clinical signs were seen in the mice. For both the rats and the mice, body weights of treated groups were comparable to the controls.

In the 500-mg/kg group (male and female rats), the liver weight to body weight ratios were significantly greater than the controls. For the mice, the liver weight to body weight ratios were significantly increased for the males in the 1000-mg/kg group and females in the 62.5-mg/kg group compared with the controls. However, in both the rats and mice, microscopic examination did not reveal any possible cause.

The other histopathologic finding in the mice attributed to the administration of 2-amino-4-nitrophenol was degeneration and necrosis of renal tubule epithelium in 5 males and 3 females in the 1000-mg/kg group. Several histopathologic findings were attributed to the administration of 2-amino-4-nitrophenol for the rats: males in both the 500- and 1000-mg/kg groups had mild to severe mineralization of the renal cortex, mild to severe degeneration of the renal tubular epithelium, and osteomalacia of moderate severity, and 2 males and 2 females in the 1000-mg/kg group had inflammation of the nonglandular portion of the stomach.[35]

The LD_{50} in rats was greater than 625 mg/kg but less than 1250 mg/kg. The LD_{50} in mice was greater than 625 mg/kg but less than 1250 mg/kg.[35]

2-Amino-5-Nitrophenol

Rats. NTP[36] orally treated 6 groups consisting of 10 F344/N rats of each sex with 2-amino-5-nitrophenol in corn oil at 1 of the following doses: 0, 100, 200, 400, 800, or 1600 mg/kg for 5 days a week for 13 weeks. Clinical observations were performed twice daily, body weights were recorded weekly, and a necropsy was performed at study termination.

Twelve rats died prior to study termination: 5 males and 2 females in the 1600-mg/kg group, 1 male and 3 females in the 800-mg/kg group, and 1 male in the 400-mg/kg group. Body weights were decreased in the 1600-, 800-, and 400-mg/kg males by 43%, 25%, and 10%, respectively. Only the 1600-mg/kg females had decreased body weights, 16%, compared with the controls. The only clinical observations attributed to treatment were loose stools and occasional mucoid feces in the 800- and 1600-mg/kg groups.

The rats in the 800- and 1600-mg/kg groups had vasculitis of the colon or cecum. The liver to body weight ratio was significantly increased compared with the controls for all animals treated with 2-amino-5-nitrophenol except for the males in the 100-mg/kg group. The LD_{50} in rats was greater than 2500 mg/kg.[36]

Mice. NTP[36] tested 2-amino-5-nitrophenol in corn oil for toxicity using $B6C3F_1$ mice of both sexes. The mice were divided (10 mice per sex per group) and orally administered 1 of the following doses: 0, 100, 200, 400, 800, or 1600 mg/kg for 5 days per week for 13 weeks.

Seven mice died prior to study termination: 4 males and 3 females in the 1600-mg/kg group. Body weights were decreased only in the 1600-mg/kg males by 11%. The only clinical observation attributed to treatment was lethargy in the 1600-mg/kg females.

The liver to body weight ratios were increased in the treated rats but not in the mice. Four male and 7 female mice in the 1600-mg/kg group had acute/chronic perivasculitis of vessels of the cecum or colon.

The LD_{50} in mice was greater than 1250 mg/kg but less than 2500 mg/kg.[36]

4-Amino-3-Nitrophenol. Quintiles England[39] performed a 13-week oral toxicity study of 4-amino-3-nitrophenol (as Imexine FN) using 80 Crl:CD(SD)Br strain rats. The rats were divided into 4 groups (10 of each sex per group); 3 of the groups received 10, 50, or 250 mg/kg/d. The fourth group, the control, received the 0.5% wt/vol carboxymethylcellulose vehicle. The animals were observed daily, and body weights and food consumption were recorded weekly. Ophthalmologic exams were performed for all animals prior to treatment and for those in the control and high-dose group during week.[13] Hematology and blood chemistry tests and urinalysis were performed during weeks 12 and 13.

At the end of the study, all animals were necropsied, and organ weights were recorded. Tissues from the control group, high-dose group, and animals that died during the study were

examined by light microscopy, whereas gross lesions and the lungs were examined in all animals.

No clinical signs were noted in any animals from any dose group. No abnormalities were observed in body weight gain, food consumption, ocular readings, hematology, or blood chemistry. Urine parameters in the 250-mg/kg/d group could not be evaluated because of the discoloration caused by the test material; however, no adverse effects were noted.

Statistically significant increases in absolute and relative liver weights occurred in males in the 250-mg/kg/d group, but these increases were within normal laboratory ranges. These authors discussed previous 28-day oral toxicity data (not provided) in which an effect was reported at 600 mg/kg/d. They concluded that the no observable effect level (NOEL) was at least 250 mg/kg/d but lower than 600 mg/kg/d.[39]

3-Nitro-p-Hydroxyethylaminophenol. A 3-month study performed by Laboratoires d'etudes et de Recherches Synthelabo (LERS)[40] evaluated the oral toxicity of 3-nitro-p-hydroxyethylaminophenol (Imexine FH) using Sprague-Dawley rats. 3-Nitro-p-hydroxyethylaminophenol was administered at doses of 0, 40, 200, or 1000 mg/kg/d for 7 days a week. There were 10 animals of each sex in each dose group. Ophthalmologic, hematologic, blood biochemistry, and urine tests were performed on the rats after weeks 4 and 13 of treatment. At the end of the study, the animals were killed and necropsied. Macroscopic and microscopic evaluations of the main organs were performed.

No mortality was observed during the treatment period. In the 1000-mg/kg/d dose group, ptyalism was observed starting at week 7 immediately after the daily treatment. This dose group also had a slight yellow-orange discoloration of the choroid with no alteration of the choroid vessels. Both sexes had slight, nonsignificant increases in liver and kidney weights. Seven of the 10 males in the 1000-mg/kg/d group had dark discoloration of the thyroid follicles, which was determined to be a nontoxic alteration.[40]

4-Hydroxypropylamino-3-Nitrophenol. In a 90-day study reported by Colipa,[23] male and female COBS CD rats received doses of 10, 30, and 90 mg/kg 4-hydroxypropylamino-3-nitrophenol.

There were no treatment-related deaths. Body weight gain was reduced in females in the lowest dose group by the end of the study. Weight gain of other groups remained the same as the control group. There were no treatment-related hematological or blood chemistry changes.

Absolute and relative thyroid weights in 30 mg/kg dose males were significantly increased compared with controls, but the absolute and relative thyroid weights in 90-mg/kg dose females were significantly decreased. After the recovery period, mean thyroid weights of 90-mg/kg dose males were significantly increased. However, no dose–response relationship could be established.

There were no significant histological findings. The researchers concluded that 4-hydroxypropylamino-3-nitrophenol did not cause systemic toxicity and that 90 mg/kg can be regarded as a NOAEL.[23]

Subchronic Dermal Toxicity

Rabbits. Burnett et al[41] conducted a study in which adult New Zealand white rabbits were divided into 12 groups (6 rabbits per sex per group). Twelve different hair dye formulations were applied topically twice a week for 13 weeks. One contained 0.4% 2-amino-4-nitrophenol, another contained 0.5% 2-amino-5-nitrophenol, and a third contained 0.3% 4-amino-2-nitrophenol. All formulations were mixed with 6% hydrogen peroxide and applied at a dose of 2 mL/kg. Three rabbits per sex per group were abraded at their dose sites on the first treatment day of each week. In addition to the test groups, 3 untreated control groups were part of the study (6 rabbits per sex per group). All surviving rabbits were killed after 13 weeks of treatment, and a necropsy was performed.

None of the rabbits had any evidence of compound toxicity throughout the study. Body weight gain was similar among all groups. Five control and 5 test rabbits died during the study; however, their deaths were attributed to bleeding procedures (no details for requirement of blood withdrawal were provided). The group treated with the hair dye containing 0.3% 4-amino-2-nitrophenol had some slight thickening of the skin. At necropsy, no gross abnormalities or microscopic lesions were seen.[41]

Chronic Oral Toxicity

2-Amino-4-Nitrophenol

Dogs. Wernick et al[42] created a composite of various chemicals found in commercially available semipermanent hair dyes. The authors used the highest concentration of that chemical found in hair dyes. 2-Amino-4-nitrophenol was added to the composite at a concentration of 0.05%. The composite mixture was used in a 2-year chronic feeding study in dogs. Eighteen male and 18 female purebred Beagle dogs were divided into 3 groups and fed the composite mixture with their feed. Dosages fed to the dogs were 0, 19.5, and 97.5 mg/kg/d and were adjusted weekly as body weights changed. Dogs were observed daily for toxic or pharmacological effects. One male and 1 female from each group were euthanized and necropsied at 6, 12, and 18 months. All surviving dogs were euthanized and necropsied at 24 months.

None of the test dogs died prior to their scheduled death, and no differences in body weight gain, clinical pathology values, ratio of organ to body weight values, and gross or microscopic evaluations were seen between the test and control animals. However, both test groups excreted blue-brown urine daily. The color of the urine returned to normal after an overnight fast.[42]

Rats. A 2-year chronic study was conducted by the NTP[35] using F344/N rats to examine the effects of 2-amino-4-nitrophenol. Corn oil or 2-amino-4-nitrophenol was orally administered

to the rats (50 per sex per group) 5 days a week for 103 weeks at doses of 0, 125, and 250 mg/kg. Animals underwent observation twice daily, recording of body weights, and necropsy at study termination. Histopathologic examinations of the high-dose and vehicle control animals were performed; other groups were added if necessary.

There were no significant differences in body weights between the treated animals and vehicle controls. Clinical observations attributed to treatment with 2-amino-4-nitrophenol were soft stool and occasional diarrhea that started 6 months after study initiation. The males in the 250-mg/kg group had significantly decreased survival compared with the vehicle control group starting after week 89. Female survival in treated groups was comparable to controls.

Histopathological findings in the kidney were chronic nephropathy in the high-dose males at a greater severity than in the controls. All treated rats had pigmentation of the small and large intestine, whereas the males in the 250-mg/kg group also had ulcers and erosions.

Mice. Three hundred $B6C3F_1$ mice were randomized into 3 groups (50 mice per sex per group) to test 2-amino-4-nitrophenol in a 2-year chronic study by NTP.[35] Administration of 2-amino-4-nitrophenol (in corn oil) was by gavage at doses of 0, 125, and 250 mg/kg for 5 days a week for 103 weeks. As with the 2-year rat study, observations were done twice daily, body weights were recorded, and all animals were necropsied at study termination. A histopathologic examination of tissues was done on high-dose and control groups and other groups as necessary.

The mice had no significant differences in body weights between treated and controls; however, females in the 125-mg/kg group had body weights that were 17% higher than the controls. No compound-related clinical signs were observed for the mice. No significant differences in survival occurred between treated groups and the control group; however, 3 female mice in the 250-mg/kg group died the same day in week 19 and all exhibited compound-related toxicity. The 250-mg/kg males had a greater incidence of renal tubule pigmentation, and males in both dose groups had increased incidence of chronic bronchopneumonia and hyperplasia of alveolar epithelium.[35]

2-Amino-5-Nitrophenol

Rats. The NTP[36] performed a 2-year chronic carcinogenicity study using F344/N rats (50 per sex per group). 2-Amino-5-nitrophenol in corn oil was orally administered to the rats at doses of 0, 100, and 200 mg/kg 5 days week for 103 weeks. All animals underwent twice-daily observations, regular recording of body weights, and necropsy at study termination. Histopathology was done on selected rats.

Survival in the low-dose and high-dose males was significantly decreased compared with controls. No differences in female survival were seen. Body weights were decreased in both the high-dose females and males; female body weight was 4% to 5% lower starting after week 93, and male body weight was 5% to 10% lower starting in week 33. Clinical signs occasionally observed were loose or poorly formed stools in the high-dose groups. All treated rats had increased incidences of acute/chronic inflammation, ulceration, and pigmentation in the large and small intestines. The low-dose males and females had increased incidences of retinal degeneration and cataracts; however, these rats were nearest to a light source.[36]

Mice. The same report by NTP[36] described a 2-year chronic carcinogenicity study using $B6C3F_1$ mice (50 per sex per group). 2-Amino-5-nitrophenol in corn oil was orally administered to the mice at doses of 0, 400, and 800 mg/kg 5 days a week for 103 weeks. All animals underwent twice daily observations, regular recording of their body weights, and necropsy at study termination. Histopathology was done on selected mice.

Survival of both the high-dose males and females was significantly decreased after weeks 20 and 22, respectively. Body weights were decreased in both the high-dose females and males: female mean body weights were 8% to 13% less than the controls from week 69 until study termination, and male body weights were 8% to 11% lower than the controls from week 29 to week 74. Low-dose female body weights were 5% to 9% lower than the controls from week 69 until study completion, whereas the body weights in low-dose male mice were greater than the controls throughout the majority of the study. Clinical observations included lethargy, prostration, cyanosis, and tremors, which usually occurred 2 hours after dose administration. These observations were more often seen in the high-dose mice rather than the low-dose mice.

All treated groups of mice had an increased incidence of acute/chronic inflammation and pigmentation of the cecum and colon. The high-dose males and females had ulcers of either the cecum or rectum. No neoplasms occurred in either the colon or rectum of the treated mice (see section titled Carcinogenicity). The high-dose mice had a greater number of increased incidences of renal tubular dilatation than did than the controls.[36]

4-Amino-2-Nitrophenol

Rats. In an NCI[37] study, Fischer 344 rats were divided into 3 groups of 50 animals per group per sex. The rats received either control diet or 4-amino-2-nitrophenol mixed into their feed at concentrations of 1250 or 2500 ppm for 103 weeks. All animals were observed twice daily, weighed at regular intervals, and necropsied at study termination. The pathological evaluation consisted of gross and microscopic examinations of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tumors of the urinary bladder occurred only in rats administered 4-amino-2-nitrophenol. Male rats had a 28% incidence at the high dose and female rats showed a 2% at the low dose and 4% at the high dose.

Body weights of the treated rats of each sex were slightly lower than the controls but did not differ significantly; no toxicity due to 4-amino-2-nitrophenol was noted. Survival rates among the treated animals were comparable to the controls.[37]

Mice. B6C3F$_1$ mice were divided into 3 groups (50 mice per sex per group) to look at possible effects of 4-amino-2-nitrophenol.[37] The mice received control feed or 4-amino-2-nitrophenol mixed with their feed at concentrations of 1250 or 2500 ppm. All mice were observed twice daily, weighed at regular intervals, and necropsied at study termination. All mice also underwent gross and microscopic examinations as described for the rat study (above). In mice, all of the tumors and hyperplasia that occurred were spontaneous type that occurred in approximately equal incidences in the control and dosed groups.

Body weights of the mice, both male and female, were slightly decreased compared with control mice. Survival of the mice in both the high-dose and low-dose groups was comparable to the controls.[37]

Chronic Dermal Toxicity

4-Amino-2-Nitrophenol. Venitt and Searle[43] reported an 80-week study in which a hair dye containing 4-amino-2-nitrophenol (concentration not given) was diluted 10-fold in 50% aqueous acetone (concentration of ingredients not provided) and applied twice weekly to clipped dorsal skin of DBAf and A strains of female and male mice (32 mice per group). The strain A mice received 0.4 mL of the diluted hair dye per application. The DBAf mice originally received 0.4 mL, but some toxicity (not described) was noted so each application of diluted hair dye was reduced to 0.2 mL per application.

Tumors of lymphoid origin and genital tract sarcomas occurred in both strains of mice at times earlier than the controls—38 weeks compared with 61 weeks in strain A mice and 41 weeks compared with 72 weeks in DBAf mice.[43]

Searle and Jones[44] tested the carcinogenicity of a commercial hair dye containing 4-amino-2-nitrophenol using 2 strains of mice. Male and female albino A/Bcr mice (26 and 16 mice per group, respectively) and gray DBAf/Bcr mice (17 and 15 mice per group, respectively) were dermally dosed with the hair dye twice weekly for 80 weeks. The hair dye was diluted with 4 parts deionized water and 5 parts aqueous acetone. A control group was dosed with aqueous acetone (50% vol/vol). The diluted hair dyes were applied to the clipped backs of the mice at a volume of 0.4 mL.

After the 24-week point, the dose was reduced to 0.2 mL for the DBAf mice because of toxic effects. The DBAf mice became emaciated between 13 and 26 weeks of exposure, and those affected were euthanized.

Toxic effects were on the urogenital tract, which were partly due to obstructive crystals seen in the urinary bladder and on the skin round the penis. The penile region was frequently distended, and in 3 mice squamous papillomas developed. The urinary bladder and seminal vesicles were grossly distended, and, microscopically, dilation of the renal tubes was noted. Gastric distention and inflammation were noted in 3 controls and in 9 treated DBAF mice.[44]

Dermal Irritation

2-Amino-3-Nitrophenol. The irritancy potential of 2-amino-3-nitrophenol from a single cutaneous application was examined using 3 female New Zealand albino rabbits. A dose of 0.5 g of the compound was applied by a gauze square to the right flank of the rabbits. The left flank was left untreated and served as the study control. A nonallergenic, nonocclusive dressing held the compound in place for 4 hours. An hour after removal of the dressing, lesions were evaluated. They were evaluated again at 24, 48, and 72 hours after the treatment. At approximately 5 hours after application, slight erythema was observed in 1 animal. This reaction was not reported at the 24-hour observation period. 2-Amino-3-nitrophenol was found to be a nonirritant.[45]

4-Amino-3-Nitrophenol. The irritancy and corrosivity potential of 4-amino-3-nitrophenol on skin was examined using 3 male New Zealand albino rabbits.[46] The test material (0.5 g, undiluted) was applied to shorn skin and covered by a semiocclusive patch for 4 hours. After the patch was removed, the test area was rinsed with distilled water and observed at 1, 24, 48, and 72 hours after removal for signs of erythema and edema. Erythema could not be determined at 1 or 24 hours after patch removal because the dyeing properties of the test material made it difficult to evaluate the skin. 4-Amino-3-nitrophenol was a nonirritant in this study.

CIT[47] tested 6% 4-amino-3-nitrophenol as Imexine FN for dermal irritation using 3 male New Zealand white rabbits. The material, prepared in 1,2-propanediol (99%), was applied to the clipped flank area by a dry compress and semiocclusive dressing for 4 hours. The application site was observed for reactions 1, 24, 48, and 72 hours after the dressing was removed. Because of the staining nature of the test material, macroscopic readings for erythema could not be conducted properly and a microscopic exam was performed. No edema was noted, and the microscopic exam revealed no unusual lesions. The researchers determined that 4-amino-3-nitrophenol was nonirritating to rabbit skin based on microscopic findings.

3-Nitro-p-Hydroxyethylaminophenol. A 6% concentration of 3-nitro-p-hydroxyethylaminophenol was evaluated by CIT[48] for dermal irritation using 3 male New Zealand white rabbits. Initially in the study, a rabbit was dosed with a single application of 0.5 mL of test material for periods of 3 minutes, 1 hour, and 4 hours on the anterior left flank, anterior right flank, and posterior right flank, respectively. The dose was applied to the clipped areas with a semiocclusive dressing. Reactions were observed 1, 24, 48, and 72 hours after application and then daily until the end of the observation period. When a persistent coloration of the skin was observed in the rabbit, an additional 2 rabbits were treated to rule out skin irritation. This time, the 0.5-mL dose was applied for 4 hours and the reactions were observed at 1, 24, 48, and 72 hours after the application. After 72 hours, the animals were killed and skin samples were taken from both flanks and analyzed microscopically.

In the first rabbit, red coloration of the skin was noted from day 1 to day 15 from the 3-minute exposure. This also occurred in the 1-hour and 4-hour exposures (all rabbits). Erythema may have been masked by the color. No dryness of the skin, crusts, or edema was observed at any exposure interval or in any rabbit. Microscopic investigation did not show evidence of skin irritation, and it was concluded that 3-nitro-p-hydroxyethylaminophenol was well-tolerated in topical applications to rabbits.[48]

Primary cutaneous irritation of 4% 3-nitro-p-hydroxyethylaminophenol was studied using 6 male New Zealand white rabbits.[49] The rabbits' right flanks were scarified on the epidermal level, whereas the left flanks were left intact. Gauze pads, with 0.5 mL of the test substance, were applied to both flanks with an occlusive patch. The patches were removed after 23 hours; at 24 hours and 72 after application, the primary irritation index was evaluated. Because the dyeing properties of the test material made observations for erythema impossible, skin biopsies were performed at 24 hours post application on both flanks of 3 rabbits. The remaining rabbits underwent skin biopsy at 72 hours post application. The histological examination found that 4% 3-nitro-p-hydroxyethylaminophenol did not produce irritation.

4-Hydroxypropylamino-3-Nitrophenol. A volume of 0.5 mL of 3% solution of 4-hydroxypropylamino-3-nitrophenol was applied occlusively to 6 white New Zealand rabbits for 4 hours.[23] After removal of the occlusive dressing and wiping of the substance, observations were made at 30 minutes, 1 hour, 24 hours, 48 hours, and 72 hours. No erythema or edema occurred at the site of exposure, and according to the European Economic Community guidelines, 4-hydroxypropylamino-3-nitrophenol was classified as nonirritating.

Dermal Sensitization

2-Amino-3-Nitrophenol. A cutaneous sensitization test in Hartley strain albino guinea pigs using the Magnusson and Kligman technique was determined to lack enough controls to make a proper judgment of 2.0 g of 2-amino-3-nitrophenol.[50] The coloring properties of the material presented difficulties in reading the skin reactions.

4-Amino-3-Nitrophenol. CIT[51] conducted an evaluation of the skin sensitization potential of 4-amino-3-nitrophenol. In the evaluation, which consisted of 2 independent experiments following a range-finding study, 28 female CBA/J mice were divided into 7 groups of 4. Five of the groups were treated with 4-amino-3-nitrophenol at concentrations of 1%, 2.5%, 5%, 10%, or 25% in the first experiment and at concentrations of 0.05%, 0.1%, 0.5%, 1%, or 2.5% in the second experiment. Two groups in each experiment received control treatments (negative and positive). In each part of the experiment, the test material was applied to the mouse ears (25 μL per ear) for 3 consecutive days. After 2 days of rest, the lymph nodes of the mice were removed and studied for proliferation with tritiated methyl thymidine. The values were then used to calculate the stimulation index (SI). Ear thickness was assessed using a micrometer before each treatment and at 24 hours after the final application.

In the first experiment, 1 mouse died in the 10% dose group on day 6. No clinical signs preceded the death. Hypoactivity/sedation and piloerection were observed in 1 animal in each the 1% and 2.5% groups on day 6. No mortality or clinical signs were observed in the second experiment. Orange discoloration of the ear skin was noted in all animals treated with more than 1% test material. No cutaneous reactions or thickening of the ears was observed in the treated groups.

The first experiment had positive lymphoproliferation responses for all test concentrations. The SI values showed a dose-dependent increase in groups treated with 1% to 10% 4-amino-3-nitrophenol and showed a decrease at 25% without evidence of cellular toxicity.

In the second experiment, a dose-related increase in SI (SI > 3) that exceeded the threshold positive value was noted at concentrations greater than 0.5%. An EC3 theoretical concentration of 0.2% was calculated. EC3 is the estimated concentration of a chemical necessary to cause a 3-fold increase in lymph node cell proliferative activity. The study concluded that 4-amino-3-nitrophenol induced delayed contact hypersensitivity and should be considered a strong sensitizer in mice.[51]

The sensitizing potential of 4-amino-3-nitrophenol was studied in a guinea pig maximization study performed by Laboratoire de Recherche et D'Experimentation (EViC-CEBA).[52] After a preliminary test to determine the maximal nonirritant concentration for the skin, female Hartley albino guinea pigs (5 in a control group, 10 in the test group) were clipped at the dorsal level.

The test group received 3 symmetrical intradermal injections of 0.1 mL that consisted of Freund's complete adjuvant (FCA) and distilled water (1:1 vol/vol), 4-amino-3-nitrophenol diluted to 10% in distilled water, and 4-amino-3-nitrophenol diluted to 10% in mixture 1:1 (vol/vol) with FCA and distilled water. Adequate controls were used.

The guinea pigs were not treated for a period of 6 days and then were reclipped at the injection sites. Because the material did not produce local irritation, the test areas of both the control and treated animals were coated with 0.5 mL of sodium lauryl sulfate at 10% in liquid petrolatum to produce irritation. After 24 hours, the treated group was patched with 0.5 mL of 4-amino-3-nitrophenol diluted to 50% for 48 hours. The control group was patched with 0.5 mL of distilled water. After an 11-day rest period, both groups of guinea pigs were clipped on the dorsolumbar region. Occlusive patches with 0.2 mL of 4-amino-3-nitrophenol at the maximal nonirritant concentration and a lower concentration were applied to all animals for a period of 24 hours. At 24 and 48 hours after challenge patch removal, the skin was observed for cutaneous reactions.

Because of the dyeing properties of the test substance, assessment of erythema was impossible. No edematous reactions were

observed. The researchers concluded that 4-amino-3-nitrophenol was not sensitizing by contact with guinea pig skin.[52]

3-Nitro-p-Hydroxyethylaminophenol. Institut Francais de Recherches et Essais Biologiques (IFREB)[53] studied the skin sensitization potential of 3-nitro-p-hydroxyethylaminophenol using Hartley albino guinea pigs (10 males and 10 females). In the induction phase, the guinea pigs were treated with 10 topical applications of 0.5 mL of pure 3-nitro-p-hydroxyethylaminophenol over the course of 24 days. The topical applications consisted of an occlusive patch behind the right shoulder blade on clipped skin. On days 1 and 10, the guinea pigs also received intradermal injections of 0.1 mL of FCA diluted to 50%.

The challenge phase began on day 35 of the study with application of 0.5 mL of pure test material to the left flank with an occlusive patch. The patch was removed after 48 hours. The skin was observed for effects at 1, 6, 24, and 48 hours after patch removal. Because of the dyeing properties of the substance, erythema could not be scored. The researchers determined that 3-nitro-p-hydroxyethylaminophenol did not produce any sensitization in the guinea pigs.[53]

CIT[54] conducted an evaluation of the skin sensitization potential of 3-nitro-p-hydroxy-ethylaminophenol. A range-finding test to define dose concentrations preceded the main experiment, which consisted of 2 parts. In each part, 28 female CBA/J mice were divided into 7 groups, and 5 of the groups were treated whereas 2 groups received control treatments (negative and positive).

In the first part, the mice were treated with concentrations of 2.5%, 5%, 10%, 25%, or 50% 3-nitro-p-hydroxyethylaminophenol. The concentrations for the second part were based on the positive results observed in the first part: 0.003%, 0.09%, 0.28%, 0.83%, and 2.5%. In each part of the experiment, the test material was applied to the dorsal surface of the mouse ears (25 µL per ear) on days 1, 2, and 3 of the experiment. Following 2 days of rest, tritiated methyl thymidine was injected into the tail vein of the mice on day 6 and the animals were killed 5 hours later. Proliferation of the cells in the lymph node draining the application site was then measured, and the values were used to calculate the SI. Ear thickness was assessed on days 1 to 3 and day 6 (after sacrifice).

No mortality or clinical signs were observed in the mice during either part of the experiment. Because of red coloring properties of the test material, erythema could not be determined. No thickening of the ears was observed. It was determined that 3-nitro-p-hydroxyethylaminophenol was not a local irritant. However, in both parts of the experiment, positive lymphoproliferation responses were recorded for all test concentrations (SI > 3), with a dose-related SI observed in the second part. The threshold value was exceeded at greater than 0.09%. An EC3 theoretical concentration of 0.07% was calculated. Chemicals that at 1 or more test concentrations provoke a 3-fold or greater lymph node cell proliferation compared with vehicle controls are classified as potential contact allergens. The study concluded that 3-nitro-p-hydroxyethylaminophenol has an extreme contact sensitization potential in mice.[54]

4-Hydroxypropylamino-3-Nitrophenol. A skin sensitization test was conducted using 40 Pirbright Bor:DHPW(SPF) albino guinea pigs treated with a 10% solution of 4-hydroxypropylamino-3-nitrophenol followed by 10% solution along with FCA.[23] A second exposure of undiluted 4-hydroxypropylamino-3-nitrophenol was followed by a final exposure of either 0.0005% or 3% 4-hydroxypropylamino-3-nitrophenol in a patch. Neither erythema nor edema was found in animals exposed to 0.0005% patch. However, at 3% concentration of 4-hydroxypropylamino-3-nitrophenol, skin coloration was observed and no results could be obtained. The researchers concluded that 4-hydroxypropylamino-3-nitrophenol was not a sensitizing compound.

Reproductive and Developmental Toxicity

2-Amino-4-Nitrophenol, 2-Amino-5-Nitrophenol. Burnett et al[24] reported on a study in which groups of 20 male Charles River CD rats (number of groups not given) were intraperitoneally administered with 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, or 4-amino-2-nitrophenol 3 times a week for 8 weeks at a dose of 20 mg/kg.[24] A group of control rats (40) was dosed with sterile water. After the 8-week dose period, the males were paired with 2 sexually mature female rats for 5 days. The females were then housed individually and euthanized 17 days later. The females were examined for the numbers of dead and live fetuses, and implantation and resorption sites were recorded.

No significant findings were reported for body weight gains, percentage of resorptions per litter, or total live fetuses compared with the controls.[24]

2-Amino-5-Nitrophenol, 4-Amino-2-Nitrophenol. Burnett et al[41] used 320 Charles River CD rats to investigate hair dyes for possible teratogenic effects. The rats were divided into 16 groups; 12 groups were topically dosed with the hair dyes (in 6% hydrogen peroxide), 1 group received acetylsalicylic acid (positive control) by gavage at a dose of 250 mg/kg on gestation days 6 through 16, and 3 groups functioned as untreated controls. One hair dye formulation contained 0.4% 2-amino-4-nitrophenol, 1 contained 0.5% 2-amino-5-nitrophenol, and another contained 0.3% 4-amino-2-nitrophenol. Hair dye was applied on days 1, 4, 7, 10, 13, and 19 of gestation. All rats were killed on gestation day 20, and caesarean sections were performed. Fetal examinations were made for visceral and skeletal anomalies.

Except for the positive control group, no signs of toxicity were seen in any of the pregnant rats or their fetuses. The administration of hair dye formulations every third day of gestation does not produce any embryotoxic or teratogenic effects.[41]

2-Amino-3-Nitrophenol. A preliminary study by CIT[55] assessed the possible embryonic and teratogenic effects of 2-amino-3-nitrophenol by oral exposure using pregnant Sprague-Dawley rats. The compound was suspended in 0.5% carboxymethylcellulose and was administered daily to 3 groups of 7 females from days 6 to 15 of pregnancy at doses of 100, 300, or 1000 mg/kg/d (total volume was 10 mL/kg/d). An additional group of 7 females was given the vehicle alone as the control. Animals were observed daily for clinical signs and mortality. On day 20, the dams were killed and the fetuses were delivered via caesarian section. Live fetuses were weighed and externally examined. The dams were examined for numbers of corpora lutea, resorptions, viable fetuses, and implantation sites.

One female in the 300-mg/kg/d dose group aborted on day 14. In the 1000-mg/kg/d dose group, mean maternal food consumption was significantly lower than the control group from day 6 to day 9 ($P < .05$). The mean body weight of the live fetuses in the 1000-mg/kg/d group was slightly lower than the control mean weights. This preliminary study concluded that 2-amino-3-nitrophenol was not maternotoxic, embryotoxic, or teratogenic.[55]

In a follow-up assessment by CIT,[56] 3 groups of 25 pregnant Sprague-Dawley rats received 2-amino-3-nitrophenol in doses of 100, 300, or 1000 mg/kg/d during days 6 through 15 of gestation. An additional group of 25 received 0.5% carboxymethylcellulose vehicle as the control. On day 20 of gestation, the dams were killed and the fetuses were delivered by caesarian section. The dams were observed for numbers of corpora lutea, resorptions, live and dead fetuses, and implantation sites. Half of the live fetuses in the first 20 litters were submitted for skeletal examination after staining with Alizarin red S. The remaining fetuses were examined for soft tissue abnormalities using Wilson's technique.

All the dams receiving test material presented lemon- or orange-colored urine from days 7 to 16. No maternal deaths occurred. One non-treatment-related abortion occurred in the 300-mg/kg/d dose group. In the 1000-mg/kg/d dose group, body weight of the dams was slightly lower than controls on days 6 to 9 as was food consumption on days 6 to 12. Food consumption was also slightly lower than controls in the 300 mg/kg/d during days 6 to 9. No macroscopic changes were observed in the dams. 2-Amino-3-nitrophenol had very slight toxicity in maternal rats at 1000 mg/kg/d, and the NOAEL was determined to be 300 mg/kg/d.

No treatment-related variations were observed in the litters, and no external abnormalities or skeletal variations were noted in the fetuses. Ventricular septal defects were observed in 2 fetuses from the 100-mg/kg/d dose group and in 1 fetus in the 300-mg/kg/d group. Two fetuses from the 300-mg/kg/d group had bilateral dilation of cerebral ventricles. These soft-tissue defects were not considered to be dose related. It was concluded that 2-amino-3-nitrophenol was not embryotoxic or teratogenic and the NOAEL was 1000 mg/kg/d.[56]

2-Amino-4-Nitrophenol and 2-Amino-5-Nitrophenol. Burnett and Goldenthal[57] performed a multigeneration reproductive study using 360 (each sex) Sprague-Dawley rats. The rats were randomly assigned to 9 groups: 3 control groups and 6 test groups. The test groups were dermally dosed with various hair dyes. The hair dye used to dose group 8 contained 0.4% 2-amino-4-nitrophenol, and group 9 was dosed with a hair dye that contained 0.5% 2-amino-5-nitrophenol. Dosing consisted of twice weekly applications of 0.5 mL of the hair dye mixed with 6% hydrogen peroxide to the back of the rats (hair had been clipped short). Dosing began 100 days prior to mating and continued to day 21 of lactation.

The F_{1a} generation was reduced to 60 rats per sex per group on lactation day 21 and used for a 2-year carcinogenicity study. The F_0 (parents) were reduced to 20 rats per sex per group and bred again to produce an F_{1b} generation. The F_{1b} generation was dosed and bred in the same manner as the F_0 generation to produce an F_2 generation. The F_2 generation was also bred and dosed but a viral infection made the data unusable.

The general health of all generations was not affected by the hair dyes. Some skin irritation (mild dermatitis) was seen intermittently throughout the study. The authors concluded that frequent topical application of hair dyes does not appear to have an adverse effect on reproductive performance or to have teratologic effects because for each generation, fertility, gestation, live birth indices, mean numbers weaned, and mean weaning weight were comparable between the test and control groups.[57]

2-Amino-4-Nitrophenol. Wernick et al[42] created a composite (15 dyes and 10 ingredients for the "base") of various chemicals that are found in commercially available semipermanent hair dyes. The authors used the highest concentration of each of the various chemicals found in hair dyes. 2-Amino-4-nitrophenol was added to the composite at a concentration of 0.05%. The composite was used in a fertility, reproductive, and teratology study in rats and a teratology study in rabbits.

Rats. The composite was mixed with the basal feed of Sprague-Dawley CD rats at concentrations of 0, 1950, and 7800 ppm. Sixty male and 120 females were divided into 6 groups (10 males per group; 20 females per group). The study was divided into 2 parts. In the first part of the study, the females were fed the basal diet only, whereas the males received the test diet for 8 weeks prior to mating and during the mating period. In the second part of the study, the females were fed the test diet 8 weeks prior to mating, during mating, during gestation, and for 21 days of lactation, and the males were fed the basal diet. In both parts, the test diet was removed when the males were present for mating.

One pregnant female was euthanized on gestation day 13 for evaluation of pregnancy, whereas all other females were allowed to deliver normally. After 21 days of lactation, all pups were euthanized. None of the male or female parents exhibited any dose-related significant difference from the controls. Parameters examined included male and female fertility, length of

gestation, numbers of females with resorption sites, live pups per litter, pup weights, and pup survival. The rats did excrete blue-brown colored urine.

These authors also tested 60 male and 60 female CFE-S rats; they were divided into 3 groups, and the males were mated to the females. Beginning on gestation day 6, the females were fed the composite (incorporated into their feed) at concentrations of 0, 1950, and 7800 ppm. Feeding of the test material continued to gestation day 15. All females were euthanized on gestation day 19.

No dose-related significant differences were observed in the parameters examined. There was 1 grossly abnormal pup in the control group and the 7800 ppm group; however, the average numbers of implantation sites, live pups, and early or late resorptions were not significantly different among the groups. The high-dose group excreted blue-brown colored urine.

Rabbits. The rabbit teratology study involved 48 female New Zealand White rabbits that had been artificially inseminated and allotted to 4 groups of 12. The rabbits received the composite (19.5 or 97.5 mg/kg/d), composite without 2-amino-4-nitrophenol (97.5 mg/kg/d), or vehicle (0.5% aqueous methylcellulose) on days 6 to 18 of gestation. All rabbits were euthanized on day 30 of gestation, and the number of pregnancies, average fetal weights, maternal weight gains, and numbers of corpora lutea, implantations, resorptions, and live and stillborn fetuses were recorded.

At study completion, none of the groups had any evidence of a teratologic effect, and fetal survival was not adversely affected by the dye composite. The high-dose group excreted blue-brown colored urine.[42]

4-Amino-3-Nitrophenol. The effects of 4-amino-3-nitrophenol on rat embryonic and fetal development during days 6 to 15 of gestation were investigated by Toxicol Laboratories.[58] Three groups of 5 mated female OFA-SD (IOPS Caw) strain rats were dosed once daily orally with suspensions of 4-amino-3-nitrophenol. Doses were 0, 100, or 300 mg/kg/d and the dose volume was 10 mL/kg body weight. The vehicle control was 0.5% wt/vol carboxymethylcellulose. Clinical signs, body weights, and food consumption were recorded. On day 20, the rats were killed and necropsy was performed. The fetuses were examined externally.

No effects were observed with regards to body weight, food consumption, or gestation in the female rats. No mortalities occurred. Also, no effects were observed in the fetuses. It was concluded that 4-amino-3-nitrophenol was not maternal toxic or teratogenic in rats.[58]

Another rat embryonic and fetal development study by Toxicol Laboratories[59] was performed using 3 groups of 24 mated female Sprague-Dawley rats that were dosed once a day by oral gavage at doses of 100, 250, or 600 mg/kg/d at a total dose volume of 20 mL/kg on days 6 to 15 of gestation. A control group of 24 females was dosed with the 0.5% aqueous carboxymethylcellulose vehicle. Clinical signs, body weight, and food consumption were recorded. All rats were killed on day 20 of gestation and necropsied. Litter parameters were noted and fetuses were examined for visceral and skeletal abnormalities.

Two females in the 600-mg/kg/d dose group were found dead after day 7 of gestation. No clinical signs were observed prior to death and the cause of death was not determined although the treatment could not be ruled out. Remaining rats in all dose groups had no treatment-related changes with the exception of yellow-orange staining of fur and urine.

Maternal body weight gain was significantly decreased ($P < .05$) in the 250- and 600-mg/kg/d dose groups after the first dosing. The 250-mg/kg/d group was similar to controls by day 10 of gestation, but the 600-mg/kg/d group saw further significant decreases ($P < .01$) in body weight gain. No effects on maternal food consumption or macroscopic abnormalities were observed in the females.

Litter parameters were within normal ranges. The fetuses did not have any evidence of treatment-related abnormalities with the exception of a dose-related increase in skeletal variance in unilateral or bilateral vestigial 14th rib in all dose groups. It was concluded that maternal toxicity occurred at 600 mg/kg/d, and although a slight transient effect of development in fetuses occurred at all dose levels, 4-amino-3-nitrophenol did not induce teratogenicity, embryolethality, or embryonic growth retardation in rats.[59]

In a follow-up developmental study by CIT,[60] pregnant female Sprague-Dawley rats received 4-amino-3-nitrophenol through daily oral gavage on days 6 to 19 of gestation. Groups consisted of 24 rats that received the material at doses of 5, 20, or 400 mg/kg/d or the 0.5% carboxymethylcellulose vehicle. Clinical signs were checked daily, and body weight gain and food consumption were recorded. On day 20, the females were killed and necropsied. Litter parameters were measured and the fetuses were examined for external, visceral, or skeletal abnormalities.

No mortality was observed during the treatment period. Three rats in the 400-mg/kg/d group exhibited ptyalism. No significant effects were observed in the litter parameters or in the fetuses at any dose. The NOAEL for maternal and prenatal exposure to 4-amino-3-nitrophenol was determined to be 400 mg/kg/d.[60]

3-Nitro-p-Hydroxyethylaminophenol. The effects of 3-nitro-p-hydroxyethylaminophenol on female rats and their offspring was studied by CIT.[34,61,62] Sprague-Dawley dams were treated orally with the test material suspended in 0.5% aqueous carboxymethylcellulose from days 6 to 15 of gestation. Two groups of 25 mated females received either 100 or 1000 mg/kg/d at a volume of 10 mL/kg/d. During gestation, the females were observed for clinical signs, and body weight gains and food consumption rates were recorded. On day 20, all dams were killed and the fetuses were delivered by caesarian section. Numbers of corpora lutea, implantation sites, resorptions, and viable fetuses were determined. Live fetuses were weighed and submitted to external, skeletal, and visceral examinations.

No treatment-related effects were observed with regard to body weight gain, food consumption, mortality, or abortion. In the 100-mg/kg/d dose group, the numbers of corpora lutea, implantations, viable offspring, and postimplantation loss were similar to the controls. Fetal weight in this group was also comparable.

In the 1000-mg/kg/d dose group, the number of viable fetuses was slightly decreased ($P < .05$) and the postimplantation loss was slightly increased ($P < .05$) compared with the controls. No treatment-related fetal anomalies or malformations were observed in the 100-mg/kg/d dose group. The 1000-mg/kg/d dose group had 2 fetuses that were initially identified as having malformations (external astomia and polydactyly).

It was concluded that 3-nitro-p-hydroxyethylaminophenol was not maternotoxic at doses of 100 and 1000 mg/kg/d, nor was it embryotoxic or teratogenic at 100 mg/kg/d. At 1000 mg/kg/d, 3-nitro-p-hydroxyethylaminophenol was slightly embryotoxic and 2 fetuses had malformations; however, upon re-review, CIT found that the polydactyly occurrence could not be confirmed and that the carpal changes were most likely an artifactual event.[34,61,62]

4-Hydroxypropylamino-3-Nitrophenol. Female Wistar-derived SPF-albino rats were mated with males of the same strain.[23] Pregnant females received 10, 30, or 90 mg/kg 4-hydroxypropylamino-3-nitrophenol in 0.5% sodium carboxymethyl cellulose once daily by oral gavage between 5 and 15 days of gestation. At day 20 of gestation, animals were killed and necropsied. After removal of the uterus, the investigators determined the number of live/dead fetuses, early/late resorptions, placentas, implantation sites, and corpora lutea for each ovary and placentas plus birth position and uteri weights. The fetuses were individually weighed, sexed, and examined grossly for externally visible deviations. One-third of fetuses were checked for organ defects by an evaluation of organ slices, and two-thirds were evaluated for skeletal defects after staining with Alizarin red.

No adverse effects on mothers, reproduction, or fetal development were observed, and the authors concluded that the dose of 90 mg/kg exhibited no effects.[23]

Genotoxicity

Available genotoxicity data are summarized in Table 4.

Shahin et al[63] used *Salmonella typhimurium* strains to detect the mutagenicity of 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-3-nitrophenol. The positive control used was 1,2-diamino-4-nitrobenzene.

2-Amino-4-nitrophenol was mutagenic with and without activation in *S typhimurium* strains TA1538 and TA98. 2-Amino-5-nitrophenol was mutagenic with or without activation for TA1537, TA1538, and TA98 and mutagenic without activation in strains TA1535 and TA100. 4-Amino-3-nitrophenol was not mutagenic.[63]

Shahin[64] tested 2-amino-3-nitrophenol, 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, and 4-amino-3-nitrophenol using *S typhimurium* strains. Positive controls used were 1,2-diamino-4-nitrobenzene and 2-aminoanthracene.

2-Amino-3-nitrophenol, 4-amino-2-nitrophenol, and 4-amino-3-nitrophenol were not mutagenic in any of the strains used in the test either with or without metabolic activation. 2-Amino-4-nitrophenol was mutagenic in stains TA1538 and TA98 with or without S9 activation. [64]

Zeiger et al[65] used the *Salmonella* mutagenicity assay to investigate 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, and 4-amino-2-nitrophenol for possible mutagenic effects. *S typhimurium* strains TA1535 and TA100 were tested with the positive control of sodium azide, TA1537 and TA97 were tested with the positive control of 9-aminocridine, and TA98 was tested with 4-nitro-o-phenylenediamine.

All 3 hair dye ingredients tested positive in all strains.[65]

2-Amino-3-Nitrophenol. CIT[66] used the Ames test to evaluate the mutagenic potential of 2-amino-3-nitrophenol. After a range-finding experiment, the strains were subjected to 5 different doses of 2-amino-3-nitrophenol (in DMSO) in 2 tests. The dose levels in first test were 125, 250, 500, 1000, and 1500 μg per plate for all strains. In the second experiment, strains TA 1535 and WP2uvrA were dosed with 125, 250, 500, 1000, or 1500 μg per plate; strains TA1537 and TA100 were dosed with 31.25, 62.5, 125, 250, or 500 μg per plate; and strain TA98 was dosed with 7.8125, 15.625, 31.25, 62.5, or 125 μg per plate.

All 4 of the *S typhimurium* strains had significant increases in the number of revertants in both parts of the tests, with and without metabolic activation. No increases were observed in the *Escherichia coli* strain. The researchers concluded that 2-amino-3-nitrophenol induced mutagenic activity.[66]

Covance Laboratories[67] used another Ames test to assay 2-amino-3-nitrophenol (in DMSO) for mutation. The experiment was divided into 2 parts: experiment 1 treated all strains with doses of 1.6, 8, 40, 200, 1000, or 5000 μg per plate, and experiment 2, which narrowed the dose interval, treated all strains with doses of 78.125, 156.25, 312.5, 625, 1250, 2500, or 5000 μg per plate. Each dose was tested with and without metabolic activation.

In both parts of the experiment, strains TA98 and TA1537, with and without metabolic activation, had statistically significant, dose-related, and reproducible increases in revertant numbers. It was concluded that there was clear evidence of mutagenic activity in *Salmonella* strains TA98 and TA1537.[67]

Toxicol Laboratories[68] examined the ability of 2-amino-3-nitrophenol to cause chromosomal or spindle damage using a mouse micronucleus test. Groups of 30 CD-1 strain mice (15 males and 15 females) received a single, intraperitoneal injection of 125, 250, or 500 mg/kg 2-amino-3-nitrophenol. Another group of 30 animals received the 0.5% carboxymethylcellulose vehicle as a control. At 24, 48, and 72 hours after dosing, 5 animals of each sex from each dose group were killed and bone marrow from the mice was extracted and prepared for microscopic examination.

Table 4. Genotoxicity Studies

Assay, Strain Per Species, Dose	Results	Reference
2-amino-3-nitrophenol		
Bacterial studies		
Salmonella typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Negative	Shahin[64] 1985
S typhimurium strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* WP2uvrA; 0-1500 μg/plate in DMSO	Positive with or without S9 in Salmonella strains; negative in *E coli*	CIT[66] 1994
S typhimurium strains TA98, TA100, TA1535, TA1537, TA102; 0-5000 μg/plate in DMSO	Positive with or without S9 in TA98 and TA1537	Covance Laboratories[67] 2003
Mammalian cell studies		
L5178Y mouse lymphoma cell mutation assay (*tk* locus); 0-525 μg/mL	Positive without S9 in doses 75-525 μg/mL	Covance Laboratories[69] 2003
Syrian hamster embryo cells; 0-15.9 μg/mL in DMSO	Positive	Covance Laboratories[70] 2003
Animal studies		
Mouse micronucleus test; CD-1 male and female mice; 0-500 mg/kg single intraperitoneal injection	Negative	Toxicol Laboratories[68] 1992
DNA damage assay; Sprague-Dawley male rats; 0-2000 mg/kg orally once daily for 2 d	Negative	Integrated Laboratory Systems[71] 2004
2-amino-4-nitrophenol		
Bacterial studies		
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; doses not reported	Positive with S9 in TA1538	Ames et al[72] 1975
S typhimurium strains TA1537, 1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Positive with or without S9 in TA1538 and TA98	Shahin et al[63] 1982
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Positive with or without S9 in TA1538 and TA98	Shahin et al[64] 1985
S typhimurium strains TA98, TA100, TA1535, TA1537, TA97; 0-1333 μg/plate in 95% ethanol and DMSO	Positive with or without S9 in all strains	Zeiger et al[65] 1987
Mammalian cell studies		
L5178Y mouse lymphoma cell mutation assay: 0-300 μg/nL in 1% DMSO	Positive with and without S9	Myhr et al[74] 1990
Chromosomal aberration assay using Chinese hamster ovary cells; 0-2999 μg/mL in DMSO	Weakly positive without S9; positive with S9	Anderson et al[73] 1990
Sister chromatid exchange assay using Chinese hamster ovary cells; 0-2670 μg/mL in DMSO	Positive with or without S9	Anderson et al[73] 1990
Animal studies		
Micronucleus assay using CFY strain mice; 5000 mg/kg in suspension of 0.5% (wt/vol) gum tragacanth containing 0.05% (wt/vol) sodium sulfite over 24 h, oral in 2 doses	Negative	Hossack and Richardson[124] 1977
Intraperitoneal injection of bone marrow and Ehrlich ascites tumor cells in CFW mice; 28.54 and 142.7 mg/kg in 0.1 mL DMSO	No significant damage	Mikstacki 1985[76]
Dominant lethal study using Charles River CD rats; intraperitoneal injection 20 mg/kg, 3 times/wk for 8 wk	No significant findings	Burnett et al[24] 1977
2-amino-5-nitrophenol		
Bacterial studies		
S typhimurium strain TA1538; dose not reported	Positive with S9	Ames et al[72] 1975
S typhimurium strains TA100 and TA98; 0-10 μmol in DMSO	Positive with or without S9 in TA98	Chiu et al[77] 1978
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Positive with or without S9 in TA1537, TA1538, TA98; positive without S9 in TA1535 and TA100	Shahin et al[63] 1982
S typhimurium strains TA98, TA100, TA1535, TA1537, TA97; 0-10 000 μg/plate in DMSO	Positive with or without S9 in all strains	Zeiger et al[65] 1987
S typhimurium strains TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, mixture of all six, TA98, TA100, TA1537; 1-100 μg/mL in 2% DMSO, final volume 0.5 mL	Positive with or without S9 in TA98	Gee et al[79] 1998

(continued)

Table 4 (continued)

Assay, Strain Per Species, Dose	Results	Reference
Mammalian cell studies		
L5178Y mouse lymphoma cell mutation assay; 0-300 μg/mL in 1% DMSO	Positive with or without S9	Myhr et al[74] 1990
Chromosomal aberration assay using Chinese hamster ovary cells; 0-1000 μg/mL in DMSO	Positive with or without S9	Anderson et al[73] 1990
Sister chromatid exchange assay using Chinese hamster ovary cells; 0-1240 μg/mL in DMSO	Positive with or without S9	Anderson et al[73] 1990
Unscheduled DNA synthesis using nonhuman fibroblasts; doses not reported	Positive	Stich et al[78] 1981
Animal studies		
Dominant lethal study using Charles River CD rats; intraperitoneal injection 20 mg/kg, 3 times/wk for 8 wk	No significant findings	Burnett et al[24] 1977
4-amino-2-nitrophenol		
Bacterial studies		
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Negative	Shahin et al[64] 1985
S typhimurium strains TA98, TA100, TA1537, TA1538, TA1535; 0-3333 μg/plate in DMSO	Positive with or without S9 in TA98, TA100, TA1537, TA1538	Dunkel and Simon[80] 1980
S typhimurium strains TA98, TA1538, TA100, TA1537; 0-5000 μg/plate in DMSO, technical and synthesized grades	Technical grade genotoxic with or without S9 in TA98 and TA1538; technical grade weakly mutagenic with or without S9 in TA100 and TA1537; synthesized material was negative	Shahin et al[81] 1982
S typhimurium strains TA98, TA100, TA1535, TA1537, TA97; 0-1000 μg/plate in DMSO	Positive with or without S9 in all strains	Zeiger et al[65] 1987
Mammalian cell studies		
Rauscher leukemia virus–infected rat embryos in vitro system; 0-17 μg/mL	Positive at 6.2-17 μg/mL; transformation at 11 μg/mL	Heidelberger[82] 1983
L5178Y mouse lymphoma cell mutation assay; 0-16 μg/mL in DMSO	Positive with or without S9	Mitchell et al[83] 1988
L5178Y mouse lymphoma cell mutation assay; 0-30 μg/mL in DMSO	Positive without S9 at 10-15 μg/mL; positive with S9 at 20 μg/mL; toxic at 30 μg/mL	Myhr and Caspary[125] 1988
Animal studies		
Dominant lethal study using Charles River CD rats; 20 mg/kg, 3 times/wk for 8 wk intraperitoneal injection	No significant findings	Burnett et al[24] 1977
4-amino-3-nitrophenol		
Bacterial studies		
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Negative	Shahin et al[63] 1982a
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Negative	Shahin et al[64] 1985
S typhimurium strains TA1537, TA1538, TA98, TA1535, TA100; 0-1000 μg/plate in DMSO	Negative	Lequesne and Mayet[86] 1978
S typhimurium strains TA1535, TA1537, TA98, TA100, TA102; 0-5000 μg/plate in DMSO	Positive with or without S9 in TA98	CIT[85] 2004
Mammalian cell studies		
Chromosomal aberration assay using Chinese hamster ovary cells; 0-0.02 mg/mL in DMSO	Negative	Darroudi[87] 1981
Human lymphocyte micronucleus assay (chromosome aberration); 0-10 mM	Positive at 48 h post mitogen with S9	CIT[88] 2004
Human lymphocyte micronucleus assay (chromosome aberration); 0-1540 μg/mL in DMSO	Positive at 48 h post mitogen with S9	Covance Laboratories[89] 2004
Animal studies		
Bone marrow micronucleus assay using Crl:CD(SD)BR rats; 0-2000 mg/kg single dose by oral gavage	Negative	Covance Laboratories[90] 2005
Bone marrow micronucleus assay using male Swiss mice; 0-300 mg/kg single intraperitoneal injection	Negative	Darroudi[91] 1982

(continued)

Table 4 (continued)

Assay, Strain Per Species, Dose	Results	Reference
3-nitro-p-hydroxyethylaminophenol		
Bacterial studies		
S typhimurium strains TA98, TA100, TA1535, TA1537, TA102; 0-5000 μg/plate in DMSO	Negative	CIT[92] 2004
S typhimurium strains TA98, TA100, TA1535, TA1537, TA1538; 0-1000 μg/plate in DMSO	Negative	Shahin[93] 1980
S typhimurium strains TA98, TA100, TA1535, TA1537, TA1538; 0-500 μg/plate in DMSO	Negative	Litton Bionetics[126] 1978
Mammalian cell studies		
Chromosomal aberration assay using Chinese hamster ovary cells; 0-0.4 mg/mL in DMSO	Negative	Darroudi[99] 1982
L5178Y mouse lymphoma cell mutation assay (*tk* locus); 0-10 mM	Positive with or without S9	CIT[94] 2004
Human lymphocyte micronucleus assay (chromosome aberration); 0-1980 μg/mL in DMSO	Positive for 48 h post mitogen stimulation with or without S9	Covance Laboratories[95] 2005
Animal Studies		
Bone marrow micronucleus assay using Crl:CD(SD)BR rats; 0-2000 mg/kg single dose by oral gavage	Negative	Covance Laboratories[96] 2005
Bone marrow micronucleus assay using male Swiss mice; 0-300 mg/kg single intraperitoneal injection	Negative	Darroudi[97] 1982
Bone marrow micronucleus assay using CD-1 mice; 0-10,000 mg/kg body weight, 2 oral doses separated by 24 h	Negative	Huntingdon Research Centre[98] 1980
4-hydroxypropylamino-3-nitrophenol		
Bacterial studies		
S typhimurium strains TA98, TA100, TA1535, TA1537, TA1538; 0-1000 μg/plate	Negative	Shahin et al[100] 1983
S typhimurium strains TA97, TA98, TA100; 1-10,000 μg/plate	Negative	Colipa[23] 1994
Repair deficient and repair competent (DNA repair test) using *E coli*; 4.88-5000 μg/mL	Negative	Colipa[23] 1994
Mammalian cell studies		
Human peripheral lymphocyte assay (chromosome aberration); 50-400 μg/mL	Negative	Colipa[23] 1994
Animal studies		
Micronucleus test using CFLP male and female mice; 1000 mg/kg single intraperitoneal injection	Negative	Colipa[23] 1994
Micronucleus test using Crl:NMRI BR male and female mice; 700-mg/kg single oral dose	Negative	Colipa[23] 1994
Unscheduled DNA synthesis using Wistar/WU rats; 2000 mg/kg for 2 h; 200 and 20,000 mg/kg for 16 h, oral	Negative	Colipa[23] 1994

DMSO, dimethyl sulfoxide; *tk*, thymidine kinase;

For a positive control, 10 mice were dosed with mitomycin C and killed after 24 hours for marrow extraction.

Bone marrow toxicity was not observed in the mice dosed with 2-amino-3-nitrophenol or the vehicle. There were no incidences of micronuclei in the dosed groups at 24, 48, or 72 hours after injection. A statistically significant increase occurred in 4 male animals at 24 hours with 0.8 micronuclei per animal; however, this incidence was not considered evidence of clastogenic activity. In this study, 2-amino-3-nitrophenol did not induce micronuclei in bone marrow up to 500 mg/kg.[68]

An assay studying the ability of 2-amino-3-nitrophenol to induce mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells was performed by Covance Laboratories[69] using the microtiter fluctuation technique. Following a range-finding test, 2 separate experiments were performed with and without S9 metabolic activation.

In the first experiment, lymphoma cells incubated without metabolic activity were dosed with 7 different concentrations of test material ranging from 37.5 to 425 μg/mL whereas cells with metabolic activation were dosed with 9 different concentrations of material ranging from 1 to 10 μg/mL. The highest concentrations with and without metabolic activation yielded 2% and 23% relative survival, respectively.

In the second experiment, the concentrations without S9 ranged from 37.5 to 525 μg/mL and the concentrations with S9 ranged from 0.125 to 10 μg/mL. The highest concentrations with and without metabolic activation yielded 22% and 14% relative survival, respectively. No statistically significant increases in mutant frequency from 2-amino-3-nitrophenol treatment occurred at any concentration with metabolic activation in either experiment, whereas statistically significant mutant frequencies were observed in the dose range 75 to

525 µg/mL without activation in both experiments (a linear trend was noted).

The test substance 2-amino-3-nitrophenol was considered mutagenic in the *tk* locus of L5178Y mouse lymphoma cells in absence of metabolic activation.[69]

Covance Laboratories[70] tested 2-amino-3-nitrophenol for the ability to induce morphological transformation using Syrian hamster embryo cells. Doses incubated in the continuous 7-day exposure ranged from 5 to 20 µg/mL after an initial range-finding experiment. The DMSO vehicle and benzo(a)-pyrene were incubated for control measurements. Plates dosed with 5, 7.5, 10, 12.5, and 15.9 µg/mL showed reduction in relative plate efficiency, and the decrease was dose dependent, ranging from 96 to 37%. These doses had significant increases in frequency of morphological transformation and were comparable to the positive controls.

The potential for 2-amino-3-nitrophenol to induce DNA damage in the cells of rat organs was examined by Integrated Laboratory Systems.[71] Male Sprague-Dawley rats (groups of 6) were treated by oral gavage once daily for 2 consecutive days with doses 500, 1000, or 2000 mg/kg body weight of the test compound. Another 2 groups of 6 received solvent and ethylmethane sulfonate as negative and positive controls, respectively.

The group mean percentage of cells with low molecular weight DNA was not significantly increased in any tissue in any of the dose groups. Liver cells in the 1000-mg/kg dose group showed a marginally significant increase in DNA migration but did not have a dose-response relationship and were not considered biologically relevant. The researchers determined that 2-amino-3-nitrophenol did not induce DNA damage in this study system.[71]

2-Amino-4-Nitrophenol and 2-Amino-5-Nitrophenol. Ames et al[72] used *S typhimurium* strain TA1538 to test 2-amino-4-nitrophenol and 2-amino-5-nitrophenol (concentration not given) for mutagenicity. Both compounds were mutagenic in the TA1538 strain but required activation with S9.

Anderson et al[73] tested 2-amino-4-nitrophenol and 2-amino-5-nitrophenol for the ability to induce chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells (with and without metabolic activation). Two positive controls were used: mitomycin C and cyclophosphamide.

2-Amino-4-nitrophenol tested positive in the sister chromatid exchange assay both with and without metabolic activation. In the chromosome aberration assay, 2-amino-4-nitrophenol was weakly positive without metabolic activation and positive with metabolic activation. 2-Amino-5-nitrophenol was positive in both the sister chromatid exchange assay and the chromosome aberration assay, with and without metabolic activation.[73]

Myhr et al[74] reported that 2 hair dye ingredients, 2-amino-4-nitrophenol and 2-amino-5-nitrophenol, tested positive in the L5178Y mouse lymphoma cell mutation assay. Tests were performed with and without S9 at concentrations of 25, 50, 100, 150, 200, and 300 mg/mL; a solvent control of 1% DMSO was included. The concentrations of 2-amino-4-nitrophenol (in DMSO) ranged from 0 to 2999 mg/mL in the aberration assay and 0 to 2670 mg/mL in the sister chromatid exchange assay. The concentration range for 2-amino-5-nitrophenol (in DMSO) was 0 to 1000 mg/mL for the aberration assay and 0 to 1240 mg/mL for the sister chromatid exchange assay.

2-Amino-4-Nitrophenol. Hossack and Richardson[75] reported that 2-amino-4-nitrophenol was not mutagenic in a micronucleus test. Rats of the CFY strain (5 males and 5 females) orally received 2-amino-4-nitrophenol in a suspension of 0.5% (wt/vol) gum tragacanth containing 0.05% (wt/vol) sodium sulfite as 2 equal doses over a 24-hour period. The total dosage over the 24-hour period was 5000 mg/kg. A control group was dosed with the vehicle only. Six hours after the second dose, the animals were euthanized and bone marrow smears were prepared from their femurs to allow determination of the incidence of micronucleated polychromatic erythrocytes. The values for the animals in the 2-amino-4-nitrophenol group were essentially the same as the control group, leading the authors to conclude there was no clear evidence of mutagenic potential.

Mikstacki[76] examined the mutagenicity of 2-amino-4-nitrophenol using the bone marrow of male CFW inbred mice (2 mice per dose and interval) and Ehrlich ascites tumor cells of mice in vivo. The mice were injected intraperitoneally with 0.4 mL of ascites tumor cells 1 day prior to dose administration with 2-amino-4-nitrophenol. Twenty-four hours after the administration of the tumor cells, the mice were intraperitoneally dosed with 2-amino-4-nitrophenol (mixed with 0.1 mL of DMSO). A positive control group was dosed with Trenimon dissolved in 0.9% NaCl. The negative control group was intraperitoneally dosed with 0.1 mL of DMSO. Animals were killed 6, 24, 48, and 72 hours after dose administration.

The positive control, 2,3,5,-triethylenoiminobenzochino-1,4, caused chromosomal damage in both the ascites tumor cells and the bone marrow cells. 2-Amino-4-nitrophenol did not cause any significant damage compared with the negative control.[76]

2-Amino-5-Nitrophenol. Chiu et al[77] tested 2-amino-5-nitrophenol for mutagenicity using *S typhimurium* strains TA98 and TA100. 2-Amino-5-nitrophenol was dissolved in DMSO at concentrations of 0.1, 1.0, and10.0 µmol. A control plate containing DMSO was also used. 2-Amino-5-nitrophenol tested positive in strain TA98 only without s9 activation.

Stich et al[78] tested 2-amino-5-nitrophenol using an unscheduled DNA synthesis (UDS) assay. In fibroblasts without metabolic activation, 2-amino-5-nitrophenol (concentration not given) tested positive.

Gee et al[79] evaluated 2-amino-5-nitrophenol (90% pure at concentrations ranging from 1 to 100 µg/mL) using different strains of *S typhimurium*. Positive controls used were N^4-aminocytidine, methyl methanesulfonate, streptonigrin, 4-nitroquinoline-N-oxide, and DMSO. In the strains TA7001 to TA7006 and TA1537, 2-amino-5-nitrophenol produced negative results;

however, in the TA98 strain, 2-amino-5-nitrophenol gave positive results in the absence of s9 activation.

4-Amino-2-Nitrophenol. Dunkel and Simon[80] tested 4-amino-2-nitrophenol using the *S typhimurium* assay. 4-Amino-2-nitrophenol was dissolved in DMSO. Positive controls used were 2-nitrofluorene (assay without activation) and 2-aminoanthracene (assay with activation). 4-Amino-2-nitrophenol was mutagenic in strains TA1537, TA1538, TA98, and TA100 with and without S9 activation.

Shahin et al[81] tested 2 forms of 4-amino-2-nitrophenol using the *S typhimurium* assay. One sample of 4-amino-2-nitrophenol was purchased as a technical grade sample (percentage purity not reported) from Aldrich-Europe; the other sample was synthesized and purified in the authors' laboratory.

The synthesized sample of 4-amino-2-nitrophenol tested nonmutagenic in all strains of *Salmonella*; however, the technical grade sample was mutagenic. The technical grade 4-amino-2-nitrophenol caused dose-dependent increases in mutation frequency in strains TA1538 and TA98, with and without metabolic activation. Technical grade 4-amino-2-nitrophenol was able to induce a weak mutagenic response in strains TA100 and TA1537. In TA1535, the response was negative. Further testing by the researchers suggests that contaminants in the technical grade sample may be responsible for the mutagenic effects.[81]

Heidelberger et al[82] reported that 4-amino-2-nitrophenol tested positive using the morphologic changes of Rauscher leukemia virus-infected rat embryos (RLV/RE) system at concentrations ranging from 6.2 to 17 μg/mL. The minimal concentration resulting in transformations was 11 μg/mL.

Mitchell et al[83] evaluated 4-amino-2-nitrophenol using the L5178Y mouse assay for mutagenicity. 4-Amino-2-nitrophenol at concentrations ranged from 0 to 16 μg/mL. 4-Amino-2-nitrophenol was mutagenic in L5178Y cells in both the absence and presence of induced S9. The concentrations of 6, 15, and 16 μg/mL were significantly mutagenic in both experiments.

Myhr and Caspary[84] reported that 4-amino-2-nitrophenol was mutagenic in the mouse lymphoma assay (L5178Y system) with and without mutagenic activation.

4-Amino-3-Nitrophenol. In a mutagenicity study performed by CIT,[85] *S typhimurium* strains were treated with 4-amino-3-nitrophenol dissolved in DMSO, with and without S9 metabolic activation, in 3 independent experiments. All but the second experiment with metabolic activation were performed using the direct plate incorporation method. The exception was performed under the preincubation method (60 minutes at 37°C). In the first and second experiments, all strains were treated with 312.5, 625, 1250, 2500, or 5000 μg per plate, with and without S9. In the third experiment, only strain TA98 was treated with 1000, 2000, 3333, 4000, or 5000 μg per plate, with and without S9. Strains were also tested with positive controls specific to strain and metabolic activity. The total treatment volume was 100 μL per plate. After 48 to 72 hours of incubation at 37°C, the revertants were scored.

No toxicity was noted in any of the dose levels or strains. Reproducible increases in the number of revertant colonies were observed in the TA98 strain, with and without S9, which indicate mutagenic activity.[85]

An Ames study using *S typhimurium* strains tested 4-amino-3-nitrophenol dissolved in DMSO at doses ranging 5 to 1000 μg per plate, with and without metabolic activation.[86] No mutagenic activity was observed.

The chromosome aberration potential for 4-amino-3-nitrophenol was evaluated using Chinese hamster ovary cells (in vitro).[87] Concentrations tested were 0.005, 0.01, and 0.02 mg/mL. DMSO served as the control. Increases of chromosomal aberration frequencies were comparable to control values following 6-, 12-, and 16-hour treatments.

CIT[88] tested 4-amino-3-nitrophenol for chromosome aberration potential using an in vitro micronucleus assay using human lymphocyte cultures. The study consisted of 2 separate experiments, both with and without S9 metabolic activation.

In the first experiment, the highest dose to be tested was determined by pH, osmolality, and solubility. In the second experiment, any toxicity that was indicated by a reduction of mitotic index (MI) in the first experiment was evaluated.

In the first experiment, the concentrations ranged from 0.08 to 10 mM, with and without metabolic activation.

Cultures treated with 4-amino-3-nitrophenol and metabolic activation had significant increases in frequency of cells with chromosomal aberrations that were reproducible.

The concentrations in the second experiment ranged from 0.128 to 2.5 mM, with and without metabolic activation.

Without S9 activation, a moderate to marked decrease in MI was observed at greater than 1.28 mM, and with S9 activation, a slight to marked decrease in MI was observed at greater than 1.02 mM in the second experiment. A significant increase in frequency of cells with chromosome aberrations was observed.

The researchers concluded that 4-amino-3-nitrophenol induced chromosome aberrations in cultured human lymphocytes with and without metabolic activation.[88]

Another study of the effects of 4-amino-3-nitrophenol on cultured human lymphocytes was performed by Covance Laboratories.[89] Using an in vitro micronucleus assay, duplicate lymphocyte cultures were exposed to the test material dissolved in DMSO, with and without S9 metabolic activation, in 2 independent experiments. Dose levels were selected by evaluating the effect of 4-amino-3-nitrophenol on the replication index (RI).

In the first experiment, cells were treated with 4-amino-3-nitrophenol 24 hours after phytohemagglutinin mitogen stimulation. Cells without S9 activation were treated for 20 hours and had a 28-hour recovery period prior to harvest. Exposure concentrations were 84.66, 132.3, 165.4, and 206.7 μg/mL. In cultures with S9 activation, the cells were treated with 788.5, 985.6, or 1232 μg/mL for 3 hours and had a 45-hour recovery period. The highest treatments with and without metabolic activation induced 62% and 69% reduction in RI, respectively.

The second experiment followed the same protocol as the first experiment except that cultures were treated 48 hours after

mitogen stimulation. The doses with S9 activation were 985.6, 1232, and 1540 μg/mL whereas the doses without S9 activation were 250.0, 300.0, and 450.0 μg/mL. The highest dose groups with and without metabolic activation induced 60% and 59% reduction in RI, respectively. The researchers concluded that 4-amino-3-nitrophenol induced micronuclei in lymphocytes 48 hours following mitogen stimulation and with S9 activation.[89]

Covance Laboratories[90] used a micronucleus assay to evaluate 4-amino-3-nitrophenol in vivo using Crl:CD (SD) BR rat bone marrow. Rats (5 per sex per dose group with an additional 5 per sex in the control and highest dose groups) received the test material via oral gavage at a single dose of 500, 1000, or 2000 mg/kg at a total dose volume of 10 mL/kg.

A vehicle control of 0.5% aqueous carboxymethylcellulose and a positive control of cyclophosphamide were included. Bone marrow was harvested from the rat femurs at 24 and 48 hours (for 2000-mg/kg dose group) to allow determination of the incidence of micronucleated polychromatic erythrocytes (PCEs). Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs).

Statistically significant increases in micronucleated PCEs were not induced by 4-amino-3-nitrophenol at any dose. Cytotoxicity occurred in bone marrow harvested at 48 hours in males of the 2000-mg/kg group.[90]

Another micronucleus assay for 4-amino-3-nitrophenol was performed using erythrocytes from male Swiss random-bred mice.[91] The test substance, dissolved in DMSO, was administered once through an intraperitoneal injection at doses of 37.5, 75, 150, or 300 mg/kg (2 mice per dose level). The mice were killed 24 or 48 hours after treatment, and the femurs were dissected for bone marrow study. An LD_{50} was observed at the 300-mg/kg dose level. No mutagenic activity was observed, but the researchers noted that the negative results could be due to detoxification within the mouse system or to not using a sufficiently high concentration because of lethality to the animal.

3-Nitro-p-Hydroxyethylaminophenol. An Ames test assayed 3-nitro-p-hydroxyethylaminophenol that had been dissolved in DMSO for mutation using *S typhimurium* strains.[92] Two independent experiments were conducted after an initial range-finding test: experiment 1 doses ranged from 78.13 to 5000 μg per plate and experiment 2 doses ranged from 19.53 to 5000 μg per plate (doses selected for each experiment tailored to each *Salmonella* strain). Each dose was tested with and without metabolic activation.

Moderate to marked toxicity was observed in each strain at differing doses in each metabolic category. Significant increases in revertant numbers were not observed in any of the strains, with or without metabolic activation, and the authors concluded that 3-nitro-p-hydroxyethylaminophenol was not mutagenic.[92]

Another Ames test assayed 3-nitro-p-hydroxyethylaminophenol (in DMSO) for mutation using doses ranging from 5 to 1000 μg per plate.[93] The number of revertants was similar to that in the controls, so it was determined that this material was not mutagenic.

An Ames test with 3-nitro-p-hydroxyethylaminophenol using *S typhimurium* strains tested lower doses (0.1, 1.0, 10, 100, or 500 μg per plate), with and without metabolic activation.[126] The initial test data exhibited unusually high spontaneous mutation, so the test was repeated in TA100 and TA1538 (with metabolic activation). Mutagenic activity was not indicated in either activation scenario.

CIT[94] assessed the ability of 3-nitro-p-hydroxyethylaminophenol to induce mutation at the *tk* locus of mouse lymphoma L5178Y cells. Following a preliminary range-finding test, 2 separate experiments were performed with and without S9 metabolic activation.

All doses with and without metabolic activation had moderate to marked toxicity in both experiments. Significant increases in mutation frequency were observed after the treatment period. The test substance 3-nitro-p-hydroxyethylaminophenol was determined to have clastogenic potential in the presence or absence of s9.[94]

Covance Laboratories[95] tested 3-nitro-p-hydroxyethylaminophenol using an in vitro micronucleus assay using duplicate human lymphocyte cultures. The study consisted of 2 separate experiments following a range-finding test, which determined the dose levels through an RI.

In the first experiment, the concentrations ranged from 55.73 to 1980 μg/mL. An RI reduction of 59% was recorded for cultures without metabolic activity. Cultures with metabolic activity had a RI reduction of 37%. The frequencies of micronucleated binucleate (MNBN) cells were not significantly different from the vehicle control and were within historical negative control ranges.

The concentrations in the second experiment ranged from 150 to 1980 μg/mL. The RI reduction was 65% without activation and 11% with activation. The frequencies of MNBN cells were significantly elevated compared with the vehicle control group, and the dose groups exceeded historical controls ($P < .001$). A dose-dependent increase in the MNBN cell numbers was observed in both metabolic regimes.[95]

In another micronucleus assay performed by Covance Laboratories,[96] 3-nitro-p-hydroxyethylaminophenol was evaluated in vivo using Crl:CD (SD) BR rat bone marrow. Rats (5 per sex per dose group with an additional 5 per sex in the control and highest dose groups) received the test material via oral gavage at a single dose of 500, 1000, or 2000 mg/kg (an additional 3 mice of each sex were dosed with 2000 mg/kg for plasma measurements). Additional groups were controls: a vehicle control of 0.5% aqueous carboxymethylcellulose and a positive control of cyclophosphamide. Bone marrow smears were prepared from rat femurs at 24 and 48 hours (for the 2000-mg/kg dose group) to allow determination of the incidence of PCEs. Cytotoxicity was assessed by scoring the number of PCEs and NCEs.

No clinical signs or mortality were observed in the rats at any concentration. Red urine and red-stained tails were observed and attributed to the staining properties of the test material. No statistically significant increases in micronucleated PCEs were noted at any dose, but the test material was

cytotoxic to bone marrow in females at 2000 mg/kg at the 24-hour harvest. Plasma analysis confirmed systemic exposure to the test article at 2000 mg/kg. The researchers concluded that 3-nitro-p-hydroxyethylaminophenol did not induce micronuclei, but bone marrow toxicity and systemic exposure occurred at 2000 mg/kg.[96]

Darroudi[97] evaluated 3-nitro-p-hydroxyethylaminophenol for mutagenicity in vivo using a mouse micronucleus test. Doses of 37.5, 75, 150, or 300 mg/kg of the test material (dissolved in DMSO) were administered to young adult male Swiss random-bred mice via 1 intraperitoneal injection. There were 4 mice per dose group. The mice were killed 24 or 48 hours after treatment, and the femurs were dissected for bone marrow study. No increases in frequency of micronuclei were observed in the mice treated with 3-nitro-p-hydroxyethylaminophenol compared with controls, but the researchers noted that the negative results could be due to detoxification within the mouse system or to not using a sufficiently high concentration because it may lead to lethality of the animal.

A mouse micronucleus test was performed by Huntingdon Research Centre[98] to assess the mutagenic nature of 3-nitro-p-hydroxyethylaminophenol. Specific pathogen-free CD-1 mice (5 mice per sex per group) received dosages of 2500, 5000, or 10 000 mg/kg body weight of test material via oral gavage in 2 equal dosages separated by 24 hours. Negative control group received 1% methylcellulose, and the positive control group received intraperitoneal injection of mitomycin C (total dosage of 8 mg/kg body weight). Mice were observed for clinical signs after the final administration for 6 hours and were then killed. Bone marrow smears were examined for presence of micronuclei. The group mean micronucleated cell count was comparable to the controls at all dose levels. It was noted that the positive control produced expected micronucleated cell counts but not the expected NCE/PCE ratio or bone marrow toxicity.[98]

Chinese hamster ovary cells exposed to 0.05, 0.1, 0.2, and 0.4 mg/mL 3-nitro-p-hydroxyethylaminonitrophenol in vitro experienced no increased frequency of chromosomal aberrations compared with controls after fixation at 6, 12, and 16 hours.[99]

4-Hydroxypropylamino-3-Nitrophenol.

4-Hydroxypropylamino-3-nitrophenol was assayed using Ames test.[23] Toxic effects were observed at 3000 μg per plate in strains TA97 and TA98 without metabolic activation. In presence of S9, 6000 μg per plate and 10 000 μg per plate were found to be inhibitory to TA98 and TA100. No mutagenic activity was observed in any of the strains with or without metabolic activation at any concentration of 4-hydroxypropylamino-3-nitrophenol.

Shahin et al[100] studied mutagenic effects of a variety of amino-nitrophenol derivatives using *S typhimurium* strains. The results showed that none of the concentrations were mutagenic for any of the species studied with or without metabolic activation. The authors proposed that mutagenic activity of nitrophenol family of compounds depends on the nature of the substituent groups and on their distribution in the molecular structure of the compound; therefore, care should be taken while establishing a structure–activity relationship for these chemicals.

Colipa[23] reported results of several genotoxicity assays.

In a DNA-repair test conducted using repair competent and repair deficient *E coli* strains, inhibition of growth was observed in both the strains with and without metabolic activation at 4.88 to 5000 μg/mL of 4-hydroxypropylamino-3-nitrophenol dissolved in DMSO. The researchers concluded that 4-hydroxypropylamino-3-nitrophenol did not have a DNA-damaging effect.

4-Hydroxypropylamino-3-nitrophenol was found to cause no chromosome-damaging effects at 50, 100, or 200 μg/mL in the absence of S9 metabolizing system or at 100, 200, or 400 μg/mL in the presence of S9 metabolizing system in human peripheral lymphocytes.

4-Hydroxypropylamino-3-nitrophenol was not found to be mutagenic in a micronucleus test using male and female CFLP mice after single intraperitoneal injections at 1000 mg/kg.

In a micronucleus test using male and female Crl:NMRI BR mice after single oral administration of 4-hydroxypropylamino-3-nitrophenol at 700 mg/kg, no cytotoxic effects on bone marrow or increase in the micronuclei were observed.

Unscheduled DNA synthesis was studied using Wistar/WU rats after single oral administration of 4-hydroxypropylamino-3-nitrophenol. For the 2-hour treatment, the dose was 2000 mg/kg, whereas for 16 hours of treatment the dosage was 200 and 2000 mg/kg 4-hydroxypropylamino-3-nitrophenol. After the exposure period, the hepatocytes from the animals were cultured in presence of ^{3}H-labeled thymidine, and incorporation of the radio label and cytotoxicity were measured. The researchers concluded that 4-hydroxypropylamino-3-nitrophenol did not induce unscheduled DNA synthesis.[23]

Carcinogenicity

2-Amino-4-Nitrophenol and 2-Amino-5-Nitrophenol.

Burnett and Goldenthal[57] dosed the F_1 generation from their multigeneration reproductive study dermally for 2 years with various hair dyes. Sixty male and 60 female Sprague-Dawley rats were randomly assigned to control and test groups. The hair dye used to dose group 8 contained 0.4% 2-amino-4-nitrophenol, and group 9 was dosed with a hair dye that contained 0.5% 2-amino-5-nitrophenol.

Dosing of the F_0 generation consisted of twice-weekly application of 0.5 mL of the hair dye mixed with 6% hydrogen peroxide to the back of the rats (hair had been clipped short). Dosing began 100 days prior to mating and continued to day 21 of lactation. The dosing of the F_1 generation followed the same pattern. At 12 months, 5 rats per sex per group were euthanized and necropsied; the remaining rats were euthanized and necropsied at week 117.

Body weights were comparable between test and control groups. Test group males had significantly decreased total

erythrocytes and hemoglobin concentration compared with 1 of the control groups. Tumors were diagnosed among the treated and untreated rats, but most were tumors commonly seen in this strain of rats. The test male rats and test female rats had significantly increased incidences of pituitary adenomas compared with the controls.

The lack of findings in the 2-year study led the authors to conclude that hair dyes do not pose a significant health risk.[57]

2-Amino-4-Nitrophenol. A 2-year chronic study was conducted by the NTP[35] using F344/N rats to examine the possible carcinogenicity of 2-amino-4-nitrophenol. 2-Amino-4-nitrophenol in corn oil was orally administered by gavage to the rats (50 per sex per group) 5 days a week for 103 weeks at doses of 0, 125, and 250 mg/kg. Animals underwent observation twice daily, recording of body weights, and necropsy at study termination. In addition, a histopathologic examination of the high-dose and vehicle control animals was performed; animals from other groups were added if necessary.

Rats in the high-dose and low-dose groups had hyperplasia of renal tubular epithelium; treated males had increased epithelial hyperplasia of renal pelvis, and high-dose males had renal cortical adenomas. The males in the high-dose group had a significant increase in the occurrence of neoplastic nodules of the liver. Only the low-dose males had a significant increase in the incidence of adenomas or carcinomas combined of the preputial gland.

There was an increased incidence of hyperplasia of the parathyroid in all treated males; no increases were observed in the females. These increases in the males were considered secondary effects of kidney disease. Only 1 male in the 250-mg/kg group had carcinoma of the colon.

The histopathologic findings led the authors to conclude that there was some evidence of carcinogenic activity in F344/N male rats as shown by increased incidences of renal cortical (tubular cell) adenoma, and no evidence of carcinogenic activity in the F344/N female rats was observed for 2-amino-4-nitrophenol.

The NTP[35] further tested the chronic effects of 2-amino-4-nitrophenol in a 2-year study using 300 $B6C3F_1$ mice that were randomized into 3 groups (50 mice per sex per group). Dose administration of 2-amino-4-nitrophenol (in corn oil) was by gavage at doses of 0, 125, and 250 mg/kg for 5 days per week for 103 weeks. Observations were conducted as with the 2-year rat study.

Histopathologic findings in the mice were mainly in the males. There was a significant increase in the incidence of hemangiomas (2/50) or hemangiosarcomas combined (3/50) in the 250-mg/kg group compared with the controls (0/50 both tumors), which was not different from historical control incidence of 11% at the study laboratory. The 250-mg/kg males had a greater incidence of renal tubule pigmentation (male: vehicle control, 4/50; low dose, 0/18 [32 kidneys not examined microscopically]; high dose, 25/50; female: none observed).

All treated rats had increased incidence of chronic and hyperplasia of alveolar epithelium. The lack of findings in the treated mice led the authors to conclude that oral administration of 2-amino-4-nitrophenol to $B6C3F_1$ mice presented no evidence of carcinogenic activity.[35]

2-Amino-5-Nitrophenol. The NTP[36] performed a 2-year chronic carcinogenicity study using F344/N rats (50 per sex per group). 2-Amino-5-nitrophenol in corn oil was orally administered to the rats at doses of 0, 100, and 200 mg/kg 5 days week for 103 weeks

Males in both the low- and high-dose groups had a significant increase in pancreatic acinar cell adenomas; however, no effect was seen on the female rats. The reduced survival of the high-dose group (200 mg/kg) markedly reduced the sensitivity of this group for detecting the presence of neoplasms. High-dose males had a marginal increase in carcinomas of the preputial gland.

Female rats had a marginally positive trend in the incidence of adenomas and/or carcinomas of the clitoral gland. Some of the treated male rats had increased incidences of hyperplasia of the bone marrow. Both male and female treated rats had increased incidences of lymphangiectasis.

The NTP concluded that under the conditions of the 2-year gavage study, there was some evidence of carcinogenic activity for male rats that receive 100 mg/kg 2-amino-5-nitrophenol, as shown by the increased incidence of acinar cell adenomas of the pancreas. The authors concluded that the use of corn oil as a gavage vehicle may have a confounding effect on the interpretation of chemical-induced proliferative lesions of the exocrine pancreas and mononuclear cell leukemia in male F344/N rats.[101] There was no evidence of carcinogenic activity for female rats receiving 100 or 200 mg/kg 2-amino-5-nitrophenol.

The 2-year carcinogenicity study was also performed using $B6C3F_1$ mice (50 per sex per group). 2-Amino-5-nitrophenol in corn oil was orally administered to the mice at doses of 0, 400, and 800 mg/kg for 5 days a week for 103 weeks. No neoplasms were found in either the colon or rectum of the treated mice. The mice did have incidences greater than the controls of renal tubular dilatation in both the high-dose males and females. There was no evidence of carcinogenic activity in the mice receiving a dose of 400 mg/kg 2-amino-5-nitrophenol. Given the reduced survival of both sexes at 800 mg/kg, this treatment group was considered inadequate for detecting a carcinogenic response.[36]

4-Amino-2-Nitrophenol. Venitt and Searle[43] reported a study in which a hair dye containing 4-amino-2-nitrophenol (concentration not given) was diluted 10-fold in 50% aqueous acetone and applied twice weekly to clipped dorsal skin of DBAf mice (for 71 weeks) and strain A mice (for 80 weeks, female and male mice 32 mice per group). The strain A mice received 0.4 mL of the diluted hair dye per application. The DBAf mice received 0.2 mL of diluted hair dye per application.

All groups of mice developed tumors of lymphoid origin; however, the mice treated with the diluted hair dye developed their tumors earlier than the control mice. Strain A tumors

developed at the 38th week in the treated mice and in the 75th week in the controls. DBAf mice developed tumors ion week 41 for the treated mice and week 72 for the control mice.[43]

Searle and Jones[44] tested the carcinogenicity of a hair dye containing 4-amino-2-nitrophenol and an azo-dye-metal complex using 2 strains of mice. Male and female albino A/Bcr (26 and 16 mice per group, respectively) and gray DBAf/Bcr (17 and 15 mice per group, respectively) were dermally dosed with the hair dye twice weekly for 80 weeks. The hair dye was diluted with 4 parts deionized water and 5 parts aqueous acetone. A control group was dosed with aqueous acetone (50% vol/vol). The diluted hair dyes were applied to the clipped backs of the mice at a volume of 0.4 mL. After the 24-week point, the dose was reduced to 0.2 mL for the DBAf mice because of toxic effects.

Both strains of mice were diagnosed with lymphoid tumors. The occurrence in the albino A/Bcr mice was 21.9% and the control incidence was 25.0%, whereas the occurrence in the DBAf mice was 9.7% compared with 3.3% of controls. The DBAf female mice had an increase in female reproductive tumors (13.3%); no tumors were reported in the control group. The applications were made using complex mixtures, so the tumors cannot be attributed to a particular dye.[44]

In an NCI[37] study, Fischer 344 rats were divided into 3 groups of 50 animals per group per sex. The rats were fed either control diet or 4-amino-2-nitrophenol mixed in their feed at concentrations of 1250 or 2500 ppm. Dose administration took place for 103 weeks. All animals were observed twice daily, were weighed at regular intervals, and underwent a gross and microscopic examination at necropsy at study termination.

Body weights of the treated rats did not differ significantly from the controls; no toxicity due to 4-amino-2-nitrophenol was noted. Survival rates among the treated animals were comparable to the control rats.

Histopathologic findings attributed to 4-amino-2-nitrophenol in the rats occurred in the urinary bladder. High-dose males were found to have higher rates of transitional-cell carcinoma, transitional-cell papilloma, or transitional-cell hyperplasia than controls.

High-dose and low-dose females were found to have more transitional-cell carcinoma than controls. No tumors of the urinary bladder were found among 220 male and 220 female historical-control rats at this laboratory. Other tumors that occurred in high-dose rats but not in the controls were epidermoid carcinoma of the salivary gland, fibrosarcoma, osteosarcoma, and chondrosarcoma.

Both the high-dose and low-dose groups had pigmentary changes in the lamina propria of the small intestine. It was concluded that 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in 3 dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol.

As with the above NCI rat study, B6C3F$_1$ mice (50 of each sex per group) were divided into 3 groups to look at possible carcinogenicity of 4-amino-2-nitrophenol. The mice were fed either control feed or 4-amino-2-nitrophenol mixed with their feed at concentrations of 1250 or 2500 ppm. All mice were observed twice daily, were weighed at regular intervals, and underwent a gross and microscopic examination at necropsy at study termination.

Body weights of the mice, both male and female, were slightly lower than those of the control mice. Survival of the mice in both the high-dose and low-dose groups was comparable to the controls.

Any tumors and hyperplasias that occurred in the mice were considered to be spontaneous types, and they occurred in both the control and dosed groups. As with the rat study, the mice in the dosed groups had pigmentation of the lamina propria of the small intestine. On the basis of rate of growth, mortality, or clinical signs, there was little evidence of toxicity of 4-amino-2-nitrophenol in the dosed rats and mice. It was concluded that under this bioassay, 4-amino-2-nitrophenol was not carcinogenic for the male and female B6C3F$_1$ mice at the doses tested.[37]

Clinical Assessment of Safety

Dermal Irritation and Sensitization Case Reports

A 42-year-old woman developed erythema and edema of the scalp, ears, and neck after applying a hair dye with 4-amino-3-nitrophenol. The standard European patch tests were negative. 4-Amino-3-nitrophenol at 2% pet elicited a positive reaction, whereas other constituents were negative. Control persons patch tested with the same substances were negative.[102]

Sosted and Menne[103] describe a case of a 50-year-old woman who developed scalp dermatitis with severe itching that spread to her face, neck, and upper thorax 1 day after using a nonpermanent hair dye that contained 3-nitro-p-hydroxyethylaminophenol and 4-amino-3-nitrophenol. The patient also developed vesicular hand eczema. The patient performed the recommended pre-exposure test without any adverse reaction. The dermatitis was treated with systemic and topical steroids. Patch testing was performed using the European Standard Series shortly after the reaction occurred and 2 years later. Screening chemicals for the hairdresser's series and local series of cosmetic allergens did not produce definite positive reactions. Patch testing of the individual hair dye ingredients in the product produced a + and ++ on day 2 and days 3 to 4, respectively, for 4-amino-3-nitrophenol and a +? and + on day 2 and days 3 to 4, respectively, for 3-nitro-p-hydroxyethylaminophenol.

Contact dermatitis from exposure to 3-Nitro-p-Hydroxyethylaminophenol was also reported by Dejobert et al.[104] in a 40-year-old atopic woman. The patient presented with edema of the face and eczema of the scalp 2 days after use of a semipermanent hair coloring product. The patient had experienced a similar episode 6 months prior. Patch tests were performed using the European standard and hairdressing series. The results of the hairdressing series were negative, but the semiopen test

with the hair dye product was positive +++/++. Another round of patch testing was then performed using the components of the hair dye in ethanol 95% diluted in water (50/50). Patch testing for 3-nitro-p-hydroxyethylaminophenol (0.14%) produced a – on day 2 and a ++ on day 3.

Epidemiology

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, whereas direct hair dyes are a preformed color. Although the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The CIR Expert Panel noted the conclusions of these reviews, including that personal use of hair colorants cannot be evaluated as to carcinogenicity and that occupation as a hairdresser or barber entails exposures that are probably carcinogenic, insufficient evidence exists to support a causal association between personal hair dye use and a variety of tumors and cancers such as acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin's lymphoma.[12] Discussion of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

The CIR Expert Panel did specifically note reports from a case-control study that suggested a possible genetically susceptible subgroup, members of which detoxify arylamines to a lower degree than the general population.[105,106] The study authors hypothesized that this subgroup may be at greater risk of bladder cancer from hair dye exposure. Helzlsouer et al[107] noted that these results were based on small sample sizes.

Several studies published since the Helzlsouer et al[107] review also have been considered.

Bladder Cancer

Andrew et al[108] reported a case-control study of New Hampshire residents whose bladder cancers were entered into a state registry from 1994 to 1998. A follow-up study by Kelsey et al[109] examined the links between those bladder cancer cases with an inactivated tumor suppressor gene (TP53) and various exposures. Huncharek and Kupelnick[110] performed a meta-analysis of 6 case-control reports and 1 cohort study. Takkouche et al[111] performed a meta-analysis of the Andrew et al[108] study and 9 other case-control or cohort studies. Ji et al[112] reported a cohort study not included in the above meta-analyses. Kogevinas et al[113] reported a bladder cancer case-control study in Spain.

Lymphoma and Leukemia

Rauscher et al[114] reported a US/Canadian case-control study of adult acute leukemia. Zhang et al[115] and Zheng et al[116] examined the relationship of hair dye use or diet with non-Hodgkin's lymphoma in a case-control study in Connecticut. Takkouche et al[111] reported a meta-analysis of reports of hematopoietic cancers, including that by Rauscher et al[114] and Zhang et al[115] and 17 other studies. Mester et al[117] reviewed 10 epidemiology studies regarding the relationship between occupational exposure in hairdressing and diseases of the malignant lymphoma group. A case-control study in Spain by Benavente et al[118] examined the association between lifetime hair dye exposure with various lymphomas, including chronic lymphocytic leukemia. de Sanjosé et al[119] reported a lymphoid tumor case control study in Europe.

Other Cancers

Takkouche et al[111] included breast cancer and childhood cancers in their meta-analysis. Efird et al[120] studied the association between the use of hair-coloring agents the month before or during pregnancy with childhood brain tumors in 1218 cases between 1976 and 1994. Heineman et al[121] studied 112 women in Nebraska newly diagnosed with brain cancer (glioma). McCall et al[122] reported on the relationship between childhood neuroblastomas and maternal hair dye use in 538 children born between 1992 and 1994 in the United States and Canada.

Other Diseases

Park et al[123] reported an occupational case-control study of neurodegenerative diseases, including Alzheimer's disease, presenile dementia, and motor neuron disease.

In considering all these data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points.

The panel stated that use of direct hair dyes, although not the focus in all investigations, appears to have little evidence of an association with adverse events as reported in epidemiology studies. However, direct hair dyes are a diverse group of chemicals, and the determination of safety may hinge on other safety test data.

The panel recognizes that hair dye epidemiology studies do not address the safety of individual hair dyes but is concerned that studies have demonstrated an association between use of oxidative/permanent hair dyes and some cancer end points. The panel, therefore, strongly supports the need to replicate these studies, along with further studies to examine the possibility of susceptible subpopulations.

Summary

2-Amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-3-nitrophenol, 4-amino-2-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are substituted aromatic compounds used as semipermanent hair colorants in cosmetic products, although they may be used as toners in permanent hair dye products.

Impurities were characterized for 2-amino-3-nitrophenol, 4-amino-3-nitrophenol, and 3-nitro-p-hydroxyethylaminophenol (>97% pure). Limited information was available for the other ingredients. Impurity levels were different for different dyes in this group but generally included heavy metals, reaction intermediates, and residual solvents.

All ingredients in this group except 2-amino-4-nitrophenol sulfate, 2-amino-5-nitrophenol and 4-amino-2-nitrophenol have reported uses in hair dyes and colors. Use concentrations reported by industry are 2-amino-3-nitrophenol (2%), 4-amino-3-nitrophenol (9%), 3-nitro-p-hydroxyethylaminophenol (10%, 5% after dilution), and 4-hydroxypropylamino-3-nitrophenol (2.6% in semipermanent and permanent hair colors).

These ingredients are known as coal tar hair dyes, in which a caution label must appear on any product in which the chemical is contained that advises the consumer to conduct a preliminary patch test with the product before use.

In vitro dermal absorption of 4-amino-2-nitrophenol in acetone using monkey abdominal skin was 64% in 1 study and in another was 45.1% and 48.2%, respectively, using human and monkey abdominal skin.

In a study using excised human cadaver skin, the permeability constant for 2-amino-4-nitrophenol was less than 3.0×10^{-5} and for 4-amino-2-nitrophenol less than 8.6×10^{-5}.

An in vitro skin diffusion study of 2-amino-3-nitrophenol found 0.26% (with hair) and 0.46% (without hair) penetration 24 hours after contact with human skin.

Dermal delivery in human skin of the oxidative and the semipermanent preparations for 3-nitro-p-hydroxyethylaminophenol was 2.50 μg Eq/cm^2 and 0.45 μg Eq/cm^2, respectively, and for 4-amino-3-nitrophenol was 3.00 μg Eq/cm^2 and 0.59 μg Eq/cm^2.

In rats, the percutaneous absorption was 0.27% 4-hydroxypropylamino-3-nitrophenol within the first 24 hours, with 82% of it excreted by the rat via urine and feces. There was no retention of 4-hydroxypropylamino-3-nitrophenol in any of the organs. Following oral delivery to rats of 4-hydroxypropylamino-3-nitrophenol in DMSO/water, 99% of the administered radiolabel was eliminated in the first 24 hours.

The oral LD_{50} values from an acute oral study using rats for 2-amino-4-nitrophenol and 2-amino-5-nitrophenol were calculated to be greater than 4000 mg/kg and to be 3300 mg/kg for 4-amino-2-nitrophenol.

The oral LD_{50} from an acute oral study using rats for 2-amino-3-nitrophenol was greater than 2000 mg/kg. A no-effect dose was observed to be less than 1000 mg/kg.

The oral LD_{50} from an acute oral study using rats for 4-amino-3-nitrophenol was greater than 500 mg/kg but less than 1000 mg/kg.

The maximum nonlethal dose of 3-nitro-p-hydroxyethylaminophenol using rats in an acute oral study was1000 mg/kg; the minimum lethal dose was 2000 mg/kg. A 10% mortality was reported in rats and no mortality in mice given 3 g/kg orally.

The 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, or 4-amino-2-nitrophenol (in a 10% aqueous solution) acute intraperitoneal LD_{50} values using rats were 246 mg/kg, greater than 800 mg/kg, and 302 mg/kg, respectively.

Ingestion of an oral 20% solution of 4-hydroxypropylamino-3-nitrophenol (10 mL/kg) resulted in no deaths or adverse effects using rats, but urine was red-stained.

2-Amino-3-nitrophenol instilled at a dose of 0.1 g in rabbit eyes was considered nonirritating. A dose of 0.1 mL of 6% 4-amino-3-nitrophenol to rabbit eyes did not produce any reactions, and the ingredient was determined to be nonirritating. 3-Nitro-p-hydroxyethylaminophenol was considered a slight irritant to rabbit eyes in 2 studies. Treatment of rabbit eyes with 0.1 mL of 3% solution of 4-hydroxypropylamino-3-nitrophenol resulted in minimal redness of the conjunctiva after 1, 24, 48, and 72 hours.

A single cutaneous 0.5-g application of 2-amino-3-nitrophenol to rabbit skin was nonirritating. 4-Amino-3-nitrophenol, in a 6% concentration, was nonirritating to rabbit skin. Dermal application of 0.5 g of 4-amino-3-nitrophenol using rabbits was nonirritating. A 6% concentration of 3-nitro-p-hydroxyethylaminophenol was well-tolerated in topical applications in rabbits. A dermal application of 4% 3-nitro-p-hydroxyethylaminophenol to rabbit skin produced no damage on histological examination. 4-Hydroxypropylamino-3-nitrophenol was classified as nonirritant at 3% when applied occlusively to rabbit or guinea pig skin.

Neither 4-amino-3-nitrophenol nor 3-nitro-p-hydroxyethylaminophenol was sensitizing in a guinea pig maximization test.

4-Amino-3-nitrophenol applied to the ears (25 μL per ear) of mice at concentrations ranging from 0.05% to 25% produced no cutaneous reactions or thickening of the ears, and lymphoproliferation occurred in doses greater than 0.5%. In the same protocol, 3-nitro-p-hydroxyethylaminophenol was not a local irritant, but the positive lymphoproliferation responses in all concentration levels indicated that this ingredient had contact sensitization potential in mice.

In a 28-day oral toxicity study of 2-amino-3-nitrophenol at 100, 300, or 1000 mg/kg/d in rats, the 1000-mg/kg/d dose group had lowered body weight gain in males, a slight to moderate increase in absolute and relative liver and spleen weights with a blackish colored spleen in both sexes, and hemosiderin-laden macrophages in both sexes. The NOAEL was 300 mg/kg/d.

In a short-term oral toxicity test in which 2-amino-4-nitrophenol was administered to 50 rats at 0, 313, 1250, 2500, or 5000 mg/kg, diarrhea resulted in all but the 313-mg/kg group. All rats in the 2500- and 5000-mg/kg groups died, and only 1 male survived in the 1250-mg/kg group. The same test was completed using the same protocol and concentrations of 2-amino-4-nitrophenol using mice. All the mice in 2500- and 5000-mg/kg groups died, as well as 2 males and all the females in the 1250-mg/kg group.

2-Amino-5-nitrophenol administered orally to 60 rats at 0, 156, 313, 625, 1250, or 2500 mg/kg for 16 days produced some lesions found at necropsy, but they were also present in the

control group. A total of 4 rats died; body weights were depressed and loose stools were found in the 3 high-dose groups.

In another short-term oral toxicity test, 60 mice received 0, 313, 625, 1250, 2500, or 5000 mg/kg 2-amino-5-nitrophenol over a period of 15 days. At the end of the study, 19 mice had died. There were no significant differences of body weights between the dosed groups and control. There were loose stools among the males in the 5000-mg/kg group, and 2 males were prostrate for the first week of the study in the 2500-mg/kg group.

A short-term dietary toxicity study was performed using rats and mice to estimate the maximum tolerated dose of 4-amino-2-nitrophenol. Neither the rats nor the mice had any compound-related deaths, and the body weights were comparable to the controls.

In a 28-day oral gavage study in which rats received 20, 100, 500, and 1000 mg/kg 4-hydroxypropylamino-3-nitrophenol, there was a decrease in white blood cell count and an increase in blood urea nitrogen at 500 mg/kg. In another study using 10, 30, and 90 mg/kg 4-hydroxypropylamino-3-nitrophenol for 90 days, there were no treatment-related hematological or blood chemistry changes—90 mg/kg was regarded as the NOEL.

In a 13-week toxicity study, 2-amino-3-nitrophenol was administered to rats at dose of 50, 200, or 800 mg/kg/d through oral gavage. The animals in the 800-mg/kg/d dose group exhibited ptyalism; loud breathing; and yellow urine, tail, and extremities. Females had lower glucose levels and higher cholesterol levels, males had increased protein levels and lower A/G ratio and kidney weights, and both sexes had higher liver and spleen weights. The 200-mg/kg/d dose group presented yellow urine, tails, and extremities; females had lower glucose and higher cholesterol levels, and males had higher protein levels and lower A/G ratio. The NOAEL was 50 mg/kg/d.

In a subchronic oral toxicity test, 60 male and 60 female mice were administered 2-amino-4-nitrophenol for 13 weeks in doses of 0, 62.5, 125, 250, 500, and 1000 mg/kg. All the rats in the 1000-mg/kg group died, 2 males and 2 females died in the 500-mg/kg group, and the rats in the 500- and 1000-mg/kg groups were observed to have diarrhea and lethargy.

Rats given oral 2-amino-5-nitrophenol at 1600, 800, and 400 mg/kg over 15 days had decreased body weights. Rats in the 800- and 1600-mg/kg groups had vasculitis of the colon or cecum, and the liver to body weight ratio was significantly increased compared with the control group. The same protocol using mice resulted in decreased body weights in the 1600-mg/kg group along with lethargy in females.

A 13-week oral toxicity study of 4-amino-3-nitrophenol administered doses of 10, 50, or 250 mg/kg/d to rats produced no clinical signs in any dose group. The NOEL is between 250 and 600 mg/kg/d.

3-Nitro-p-hydroxyethylaminophenol given orally over 3 months to rats at 0, 40, 200, or 1000 mg/kg/d resulted in few effects. Rats in the 1000-mg/kg/d dose group exhibited ptyalism starting at week 7.

In a 13-week dermal toxicity test, 0.4% 2-amino-4-nitrophenol, 0.5% 2-amino-5-nitrophenol, and 0.3% 4-amino-2-nitrophenol administered topically to rabbits at 2 mg/kg did not produce any evidence of compound toxicity throughout the study. Dogs fed 2-amino-4-nitrophenol at 0.0, 19.5, and 97.5 mg/kg/d of this composite mixture along with their feed had no differences compared with the control group.

In a 2-year chronic study, rats and mice were fed diets that included 0, 125, and 250 mg/kg 2-amino-4-nitrophenol. In both species, there were no differences in body weight. The male rats in the 250-mg/kg group had a decreased survival rate compared with controls; however, there were no differences in survival rates between groups of mice. The males in the high-dose group had ulcers and erosive lesions. In the high-dose group of mice, the males had a greater incidence of renal tubule pigmentation, and all treated males had increased incidence of chronic bronchopneumonia and hyperplasia of alveolar epithelium.

In a 2-year chronic study, 2-amino-5-nitrophenol was orally administered to rats (0,100, and 200 mg/kg) and significantly decreased survival rates. Body weights were decreased in the high-dose groups and the rats had loose stools. In another 2-year chronic test, mice were given 2-amino-5-nitrophenol orally in concentrations of 0, 400, and 800 mg/kg. As in the previous study, similar results were seen in this study, including decreases in survival and body weight gains.

A 2-year dietary study was performed using rats with 4-amino-2-nitrophenol at concentrations of 1250 and 2500 ppm. Body weights were slightly decreased but not significantly so compared with the controls. The survival rate was comparable to the controls. Carcinomas were observed in the rats. The same researchers performed a 2-year dietary study in mice with 4-amino-2-nitrophenol at the same concentrations. Body weights in the mice were also slightly decreased, and the survival rates in the dosed groups were comparable to the controls. No carcinogenic effects were observed.

4-Amino-2-nitrophenol, present in hair dye formulations at unreported concentrations, was tested and applied to the clipped backs of mice. Some mice became emaciated between 13 and 26 weeks of exposure, and those affected were euthanized. Toxic effects were on the urogenital tract, in which obstructive crystals were seen in the bladder and on the skin around the penis.

Reproductive, developmental, and teratogenic studies for 2-amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-3-nitrophenol, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol reviewed in this report did not reveal any adverse effects on reproductive performance or development. No reproductive or developmental studies were available for 4-amino-2-nitrophenol.

2-Amino-3-nitrophenol was positive for genotoxicity in 2 of the 3 Ames tests described in this report (with and without metabolic activation). It was also positive using a L5178Y mouse lymphoma cell mutation assay without metabolic activation in doses ranging from 75 to 525 μg/mL and in a Syrian

hamster embryo cell study. Tests for genotoxicity in mouse micronuclei and DNA damage in Sprague-Dawley rats were negative.

Ames tests, a L5178Y mouse lymphoma cell mutation assay, and Chinese hamster ovary chromosomal aberration and sister chromatid exchange assays described in this report were positive for genotoxicity with and without metabolic activation for 2-amino-4-nitrophenol. A micronuclei assay and bone marrow/tumor cell test in mice did not show signs of mutagenicity with this ingredient.

Genotoxicity tests for 2-amino-5-nitrophenol described in this report were all positive for mutagenicity. 4-Amino-2-nitrophenol genotoxicity test results were also positive, except for 1 Ames test.

4-Amino-3-nitrophenol was positive for genotoxicity in 1 of the 4 Ames tests described in this report. It was also positive in human lymphocyte micronuclei assays but not in a Chinese hamster ovary cell chromosomal aberration assay or in a rat bone marrow micronucleus assay.

A L5178Y mouse lymphoma cell mutation assay and a human lymphocyte micronucleus assay were positive for genotoxicity in 3-nitro-p-hydroxyethylaminophenol, but there was no mutagenicity in Ames tests, rat and mouse bone marrow micronucleus assays, or a Chinese hamster ovary cell chromosome aberration test.

4-Hydroxypropylamino-3-nitrophenol was negative in all genotoxicity tests reported in this safety assessment.

There was an increased incidence of acinar cell adenomas of the pancreas in male rats that received 100 mg/kg 2-amino-5-nitrophenol. The significance of the acinar cell adenomas of the pancreas was confounded by the use of corn oil as a vehicle that has been shown to promote these tumors in the strain of rats used in the study. There was no evidence of carcinogenic activity for female rats receiving 100 or 200 mg/kg 2-amino-5-nitrophenol. In another study, it was found that there was no evidence of carcinogenic activity in mice receiving a dose of 400 mg/kg 2-amino-5-nitrophenol, and given the reduced survival rate of both sexes at 800 mg/kg the number of animals was inadequate to detect a carcinogenic response.

When 4-amino-2-nitrophenol was fed to rats in concentrations of 1250 and 2500 ppm, high-dose males were found to have either transitional-cell carcinoma, transitional-cell papilloma, or transitional-cell hyperplasia. No tumors of the bladder were found among 220 male and 220 female historical-control rats. Other tumors that occurred in high-dose rats but not in the controls were epidermoid carcinoma of the salivary gland, fibrosarcoma, osteosarcoma, and chondrosarcoma. It was concluded that under this bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, including transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in 3 dosed female rats may have been associated with administration of the 4-amino-2-nitrophenol. When the same study was conducted with mice instead of rats, it was concluded that under this bioassay, 4-amino-2-nitrophenol was not carcinogenic for the male and female mice tested at 1250 and 2500 ppm.

In 2 separate studies, mice were treated dermally with 4-amino-2-nitrophenol. In both studies, all of the mice (even control groups) developed lymphoid tumors. However, in the first study, it was discovered that the mice treated with the chemical developed the tumors earlier (38th week) than the control mice (75th week). Also, in the second study it was found that the female mice had an increase in female reproductive tumors, whereas the control groups did not.

4-Amino-3-nitrophenol in a hair dye application was reported to have caused erythema and edema in a case study of a 42-year-old woman. Another case study described a 50-year-old woman who developed scalp dermatitis; severe itching of the face, neck, and upper thorax; and vesicular hand eczema 1 day after using a nonpermanent hair dye that contained 3-nitro-p-hydroxyethylaminophenol and 4-amino-3-nitrophenol.

Available hair dye epidemiology studies are insufficient to conclude a causal relationship between hair dye use and cancer or other diseases.

Discussion

Overall, the available toxicity studies for these amino nitrophenol hair dyes do not suggest safety concerns except for the potential carcinogenicity of 4-amino-2-nitrophenol. Early onset of lymphoid tumors in mice and the increased incidence of transitional-cell carcinoma in rat bladders were noted. In addition, 4-amino-2-nitrophenol is genotoxic in Ames tests, Rauscher leukemia virus–infected rat embryo assays, and L5178Y mouse lymphoma cell mutation assays. The panel noted the absence of reproductive and developmental toxicity data regarding 4-amino-2-nitrophenol. It may be that the effects noted are related to the presence of an impurity. It also may be that there is so little dermal penetration that these effects are not realized under use conditions.

For the Expert Panel to reach a conclusion for 4-amino-2-nitrophenol, additional data are needed that describe any impurities and percutaneous absorption for this ingredient under conditions used in cosmetic applications. If there is significant dermal absorption, dermal reproductive and developmental toxicity data may be needed.

Increased incidences of pancreatic acinar cell adenomas were seen in male rats treated with 2-amino-5-nitrophenol. However, there were no incidences of acinar cell adenomas in female rats or in a similar study with mice. The panel noted that corn oil was used as a vehicle in the study with 2-amino-5-nitrophenol. This vehicle has been shown to promote the types of tumors found in male rats of the strain used in the NTP study. No adverse reproductive or developmental effects were noted for this ingredient.

Reproductive and developmental studies for the remaining ingredients in this group did not indicate toxicity.

The Expert Panel recognizes that 2-amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 4-amino-2-nitrophenol, 4-amino-3-nitrophenol, 2-amino-4-nitrophenol sulfate, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are used as hair dye ingredients and may

be sensitizers. However, hair dyes containing these ingredients, 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, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

Conclusion

Based on the data contained in this report, the CIR Expert Panel concludes that 2-amino-3-nitrophenol, 2-amino-4-nitrophenol, 2-amino-4-nitrophenol sulfate, 2-amino-5-nitrophenol, 4-amino-3-nitrophenol, 3-nitro-p-hydroxyethylaminophenol, and 4-hydroxypropylamino-3-nitrophenol are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment, but the data are insufficient to make a determination of safety for 4-amino-2-nitrophenol.

Authors' Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th Street, Suite 412, Washington, DC 20036, USA.

Declaration of Conflicting Interests

No potential conflict of interest relevant to this article was reported. F. Alan Andersen and Valerie Robinson are employed by the Cosmetic Ingredient Review.

Financial Disclosure/Funding

The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review Program is financially supported by the Personal Care Products Council.

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