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Final Report on the Safety Assessment of Toluene-2,5-Diamine, Toluene-2,5-Diamine Sulfate, and Toluene-3,4-Diamine

ABSTRACT

The diaminotoluenes 2,5-TD, 2,5-TDS, and 3,4-TD are used as colorants in permanent hair dyes and tints. 2,5-TD is used at concentrations up to 1% and 2,5-TDS is used up to 5%. The major routes of excretion after cutaneous absorption by rats were through the urine and feces. The oral LD_{50} of 2,5-TDS in rats was 98 mg/kg. There was no evidence of percutaneous toxicity to rabbits when 6% 2,5-TDS was applied to intact or abraded skin. In a two-year study, no toxicity was reported in rats receiving biweekly cutaneous applications of formulations containing 3% or 4% 2,5-TD. Mice were also unaffected by 3% 2,5-TDS in an 18-month study, or by 3% 2,5-TD in a two-year study. Rabbits had a slight dermal irritation response after exposure to 2.5% 2,5-TDS, but no irritation occurred in guinea pigs after exposure to 10% solutions of 2,5-TDS or 3,4-TD. 2,5-TD (2.5%) caused mild, transitory conjunctival inflammation in rabbits. The results of sensitization tests of 2,5-TD, 2,5-TDS, and 3,4-TD indicated that each of these diaminotoluenes were sensitizers to laboratory animals and humans. 2,5-TDS was toxic to pregnant rats and their embryos at oral doses of 80 mg/kg/day. Doses between 10 and 50 mg/kg/day did not cause congenital or maternal abnormalities. *o*-Toluenediamine, a mixture of 2,3-TD and 3,4-TD (40:60), was not teratogenic when administered orally to rats or rabbits. Cutaneous exposure to hair dye formulations containing 3% 2,5-TDS caused a statistically significant increase in fetal skeletal anomalies in rats. Rats treated with 6% 2,5-TDS did not have this adverse response. In a two-generation reproduction study, mice receiving dermal applications of hair-dye formulations containing either 3% or 6% 2,5-TDS had no signs of pharmacotoxicity, teratogenicity, or reproductive abnormalities. The results cited from various mutagenicity assays of 2,5-TD, 2,5-TDS, and 3,4-TD varied in accordance with the assay system and protocols used. 2,5-TD and 2,5-TDS were noncarcinogenic to rats and mice in both oral and dermal exposure studies. On the basis of the animal and clinical data presented in this report, and the required labeling, it is concluded that Toluene-2,5-Diamine Sulfate and Toluene-3,4-Diamine are safe as cosmetic ingredients in the present practices of use.

INTRODUCTION

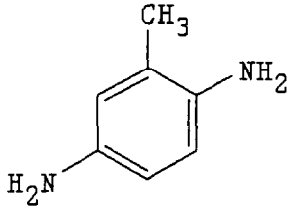
TOLUENE-2,5-DIAMINE (2,5-TD) and Toluene-3,4-Diamine (3,4-TD) are two of six isomers of the diaminotoluenes. A comprehensive review of diaminotoluenes and some selected isomers was conducted by the World Health Organization (WHO, 1987) and the Environmental Protection Agency (EPA, 1984). However, it was felt that in order to evaluate 2,5-TD, its sulfate, and 3,4-TD it was necessary to focus a report specifically on them and their use in cosmetics. The information included in the WHO and EPA reviews was used as a partial guide for this report.

CHEMISTRY

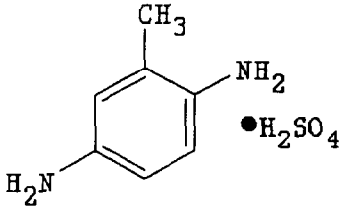
Definition and Structure

2,5-TD (CAS No. 95-70-5) and 2,5-TDS (CAS Nos. 615-50-9 and 6369-59-1) are the substituted aromatic amine and its salt which conform to the structural formulas (Estrin et al., 1982):

2'5-TD

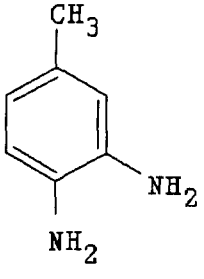


2'5-TDS



Other names for 2,5-TD (and its sulfate) are: 1,4-benzenediamine, 2-methyl-(sulfate); 2,5-diaminotoluene (sulfate); 2-methyl-1,4-benzenediamine (sulfate); *p*-toluenediamine (sulfate); and *p*-tolyenediamine (sulfate) (Estrin et al., 1982; Sax 1979).

3,4-TD (CAS. No. 496-72-0) is the substituted aromatic amine that conforms to the formula:



Other names for 3,4-TD are: 1,2-benzenediamine, 4-methyl-; 3,4-diaminotoluene; 4-methyl-1,2-benzenediamine; and 4-methyl-o-phenylenediamine (Estrin et al., 1982).

Properties

2,5-TD is a colorless, crystalline plate which is soluble in water, ethanol, ether, and hot benzene. It is slightly soluble in cold benzene (IARC, 1978; Sax, 1979; Weast,

1982). The sulfate form of 2,5-TD is a white powder that is soluble in water and ethanol. The molecular weight of 2,5-TD is 122.2 (Anonymous, 1985; Sax, 1979; Weast, 1982), and 220.2 for its sulfate (IARC, 1978). The melting and boiling points for 2,5-TD are 64°C and 273–274°C, respectively (Anonymous, 1985; IARC, 1978; Sax, 1979; Weast, 1982).

3,4-TD forms a colorless crystal with a molecular weight of 122.17. It is soluble in water and has a melting point of 89–90°C and a boiling point of 265°C (sublimes) (Weast, 1982). The absorption maxima of 2,5-TD were 201, 237, and 285 nm; and in a duplicate experiment, 198, 236, and 302 nm (Mederos et al., 1986). 3,4-TD in methanol absorbs at 297 nm (Grasselli, 1973).

Methods of Manufacture

In general, diaminotoluenes are manufactured from dinitrotoluenes using catalytic hydrogenation procedures or by the reaction of iron and hydrochloric acid with dinitrotoluenes (WHO, 1987).

According to the International Agency for Research on Cancer (IARC, 1978), 2,5-TD is manufactured by:

> . . . reductive cleavage of 4-amino-2,3'-dimethylazobenzene (*ortho*-aminoazotoluene[1]) with tin and hydrochloric acid (Prager et al., 1930); reductive cleavage may also be carried out with zinc dust and hydrochloric acid (Thirtle, 1968). It can be prepared by the electrolytic reduction of 2,5-dinitrotoluene (Tallec & Gueguen, 1966) or by condensing 2-amino-1-methylbenzene and toluene-4-sulphonyl chloride to 4-toluenesolphono-2-toluidide, which is then coupled with diazotized aminobenzenesulphonic acid and reduced (Wlodarski, 1971).

Impurities

Commerically produced 2,5-TD contains various concentrations of the other diaminotoluene isomers: 2,3-; 2,4-; 2,6-; 3,4-; and 3,5-diaminotoluenes (WHO, 1987). Of these other five isomeric diaminotoluenes, 2,4-diaminotoluene is an animal carcinogen (IARC, 1987).

According to the International Agency for Research on Cancer (IARC, 1978):

> 2,5-diaminotoluene sulphate is available in the US as a commercial grade with the following typical specifications: purity, 95% min.; residue on ignition, 0.2% max.; loss on drying, 0.5% max.; and iron content, 25 mg/kg max. (Ashland Chemical Co., 1975).

In Japan, 2,5-TD is available with a minimum purity of 95%, and contains nitroaminotoluene as an impurity (IARC, 1978). The location of the nitro and amino groups was not specified.

Analytical Methods

Methods for separation and/or determination of 2,5-TD and 3,4-TD in hair dyes are presented in Table 1 (WHO, 1987).

TABLE 1. ANALYTICAL METHODS FOR DETERMINING 2,5-TD AND 3,4-TD IN HAIR DYES

Procedure	Ingredient	Detection limit	Reference
Gas-liquid chromatography/flame ionization detector	2,5-TD	5 mg/l	Choudhary (1980)
Thin-layer chromatography	2,5-TD & 3,4-TD	0.2 mg/l	Kottemann (1966)
High-performance liquid chromatography/ultraviolet detection	2,5-TD & 3,4-TD	—	Liem & Rooselaar (1981)

Source: WHO, 1987

USE

Cosmetic Use

United States. 2,5-TD, 2,5-TDS, and 3,4-TD are used in colorants in permanent hair dyes and tints (Nikitakis, 1988). Permanent hair dyes are formed by mixing three classes of chemicals: primary intermediates, couplers, and an oxidant, usually hydrogen peroxide. When mixed, these chemicals undergo oxidation and coupling reactions to form colored material inside the hair shaft. The colors are not removed by shampooing. Subsequent dyeing is necessitated by the need to color new hair growth rather than because of fading of already colored hair (Corbett et al., 1973; Wilkinson and Moore, 1982).

2,5-TD and 2,5-TDS are used as primary intermediates in permanent hair dye formulations to produce black, drab and warm browns, and shades of blond and grey (Wall, 1972). Since most hair dye formulations are proprietary, exact concentrations are not available (IARC, 1978). 2,5-TDS is also used in formulas for bleach toners for silver (at levels of 0.100%), smoke (0.04%) and platinum blond (0.08%) (Tucker, 1968; Wall, 1972).

3,4-TD is a primary intermediate used to produce drab browns, warm browns, reds, drab blonds, and gold blonds (Wall, 1972).

Cosmetic product formulation data made available by the Food and Drug Administration (FDA) are compiled through voluntary filing of such information in accordance with Title 21, Part 720.4 of the Code of Federal Regulations (1984). Data submitted in 1984 and 1989 to the FDA by cosmetic firms participating in this voluntary cosmetic registration program indicated that 2,5-TD was used in five hair dyes, and 2,5-TDS was used in a total of 107 hair coloring formulations (Tables 2 and 3). No cosmetic formulation data on 3,4-TD were submitted to the FDA. Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to tenfold error in the assumed ingredient concentration.

Permanent hair coloring formulations containing 2,5-TD, 2,5-TDS, or 3,4-TD are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and

Table 2. Product Formulation Data for Toluene-2,5-Diamine[a]

Product category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
		0.1–1	0–0.1
Hair dyes and colors	5	3	2
1984 totals	5	3	2

[a] CIR requests that the cosmetics industry provide current formulation data on each product category.

Source: FDA, 1984.

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. Forty percent of women in the United States are estimated to be regular users of hair dyes (Corbett et al., 1973). Under normal conditions of use, skin contact with the hair dye is restricted to 30 minutes.

Most permanent hair dyes on the market contain coal tar hair dyes. These dyes are no longer produced from coal but come from petrolatum. Although the term "coal tar" is archaic, it is still used in legal documents (Consumer Reports, 1979; Menkart and Lanman, 1977). Coal tar hair dyes are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

Patch test instructions call for a 24-hour patch with the intermediates and hydrogen peroxide mixed in the same manner as in use (Corbett et al., 1973; Wilkinson and

Table 3. Product Formulation Data for Toluene-2,5-Diamino Sulfate[a]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)		
			>1–5	>0.1–1	≤0.1
Hair dyes and colors	1095	107	13	74	20
1989 Totals		107	13	74	20

[a] CIR requests that the cosmetics industry provide current formulation data on each product category.

Source: FDA, 1989.

Moore, 1982). The irritation test is to be performed prior to each and every application of the dye. In actual practice, many beauty salons do a 24-hr patch test prior to the initial hair dyeing procedure, but omit the test on subsequent applications (Fisher, 1974).

International Use. Ingredient labelling is required for the use of 2,5-TD, 2,5-TDS, and 3,4-TD in hair dyes in Japan (CTFA, 1983; IARC, 1978).

The United Kingdom approves the use of 2,5-TD in hair dyes under the Pharmacy and Poisons Act of 1933, which stipulates that all hair dyes containing toluenediamines, phenylenediamines, or other alkylated benzene diamines or their salts must bear a label with the words: "Caution. This preparation may cause serious inflammation of the skin in certain persons and should be used only in accordance with expert advice." This requirement was repeated in the Poison Rules, 1970 (Wilkinson and Moore, 1982).

Noncosmetic Use

The diaminotoluenes are used as intermediates in the production of dyes used for textiles, furs, leathers, biological stains and indicators, spirit varnishes and wood stains, and pigments (WHO, 1987).

2,5-TD is used as an intermediate in the production of two dyes: CI Basic Red 2 and CI Acid Brown 103. CI Basic Red 2 is used to dye fabrics, paper, in spirit inks, biological stains, and solvent dyes. Both dyes are used to color leather (Anonymous, 1985; IARC, 1978). 2,5-TD also has been used as an artificial electron donor in photosystem 1 studies (Anonymous, 1985).

BIOLOGICAL PROPERTIES

Absorption and Excretion

The absorption of 2,5-TD through the skin of dogs was studied by Kiese et al. (1968). A 50 ml solution composed of a typical hair dye formulation and water was used to suspend 1.4 g of 2,5-TD. The gel was applied to the abdominal skin of dogs for 3 hours (the number of dogs was not specified). The amount of 2,5-TD absorbed into the blood was determined using the Schiff base method. One caveat to this method was that it also indicated derivatives produced from 2,5-TD by oxidation *in vivo*. During the 3-hr exposure, the concentration of 2,5-TD increased to 1 μg/ml of blood. The concentration increased more rapidly during the later stage of the experiment than during the earlier stage. After the gel was removed and the area washed, the concentration in the blood decreased slowly.

In order to determine the amount of 2,5-TD which had been absorbed through the skin, the absorption was imitated by a continuous intravenous infusion of 2,5-TD. An infusion of 0.022 mg 2,5-TD/kg/min was administered for 3 h. During the first two hours, the concentration in the blood was greater than that seen in the absorption experiments; after three hours of infusion, the concentrations were the same. Since each dog weighed approximately 10 kg, it was calculated that 40 mg of 2,5-TD had been infused over the 3-h period. Thus, it was assumed that the same amount was absorbed through the skin of the dogs in 3 hours.

The amount of 2,5-TD excreted in the urine of dogs after a cutaneous application also was investigated using the Schiff base method. A 50 ml application of gel

containing 1.25 g 2,5-TDS was applied to the skin of an unspecified number of dogs for 3 hours. Over 24 h, an average of 0.41 mg of 2,5-TD was found in the urine; 0.1 mg and trace amounts were detected over the following two days (Kiese et al., 1968).

In a similar series of studies regarding the concentrations of 2,5-TD absorbed in the blood and excreted in the urine, the addition of 3% hydrogen peroxide reduced absorption of 2,5-TD. After a 3-h cutaneous exposure to a gel containing 3% hydrogen peroxide and 1.4 mg of 2,5-TD, no 2,5-TD was detected in the blood of dogs. An intravenous infusion of 0.0015 mg of 2,5-TD/kg/min for 3 hours produced a detectable concentration of 2,5-TD. Thus, the absorption through the skin was <3 mg. Absorption of 2,5-TD was proved by the detection of small amounts (60–70 μg) in the urine (Kiese et al., 1968).

The extent of cutaneous absorption was further investigated by Hruby (1977) in rats using radioactive 2,5-TD, [Me-^{14}C] 2,5-TD. The skin of nine female and nine male Sprague-Dawley rats was treated with 0.5 g of a formulation containing 7.5 mg of radioactive 2,5-TD (specific activity: 77.8 mCi/g 2,5-TD). Immediately before application, the formulation was mixed with an equal volume of 6% hydrogen peroxide. After 30 minutes of exposure, the gel was removed and the skin washed. The animals were observed for 24 hours and then terminated. The treated skin was removed and the carcass was homogenized. Over the 24 hours following application, 0.207% of the total dose was absorbed. The majority of the radioactivity (0.14% of the total dose) was found in the urine. Approximately 0.063% of the radioactivity was found in the total-body homogenate.

An identical experiment was conducted using radioactive compound with a specific activity of 22.2 mCi/g. A total of 0.211% of the total dose was absorbed in 24 h; 0.08% was found in the urine, and 0.13% in the total-body homogenate (Hruby, 1977).

A 1 ml subcutaneous administration of 0.4% aqueous [^{14}C]2,5-TD hydrochloride (specific activity: 12.8 mCi/g 2,5-TD) was given to 18 Sprague-Dawley rats. The excretion products were collected daily for five days, and then the animals were sacrificed and their bodies homogenized. Over 65% of the radioactivity was excreted in the urine within 24 h. After 5 days, approximately 15% of the radioactivity was found in the feces and 6.9% was detected in the total-body homogenate (Hruby, 1977).

The extent of cutaneous absorption also was studied using dogs. Fifty milliliters of a formulation containing 1.4 g 2,5-TD ([Me-^{14}C] 2,5-TD, specific activity: 32.0 mCi/g 2,5-TD) was applied for 3 hours to the lateral abdominal region of six Beagle dogs. Blood samples, urine, and feces were collected regularly. Peak concentrations of radioactivity (925.5 pCi/ml) were reached in the blood at 6 h. Over 4 days, 0.092% and 0.840% of the administered dose was excreted in the urine and feces, respectively. The site of application retained 1.79 mCi ^{14}C activity on day 5 (Hruby, 1977).

Three weeks later, the same dogs were used for an intravenous administration. The infusion consisted of 0.224 g 2,5-TD hydrochloride 0.14 g 2,5-TD with a specific reactivity of 15.2 mCi/g 2,5-TD) dissolved in 27.0 ml deionized water. Each dog was infused at a constant rate with 9 ml/h of the solution for 3 hours. Within 2 hours of the start of infusion, peak blood concentrations of radioactivity were achieved. Sixty percent of the radioactivity was found in the urine and 19% in the feces over 4 days. The bulk of this radioactivity was found after the first 24 h (Hruby, 1977).

Eighteen Sprague-Dawley rats were given 1 ml oral doses of a 1.6% aqueous solution of radioactive 2,5-TD hydrochloride (specific activity: 15.8 mCi/g 2,5-TD) by stomach tube. The excretion products were monitored daily and the animals were terminated on day 5. The gastrointestinal tract from the cardia to the anus was removed

and homogenized separately from the rest of the body. Within 24 hours, over 70% of the radioactivity was excreted in the urine. After 5 days, a total of 10% was detected in the feces, 1.2% in the body homogenate, and 1.4% remained in the gastrointestinal tract (Hruby, 1977).

Kiese and Raucher (1968) assessed the absorption of 2,5-TD through human skin. A simple hair dye containing 2.5 g of 2,5-TD was applied to the hair and scalp of five human subjects for 40 minutes. All urine produced in hours 1–4, 5–8, 9–24, 25–36, and 37–48 following application was collected and analyzed using the Schiff base method. Since humans excrete 2,5-TD as *N,N'*-diacetyl-*p*-toluenediamine (*N,N'*-dpt) rather than as a free amine, ethyl acetate, instead of chloroform, was used as the solvent for extracting this compound from the urine. The greatest rate of excretion was observed during the 5–8-h period following the dyeing. On average, the total amount of *N,N'*-dpt excreted was 3.66 mg, which is equivalent to 2.17 mg of 2,5-TD.

In order to estimate the amount of 2,5-TD absorbed from the amount of the *N,N'*-dpt found in the urine, *N,N'*-dpt concentrations were determined using the same methods after subcutaneous injection of a known amount of 2,5-TD. Three women and three men were injected with 2.6 ml of a solution containing 5.54 mg 2,5-TD. As in the hair dyeing experiment, the rate of excretion was highest during the first few hours after the injection. An average of 4.5 mg of *N,N'*-dpt, or more specifically, 2.64 mg of 2,5-TD, was isolated from the urine; thus, 47.6% of the injected dose was excreted as *N,N'*-dpt. The authors calculated that 4.6 mg of 2,5-TD was either absorbed during the dyeing process or liberated *in vivo* from a compound formed during the preparation of the hair dye and absorbed through the skin (Kiese and Raucher, 1968).

ANIMAL TOXICOLOGY

Acute Toxicity

Oral studies. According to the EPA (1977), the following LD_{LO}s have been reported for Toluene-2,5-Diamine:

Oral: unspecified mammal: 3600 mg/kg
Subcutaneous: rat: 50 mg/kg; rabbit: 100 mg/kg.

The oral LD_{50} of Toluene-2,5-Diamine sulfate in an oil-in-water emulsion in rats was reported as 98 mg/kg; the intraperitoneal LD_{50} of the compound in dimethylsulfoxide in rats was 49 mg/kg (EPA, 1977).

A 10% concentration of 2,5-TD was administered to CFY strain rats by oral intubation. Using a geometric dose progression of 1.6, the LD_{50} value was calculated to be 102 mg/kg. The 95% confidence limits were 69–152 mg/kg. Necropsies were performed on the surviving rats. No abnormalities indicative of residual systemic effects were observed (Lloyd et al., 1977).

Subcutaneous studies. In a study of the ulcerogenic activity of 3,4-TD, Sprague-Dawley rats were given subcutaneous injections of 3,4-TD dissolved in 10% ethanol at doses ranging from 62.5–500 mg/kg. The rats were observed for 24 h. No deaths occurred from doses of 300 mg/kg or lower. Mortality increased progressively at greater doses; groups given 350, 400, 450, and 500 mg/kg had mortalities of 5, 30, 50, and

94%, respectively. The incidence of duodenal lesions increased progressively up to 80% as doses increased to 400 mg/kg. At the two highest doses (450 and 500 mg/kg), the incidence decreased to 20 and 27%, respectively (Perkins and Green, 1975).

Subchronic Toxicity

Oral studies. Thirteen female Sprague-Dawley rats were fed 3,4-TD in 2 ml water (individual dose: 50 mg/100 g body weight) twice a day for 5 days by stomach tube. Seven rats died before termination, and 6 of these had perforated duodenal ulcers (Selye, 1973). An oral dose of 33.3 mg in 2 ml water also caused duodenal ulcers (Selye and Mecs, 1974).

Dermal studies. Five hair dye formulations were tested for percutaneous toxicity in New Zealand white rabbits. These formulations contained 0.25, 0.4, 0.6, 3.0, and 6.0% 2,5-TDS, as well as various other hair dye constitutents. Dosages of 1 mg/kg were applied for one hour to either intact or abraded skin of 12 animals twice a week for 13 weeks. The animals were monitored for changes in body weight, for changes in urine composition, and for either hematologic or clinical chemistry abnormalities. No evidence of systemic toxicity was reported (Burnett et al., 1976).

Chronic Dermal Toxicity

Kinkel and Holzmann (1973) conducted a long-term percutaneous study of 2,5-TD in rats. Two groups of 50 male and 50 female Sprague-Dawley rats were treated with solutions containing 2,5-TD. One solution contained 4% 2,5-TD and the second solution contained 3% 2,5-TD and two other important hair dye constituents, resorcinol and *m*-diaminoanisole (each at a concentration of 0.75%). A third group of rats served as untreated normal controls, while a fourth group of rats (25 male and 25 female) were treated with the vehicle alone. In order to simulate typical hair-dyeing techniques, the solutions and the vehicle were mixed with 6% hydrogen peroxide immediately before application. Solutions of 0.5 g were applied to the skin of the rats for 30 minutes twice weekly for two years. The rats were weighed weekly and observed regularly for behavioral changes. No cutaneous irritation was evident in any of the rats during the study, and no marked differences in body weight changes were observed among the groups. The mean lifespan and mortality rate were comparable in the experimental and control groups. The behavior of all the rats was normal.

Three hair dye formulations containing 3% 2,5-TDS were tested for cutaneous toxicity in an 18-month study. The formulations contained various concentrations of other chemical intermediates (*p*-phenylenediamine, resorcinol, *m*-phenylenediamine, 2,4-diaminoanisole sulfate, and 2,4-TD), an were mixed with 6% hydrogen peroxide just prior to use to simulate conventional hair-dyeing techniques. Nine groups of Swiss-Webster mice were given the following treatments: six groups were treated with 0.05 ml of the different formulations once weekly or once every other week, two groups had the vehicle alone applied at the two dosing frequencies, and one positive control group received weekly applications of 0.05 ml of a solution of 7,12-dimethylbenz-[a]anthracene in acetone (total dose during 18 months was ~1.9 mg). One group of 250 mice served as an untreated control group.

No signs of systemic toxicity were observed in any of the dye-treated groups. The average body weight for each of the groups was comparable throughout the study. Survival varied from 58 to 80% except in the positive controls, in which only 21% of the

mice were alive at the termination of the study. Moderate alopecia was reported in 50% of the animals in each of the three groups receiving the dyes once weekly. This occurred during the first five months of the study and was not observed in any of the other groups of mice. The alopecia diminished as the study progressed and the hair coat appeared normal after 11 months. Sections of the treated skin were normal when examined microscopically (Burnett et al., 1975).

A two-year toxicity study of hair dye formulations containing 3% 2,5-TD was conducted using Swiss-Webster mice. The formulations also contained 2,4-TD, *p*-phenylenediamine, and resorcinol. Once a week, 0.05 ml of the solutions was applied to the skin of the mice, and the mice were evaluated for signs of toxicity. Survival rates varied greatly in both the experimental and control groups. The average body weight gains for the treated mice were not significantly different from the untreated controls. No abnormal proliferation and maturation of squamous epithelium of the skin were reported (Giles et al., 1976).

Dermal Irritation and Sensitization

An assessment of dermal irritation was conducted using New Zealand White rabbits. The assessment was based on the procedures prescribed in the Code of Federal Regulations (CFR, Title 16, Sec. 1500.42). 2,5-TD was applied as a 2.5% (w/v) preparation to the intact and abraded skin of three rabbits. Slight erythema and edema were observed at the intact site of all the animals. One rabbit had very slight erythema at the abraded site. The primary irritation index was estimated at 0.3. No signs of irritation were observed after 72 h (Lloyd et al., 1977).

Hartley albino guinea pigs were used to study the cutaneous irritation and sensitization potential of 2,5-TDS and 3,4-TD. Concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10% 2,5-TDS or 3,4-TD were mixed with white petrolatum and applied under occlusive patches to the flanks of 10 animals for 48 h. No animal treated with 2,5-TD had irritation. However, 30% of the guinea pigs treated with 10% 3,4-TD had cutaneous irritation. No irritation was caused by 3,4-TD at lower concentrations (Ishihara et al., 1985).

During the two-week induction phase of the sensitization experiment, a 1% concentration of either 2,5-TDS or 3,4-TD in white petrolatum was applied under occlusive patches to the nape of 10 guinea pigs for 48 h three times per week. The animals were given a two-week nontreatment period before they received challenge applications of either 1% or 0.1% 2,5-TDS and 3,4-TD. The challenge applications were made under occlusive patches to the flanks of the test animals for 48 h, and the animals were observed 24 and 48 h after the test materials had been removed. The sensitization rates of 0.1% and 1% 2,5-TDS were reported to be 10 and 40%, respectively. 3,4-TD was a strong sensitizer; sensitization rates were 90 and 100% at concentrations of 0.1 and 1.0%, respectively (Ishihara et al., 1985).

Ocular Irritation

Lloyd et al. (1977) conducted an eye irritancy test on New Zealand White rabbits. The study was done in accordance with the Code of Federal Regulations (Title 16, Sec. 1500.42). A 2.5% (w/v) solution of 2,5-TD was instilled into the conjunctival sacs of three rabbits. After 10 seconds of exposure, the eyes were rinsed with water. Mild conjunctival inflammation was observed; however, these reactions did not persist for more than 24 h.

Teratogenicity and Reproduction Studies

2,5-TDS was administered by gavage at doses of 10, 50, and 80 mg/kg/day to pregnant rats, and at doses of 10, 25, and 50 mg/kg/day to pregnant rabbits. The rats were dosed on days 6–15 of gestation, and the rabbits on days 6–18. The rats and rabbits were sacrificed on days 19 and 28, respectively, and the mothers and fetuses were examined for abnormalities. Evidence of maternal toxicity and embryotoxicity was reported in the rats receiving 80 mg/kg/day of 2,5-TDS. Neither congenital nor material abnormalities were observed in either the rats treated with the smaller doses of 2,5-TDS or in any of the rabbits (Spengler et al., 1986).

The teratogenic potential of 2,5-TD was studied in pregnant JCL:ddN mice. A 50 mg/kg dose of 2,5-TD dihydrochloride dissolved in distilled water was injected subcutaneously into eight groups of 10 or 11 mice on one of days 7–14 of pregnancy. The mice were terminated on day 18 of pregnancy, and the uteri and fetuses were examined for abnormalities. The greatest teratogenic effects were observed in the mice dosed on day 8; 18% of the fetuses had craniofacial malformations. These mice also had a greater incidence of fused or distorted thoracic vertebrae associated with absent or fused ribs. No such observations were made in the control mice, and did not occur at all or occurred to a much lesser extent in mice treated on the other seven days. All of the mice survived to termination, and no significant differences in the incidence of intrauterine deaths were found between groups and the untreated controls (Inouye and Murakami, 1977).

Based on these results, a more detailed analysis of the teratogenicity of 2,5-TD was carried out on an additional two groups of mice. On day 8 of pregnancy, a larger dose of 2,5-TD dihydrochloride (75 mg/kg) was administered subcutaneously to a group 10 mice, and 50 mg/kg was administered intraperitoneally to a group of 12 mice. Six dams from the subcutaneously treated group and four dams from the intraperitoneal group died before termination. Nearly half of the fetuses in both groups were either dead or were reabsorbed. Thirty-five percent of the fetuses from the subcutaneously treated mice, and 45% of those from the intraperitoneally treated mice had craniofacial and skeletal malformations (Inouye and Murakami, 1977).

Pregnant outbred albino (CD-1) mice were given subcutaneous injections of 16, 32, 48, or 64 mg/kg/day 2,5-TDS on days 6–15 of gestation. The mice were sacrificed on day 18, and maternal and fetal abnormalities were recorded. 2,5-TDS was lethal to four of 31 dams, and nine of 11 dams receiving dosages of 48 and 64 mg/kg/day, respectively. A significant trend ($p < 0.05$) toward maternal weight reduction with increased dosage was reported. Also, a significant decrease ($p < 0.05$) in fetal weight was reported for dams receiving dosages above 32 mg/kg/day. The average percentage of malformed fetuses was very small and did not increase significantly at the greater dosages (Marks et al., 1981).

Five hair dye formulations containing various levels of 2,5-TDS (0.25, 0.4, 0.6, 3, and 6% 2,5-TDS) were cutaneously tested for teratogenic effects on pregnant Charles River CD rats. Four of these formulations were permanent hair dyes, which were mixed with hydrogen peroxide before application, and one formulation (containing 0.25% 2,5-TDS) was a semipermanent dye. Five groups of 20 rats were given 2 ml/kg topical applications on days 1,4, 7, 10, 13, 16, and 19 of gestation. A positive control group received acetylsalicylic acid by gavage, and three negative control groups received no treatment. All animals were sacrificed on day 20. No significant differences in the mean number of corpora lutea, live fetuses, and resorptions per pregnancy were reported for

the experimental animals. There were no significant changes in soft-tissue anomalies between the fetuses of the treated groups and the negative controls. A statistically significant increase in skeletal anomalies (9 of 169 fetuses) was reported for the group of rats receiving the hair dye formulation containing 3% 2,5-TDS. No adverse changes occurred from hair dye formulations containing 6% 2,5-TDS (Burnett et al., 1976).

Becci et al. (1983) studied the teratogenic potential of o-toluenediamine, a mixture of 2,3-TD and 3,4-TD (40:60), in rats and rabbits. Groups of pregnant Sprague-Dawley rats (22–25 animals) were given daily oral doses of 10, 30, 100, and 300 mg/kg on days 6–15 of gestation. o-Toluenediamine was dissolved in corn oil at appropriate concentrations so that the mixture was administered at 10 ml/kg/day to each group. Positive control rats were given aspirin (2.5% suspension administered at 250 mg/kg/day) and negative controls received corn oil (10 ml/kg/day). The rats were observed daily and weighed regularly. On day 20 of gestation, the dams were killed and the uterine contents examined.

The mean weight gain of the high-dose (300 mg/kg/day) dams and their fetuses was significantly different from that of the negative control group. The fetuses had an elevated incidence of missing sternebrae and of incomplete ossification of vertebrae. An elevated incidence of the latter malformation also occurred in the 100 mg/kg-treated group. The authors noted that these malformations were indicative of delayed fetal development. Weight gain was normal in the dams and fetuses of the lower dosage groups, and the number of live fetuses, implantation sites, and resorption sites were normal for all groups. The fetuses from the rats of the lower dosage groups had neither skeletal nor soft tissue malformations related to treatment with o-toluenediamine. The authors concluded that o-toluenediamine was not teratogenic to Sprague-Dawley rats. The adverse effects on the fetus occurred only at dosages that were toxic to the dam.

A similar study was also conducted using Dutch-Belted rabbits. Doses of 3, 10, 30, and 100 mg/kg/day were administered orally to groups of pregnant rabbits (15–16 animals) on days 6–18 of gestation. o-Toluenediamine was dissolved in corn oil at appropriate concentrations so that 2 ml/kg/day of the mixture was administered. A positive control group was given 6-aminonicotinamide as a 0.125% aqueous solution (2.5 mg/kg on day 9). Negative control animals were given corn oil alone (2 ml/kg/day). On day 29 all the rabbits were terminated and the uterine contents examined.

The mean weight gain of the high-dose (100 mg/kg/day) rabbits and their fetuses was significantly smaller than that of the negative control animals. The dams also had a greater number of resorption sites. The mean number of fetuses per dam was decreased in this group, but the difference was not statistically significant. None of the dams or fetuses from the lower dose groups had any of these changes. No fetuses from any of the treatment groups had skeletal malformations. The authors concluded that o-toluenediamine was not teratogenic. Increased resorption rates and delayed fetal development occurred only at dosages that were toxic to the dam (Becci et al., 1983).

Burnett and Goldenthal (1988) conducted a two-generation reproduction study using rats that received cutaneous applications of two oxidative hair-dye formulations containing 3 and 6% 2,5-TDS. Each formulation (0.5 ml) was applied to the skin of 40 Sprague-Dawley rats twice weekly. When the rats were 100 days old, they were mated to produce an F_1 generation. These offspring were subsequently used to produce an F_2 generation using the same procedures. Observations were made during the growth, mating, gestation, and lactation phases of the F_0 parents to the weaning of F_1 and F_2 litters. Comparisons of male and female fertility, gestation, and fetal viability indices and body weights were made between the experimental groups and untreated control

rats. Mild dermatitis was noted during the treatment period in each generation. However, all the rats appeared to be healthy throughout the study; no signs of pharmacotoxicity, teratogenicity, or reproductive abnormalities were reported.

MUTAGENICITY

2,5-TDS was nonmutagenic when tested in CFY rats with the micronucleus test. A 120 mg/kg dose of 2,5-TDS was administered by gastric intubation to five male and five female rats as two equal doses separated by a 24-h interval. The animals were sacrificed 6 h after the second dose was administered and bone marrow slides were prepared. The mean incidence of micronucleated cells per 2,000 polychromatic erythrocytes per rat was 0.9; this value was similar to that obtained with a vehicle control group, and did not fall outside the laboratory standard range for negative control groups (Hossack and Richardson, 1977).

Soares and Lock (1980) conducted a recessive spot test with 2,5-TD. T stock and C57BL/6J (or C57BL/10J) strains of mice were used in the following matings: C57BL/6J (or C57BL/10J) males X C57BL/6J females, and T stock males X C57BL/6J females. The pregnant females were given intraperitoneal injections of 30 mg/kg 2,5-TD or Hanks' balanced salt solution (to serve as controls). The total volume of each injection was 0.1 ml. There were no significant differences in number or morphology of offspring between the experimental and control groups. No evidence of mutagenicity was reported, as none of the offspring from the experimental groups had recessive spots.

A dominant lethal test of 2,5-TD was conducted on Charles River CD rats. 2,5-TD was administered as a 0.2% aqueous solution in a dose volume of 1 ml/100 g body weight. Twenty male rats were given intraperitoneal injections (20 mg/kg) three times per week. After eight weeks, the rats were mated for five days. The female rats were terminated 17 days later and examined for evidence of increased postimplantation fetal loss. No significant differences were reported between the experimental and control groups (Burnett and Corbett, 1977).

Ames et al. (1975) tested 169 different permanent hair-dyes for mutagenicity using *Salmonella typhimurium* strain TA1538. Of 169 dyes, 150 (89%) were mutagenic both before and after mixing with hydrogen peroxide. Some of these dyes were mutagenic alone, and others required microsomal (S9) activation. 2,5-TD, a common constituent in these hair dyes, was weakly mutagenic when activated with S9. When oxidized with hydrogen peroxide and spot tested with S9, 2,5-TD was strongly mutagenic; there was a 40-fold increase in revertants.

2,5-TD was tested for its ability to enhance the transformation of primary hamster embryo cells (HEC) by simian adenovirus 7 (SA7) and for its ability to transform secondary HEC. Cultures of primary HEC were treated with 2,5-TD (at a concentration range of 3.13–50 μg/ml) 18 h to the addition of SA7, or 48 h immediately after absorption of SA7 (at a concentration range of 1–200 μg/ml). For both protocols, 2,5-TD was active, producing an absolute increase in the number of virus-transformed foci per plate. A dose–response relationship was observed in one set of cells treated prior to the addition of the virus. 2,5-TD also transformed secondary HEC (at concentrations of 5 and 10 μg/ml), but did not produce a clear dose-response relationship. The authors concluded that 2,5-TD has ". . . a low but definite potential for inducing genetic damage" (Greene and Friedman, 1980).

3,4-TD was also active in the transformation protocols of Greene and Friedman (1980). 3,4-TD was administered at concentrations of 12.5–200 μg/ml to primary HEC prior to the addition of SA7, or at concentrations of 10–200 μg/ml after the addition of SA7. A dose–response relationship was observed in both protocols, and an absolute increase in the number of foci per dish at all dosages occurred when 3,4-TD was administered prior to SA7 addition. 3,4-TD (at concentrations 2.5–10 μg/ml) also enhanced secondary HEC transformation. However, at higher concentrations (15–50 μg/ml) no transformation occurred.

3,4-TD was not active in the standard Ames plate incorporation testing using *S. typhimurium* strains TA98 and TA100 with and without metabolic activation (Greene et al., 1979).

Florin et al. (1980) used the Ames test to evaluate the mutagenic potential of 3,4-TD (3 μmol/plate), using *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 both with and without metabolic activation. 3,4-TD was nonmutagenic.

The mutagenic potential of 3,4-TD was tested alone and with the addition of H_2O_2 using the Ames test. *S. typhimurium* strain TA98 was used both with and without metabolic activation with S9. 3,4-TD and its oxidation products were not mutagenic in either system (Watanabe et al., 1989).

The DNA repair induced by 2,5-TD was determined in rat and hamster hepatocytes using the primary hepatocyte culture/DNA repair assay. Hepatocytes obtained from male Sprague-Dawley rats and male Golden Syrian hamsters were treated with 2,5-TD at molar concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and 2×10^{-7}. Two sets of assays were done. An average net nuclear count of 5 or greater was reported as a positive response, since these values differed from the control net nuclear counts by more than two standard deviations. The 10^{-4} M treatment was toxic in both types of hepatocytes. At 10^{-5} M, 2,5-TD produced a positive response (net nuclear count: 8.2 and 5.9) in the rat hepatocytes, and a weak positive response (net nuclear count: 4.1 and 2.5) in the hamster hepatocytes. The lower concentrations of 2,5-TD had negative responses. The primary purpose of this study was to determine relative differences in DNA repair responses between the rat and hamster hepatocytes, not to evaluate the genotoxic status of 2,5-TD; thus, no statistical comparisons between data from control and experimental hepatocytes were performed (Kornbrust and Barfknecht, 1984).

2,5-TDS significantly inhibited the incorporation of [^{125}I]iododeoxyuridine into murine testicular DNA in a dose-dependent manner. Groups of 10 mice were given intraperitoneal injections of 2,5-TDS at doses of 40, 44.5, 49.5, and 55 mg/kg. Control mice were given 100 mg/kg of dimethylnitrosamine perorally. After three hours, 10 μCi [^{125}I]iododeoxyuridine was injected intraperitoneally. The testes were removed 30 minutes later for analysis. As the doses increased, the radioactivity incorporated into the testicular DNA decreased; the radioactivity in the DNA decreased from 1.77 dpm/μg DNA (control animals) to 0.99 dpm/μg DNA (high-dose animals 55 mg/kg). This inhibition of DNA synthesis was not the result of a drop in body temperature, as the rectal temperature was not significantly reduced; and 2,5-TDS did not inhibit the radioisotope from reaching the testes, as the soluble radioactive pool remained unchanged after treatment. Since 2,5-TDS was capable of reaching the testes and permeating target cell membranes at this site, 2,5-TDS might represent a genetic health hazard to animals (Greene et al., 1981).

The same methods were also used to study the effect of 3,4-TD on murine testicular DNA synthesis. Doses of 200, 229, 262, and 300 mg/kg 3,4-TD were administered intraperitoneally to mice. As seen in the 2,5-TDS treated mice, the amount of

radioactivity incorporated into the testicular DNA decreased as the dosage of 3,4-TD increased. Radioactivity decreased from 0.79 dpm/μg (control) to 0.44 dpm/μg (high dose: 300). The authors concluded that 3,4-TD was also a potential genetic health hazard to animals (Greene et al., 1981).

A summary of the preceding data is recorded in Table 4.

CARCINOGENICITY

A bioassay of 2,5-TDS for possible carcinogenicity was conducted by the National Cancer Institute (1978). Fifty Fischer 344 rats of each gender and 50 B6C3F1 mice of each gender were given doses of 2,5-TDS in their feed for 78 weeks. The rats received 2,5-TDS at concentrations of either 0.06% (the dose was 0.05% for the first 15 weeks) or 0.2%, and the mice received either 0.06% or 0.1%. The animals were monitored for changes in body weight and rate of survival, and clinical observations were made regularly. Gross and microscopic examination of major tissues, organs, and gross lesions were conducted on all the animals, either at interim death or at termination of the study.

There was no evidence that 2,5-TDS accelerated the mortality of either sex of either species. In both the low- and high-dose male rats, the incidence of interstitial cell neoplasms of the testis was statistically significant (43 of 48 rats in the low dose group, and 47 of 48 rats had such neoplasms). However, the development of these neoplasms was not attributed to 2,5-TDS since spontaneous incidence of these neoplasms is known to be both high and variable in male Fischer 344 rats. The incidence of neoplasms in treated female rats was not significantly different from control rats.

In the study using the B6C3F1 mice, there was no significant increase in the development of neoplasms in the male mice of either treatment group. The high-dose-treated female mice had an elevated incidence of alveolar/bronchiolar adenomas and carcinomas (8 of 45 mice had one or both types of neoplasms). The authors noted that the female high-dose and low-dose–treated mice were received in separate shipments and were housed in different rooms. Thus, it was concluded that the data did not provide sufficient evidence of a compound-related effect (NCI, 1978).

Two groups of 50 male and 50 female Sprague-Dawley rats were treated with hair dyes containing 2,5-TD in a 2.5 year percutaneous study. One solution contained 4% 2,5-TD alone and the second solution contained 3% 2,5-TD with other important hair dye constituents (resorcinol and *m*-diaminoanisole). The hair dyes were mixed with 6% hydrogen peroxide immediately before application, and 0.5 g of the solutions was applied to the skin of the rats for 30 min twice weekly for two years. Fibroadenomas, fibromas, lymphosarcomas, and round-cell sarcomas were reported during the second half of the life of both groups of rats. The incidence of the neoplasms, and the proportion of male and female rats affected were not significantly different from that observed in vehicle and untreated control rats. None of the neoplasms in the experimental animals developed at the site of application. The authors concluded that neither of these hair dyes induced carcinogenic effects (Kinkel and Holzmann, 1973).

In conjunction with the 18-week toxicity study and the two-generation reproduction study, described earlier in this report, Burnett et al. (1975) and Burnett and Goldenthal (1988) reported that no evidence of carcinogenic activity was observed from topical applications of hair dyes containing 3% and 6% 2,5-TDS. In the toxicity study, the incidence of alveologenic adenomas and carcinomas in the three groups of

Table 4. Mutagenicity Studies

Ingredient	*Strain/cells tested*	*Methodology*	*Results*	*References*
2,5-TDS	CFY rats	Micronucleus test	Negative	Hossack and Richardson (1977)
2,5-TD	T stock and C57BL/6J mice	Recessive spot test	Negative	Soares and Lock (1980)
2,5-TD	Charles River CD rats	Dominant lethal test	Negative	Burnett and Corbett (1977)
2,5-TD	*S. typhimurium* TA1538	Ames test with S9 activation	Weakly mutagenic	Ames et al. (1975)
2,5-TD mixed with H_2O_2	*S. typhimurium* TA1538	Ames test with S9 activation	Strongly mutagenic	Ames et al. (1975)
2,5-TD	Hepatocytes from Sprague-Dawley rats and Golden Syrian hamsters	Primary hepatocyte culture/DNA repair assay	Positive	Kornbrust and Barfknect (1984)
2,5-TD & 3,4-TD	Primary hamster embryo cells (HEC)	Enhancement of transformation by simian adenovirus 7 (SA7)	Positive	Greene and Friedman (1980)
2,5-TD & 3,4-TD	Secondary HEC	Transformation of secondary HEC	Positive	Greene and Friedman (1980)
2,5-TDS & 3,4-TD	Mice	Effects on murine testicular DNA synthesis	Inhibited DNA synthesis	Greene et al. (1981)
3,4-TD	*S. typhimurium:* TA98 and TA100	Ames test with and without S9 activation	Negative	Greene et al. (1979)
3,4-TD	*S. typhimurium:* TA98, TA100, TA1535, and TA1537	Ames test with and without S9 activation	Negative	Florin et al. (1980)
3,4-TD & 3,4-TD mixed with H_2O_2	*S. typhimurium:* TA98	Ames test with and without S9 activation	Negative	Watanabe et al. (1989)

Swiss-Webster mice treated with 3% 2,5-TDS was comparable to that reported in control groups. The incidence, type and distribution of other neoplasms were not significantly different (Burnett et al., 1975).

In the multigeneration study, 120 F_1 generation Sprague-Dawley rats were given topical applications of the same hair dyes as their parents (containing either 3% or 6% 2,5-TDS) twice a week for two years. A wide variety of nonneoplastic lesions was observed in both the experimental and control groups. The incidence of neoplasms was highly variable in all of the groups and could not be directly correlated to hair dye exposure (Burnett and Goldenthal, 1988).

Hair dye containing 3% 2,5-TDS, 1.5 *p*-phenylenediamine, 0.4% resorcinol, and either 0.2% or 0.6% 2,4-TD was used in a carcinogenicity study conducted by Giles et al. (1976). For two years, 28 Swiss-Webster mice of each gender were given 0.05 ml applications of the dye mixed with 6% hydrogen peroxide once a week for two years. The type, number, incidence and distribution of both benign and malignant neoplasms observed in the experimental groups was comparable to that of the control groups. However, IARC (1978) reported that ". . . a large number of animals were unaccounted for in the final analysis of tumor incidence, thus making the published data inadequate for the evaluation of the carcinogenicity of this chemical [2,5-TDS]." In its overall evaluation of 2,5-TD, the IARC concluded that there was inadequate evidence to regard this amine as carcinogenic to animals (IARC, 1987).

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

Lynde and Mitchell (1982) patch tested 66 hairdressers to 1% 2,5-TD in petrolatum. Details of their methods were not given. Five subjects had positive reactions.

Patches of 2,5-TD (1% in petrolatum) were applied to the skin of 9 beauticians with allergic contact dermatitis. Readings were taken 72 h after the patch was applied. Positive reactions were reported in 7 of the beauticians (Matsunaga et al., 1988).

2,5-TD was tested for its sensitization potential using 31 men and the maximization test. Two percent aqueous 2,5-TD was used for induction and challenge. None of the subjects were sensitized (Epstein and Taylor, 1979).

EPIDEMIOLOGY

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

A variety of published studies have assessed the association between occupational exposure to and use of hair dyes and the risk of cancer. These studies do not note which specific hair dye ingredients were involved in the human exposure. A summary of reports of how occupational exposure to hair dye affects the risk of bladder cancer (Anthony and Thomas, 1970; Cole et al., 1972; Dunham et al., 1968; Wynder et al., 1963) and lung cancer (Garfinkel et al., 1977; Menck et al., 1977), or use of hair dyes affects the risk of bladder cancer in men or women (Jain et al., 1977) and breast cancer

in women (Hennekens et al., 1979; Kinlen et al., 1977; Nasca et al., 1979; Shafer and Shafer, 1976; Shore et al., 1979; Wynder and Goodman, 1983) has been published in previous Cosmetic Ingredient Review reports on *p*-phenylenediamine, 2-nitro-*p*-phenylenediamine, and 4-nitro-*o*-phenylenediamine (Elder, 1985a,b). In the small case-controlled study by Shore et al. (1979), a positive correlation between hair dye and breast cancer was reported. When their study was extended to include 398 breast cancer cases, the same investigators could not implicate hair dye use as an important cause of human breast cancer (Koenig et al., 1991). The latter study indicated that beauticians who work for five or more years in this occupation have an increased breast cancer risk. However, the increased risk was not a strong finding, and "if beauticians are at increased breast cancer risk, exposures other than hair dyes may be responsible" (Koenig et al., 1991).

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

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

SUMMARY

2,5-TD, 2,5-TDS, and 3,4-TD are diaminotoluenes used as colorants in permanent hair dyes and tints. 2,5-TD is used at concentrations up to 1%, and 2,5-TDS is used up to 5%. No formulation data were available for 3,4-TD.

2,5-TD penetrated the skin of rats, dogs, and humans. The extent of penetration varied according to the formulation. The major routes of excretion after cutaneous absorption were through the urine and feces.

The oral LD_{50} levels of 2,5-TDS in an oil-in-water emulsion and 10% 2,5-TD in rats were 98 and 102 mg/kg, respectively. The intraperitoneal LD_{50} of 2,5-TDS for rats was 49 mg/kg.

Subcutaneous doses of 450 and 500 mg/kg of 3,4-TD dissolved in 10% ethanol caused mortality rates of 50% or greater in Sprague-Dawley rats. 3,4-TD also markedly increased the incidence of duodenal lesions when given at doses between 250 and 400 mg/kg.

In a subchronic toxicity study, 3,4-TD was toxic to Sprague-Dawley rats. More than 50% of the rats given twice-daily oral doses (50 mg/100 g body weight) died within 5 days. Most of these animals had perforated duodenal ulcers.

There was no evidence of percutaneous toxicity to New Zealand White rabbits when 6% 2,5-TDS was applied to intact or abraded skin during a 13-week study. In a two-year study, no toxicity was reported in rats receiving bi-weekly cutaneous applications of formulations containing 3–4% 2,5-TD. Swiss-Webster mice were also unaffected by 3% 2,5-TDS in an 18-month study, or by 3% 2,5-TD in a two-year study.

New Zealand White rabbits suffered from slight dermal irritation after exposure to 2.5% 2,5-TDS, but no irritation occurred in guinea pigs after exposure to 10% solutions

of 2,5-TDS or 3,4-TD. The sensitization rates of 0.1% and 1% 2,5-TDS to guinea pigs were 10% and 40%, respectively. 3,4-TD was a strong sensitizer, having rates of 90% and 100% at concentrations of 0.1% and 1%, respectively.

2,5-TD (2.5%) caused mild, transitory conjunctival inflammation in rabbits. 2,5-TDS was toxic to pregnant rats and their embryos at oral doses of 80 mg/kg/day. Doses between 10 and 50 mg/kg/day did not cause congenital or maternal abnormalities.

o-Toluenediamine, a mixture of 2,3-TD and 3,4-TD (40:60), was not teratogenic when administered orally to Sprague-Dawley rats or Dutch-Belted rabbits. There was an increase in resorption rate and skeletal abnormalities, but these changes occurred only at concentrations which were toxic to the dams (300 mg/kg/day for rats, and 100 mg/kg/day for rabbits).

Subcutaneous doses of 32–70 mg/kg/day, of 2,5-TD dihydrochloride were toxic to dams and their fetuses, and caused fetal, craniofacial malformations.

Cutaneous exposure to hair dye formulations containing 3% 2,5-TDS caused a statistically significant increase in fetal skeletal anomalies in rats. Rats treated with 6% 2,5-TDS did not have this adverse response.

In a two-generation reproduction study, mice receiving cutaneous applications of hair-dye formulations containing either 3% or 6% 2,5-TDS had no signs of pharmacotoxicity, teratogenicity, or reproductive abnormalities.

2,5-TD was nonmutagenic in a recessive spot test or a dominant lethal test. It was weakly mutagenic in the Ames test using *S. typhimurium* strain TA1538 with S9 activation, and was strongly mutagenic when oxidized with hydrogen peroxide and spot tested with S9. 2,5-TDS was nonmutagenic in the micronucleus test using CFY rats.

3,4-TD was not active in the Ames plate incorporation test or spot test. Its oxidation products were also nonmutagenic.

Primary HEC were transformed by 2,5-TD and 3,4-TD both prior to and after the addition of SA7. Dose–response relationships were demonstrated. 2,5-TD and 3,4-TD also transformed secondary hamster embryo cells, but did not demonstrate a dose–response relationship.

2,5-TDS and 3,4-TD were identified as potential genetic health hazards to animals. Murine testicular DNA synthesis decreased in a dose dependent manner when these ingredients were injected intraperitoneally into mice.

A bioassay of 2,5-TDS for carcinogenicity was negative in rats fed either 0.06% or 0.2% 2,5-TDS, and mice fed either 0.06% or 0.1% 2,5-TDS. No evidence of carcinogenicity was reported in several studies of rats and mice treated with cutaneous applications of hair dyes containing either 3% or 4% 2,5-TD, and either 3% or 6% 2,5-TDS.

2,5-TD was a sensitizer in clinical studies.

DISCUSSION

2,5-TD and 2,5-TDS are used as colorants in permanent hair dyes at concentrations up to 5 and 1%, respectively. The oxidative or permanent hair dyes containing 2,5-TD or 2,5-TDS, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and appropriate "patch test" instructions for determining whether the product caused skin

irritation. The patch test, in which the intermediates and hydrogen peroxide are mixed in the same manner as in use, is to be performed prior to each and every application of the hair dye. 3,4-TD is not reported to be presently used in cosmetics.

The toluene diamines exhibit low to medium acute toxicity in rats and rabbits. 2,5-TD is absorbed up to 0.18% through human skin. 2,5-TD is a mild eye irritant. The Panel noted that 3,4-TD has not been tested for ocular irritation. 2,5-TD (2.5%), 2,5-TDS (5%), and 3,4-TD (5%) were not dermal irritants to rabbits or guinea pigs. However, 2,5-TD (1%) is a sensitizer, and 3,4-TD (1%) a strong sensitizer in studies using guinea pigs. Photosensitization data were not available for these toluene diamines. 2,5-TD is mutagenic in the Ames assay, especially in the presence of H_2O_2, and is active in unscheduled DNA synthesis in the rat liver DNA assay. There is inadequate evidence to regard 2,5-TD as carcinogenic to animals. 3,4-TD is inactive in the Ames mutagenicity test.

The Panel cautioned that 2,5-TD may be a sensitizer to hairdressers and beauticians who have frequent contact with hair dyes.

Although there were no available studies regarding either the chronic toxicity or carcinogenic potential of 3,4-TD, the Panel agreed that the available data were sufficient to make a safety evaluation.

CONCLUSION

On the basis of the animal and clinical data presented in this report, the CIR Expert Panel concludes that Toluene-2,5-Diamine and Toluene-2,5-Diamine Sulfate are safe as cosmetic ingredients in the present practices of use. Although Toluene-3,4-Diamine is not reported to be presently in use, the available data are supportive of its safety at the same use levels as Toluene-2,5-Diamine and its sulfate.

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