Final Amended Report of the Cosmetic Ingredient Review Expert Panel

Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

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The 2007 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina L. Burnett, CIR Scientific Analyst/Writer.

Cosmetic Ingredient Review
1101 17th Street, NW, Suite 412 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊ cirinfo@cir-safety.org

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Washington, DC 20036

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Abstract: 2,4-Diaminophenoxyethanol HCl is an aromatic amine salt that is an odorless white, slightly gray, or lavender gray powder. 2,4-Diaminophenoxyethanol Sulfate also is an aromatic amine salt for which no chemical, physical, or toxicological data were available. 2,4-Diaminophenoxyethanol HCl is soluble in water and DMSO up to 10% (w/w), but is insoluble in solvents such as acetone and propylene glycol. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are used as couplers mixed with primary intermediates in permanent (oxidative) hair dyes. Both ingredients are used in concentrations up to 2%. These ingredients are accepted for use in the European Union and Japan. Dermal absorption of 2,4-Diaminophenoxyethanol is low. The results of 3 acute oral toxicity studies found 2,4-Diaminophenoxyethanol HCl to have an oral LD_{50} ranging from 1160 to 1760 mg/kg in mice and 1000 to 1191 mg/kg in rats. No clinical signs were observed in one subchronic toxicity test using mice and rats and only discoloration was observed in rats in another study. In another subchronic oral toxicity study using rats, a NOEL of 20 mg/kg/day was reported for 2,4-Diaminophenoxyethanol HCl. At 100 mg/kg/day, a higher incidence of hemosiderosis (without associated hematological changes), increased salivation, lower weight gain in males, colored urine, traces of nitrites and bilirubin in urine. 2,4-Diaminophenoxyethanol HCl was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits in two studies. When tested neat using rabbits, it was found to be an ocular irritant. 2,4-Diaminophenoxyethanol HCl was slightly irritating to the skin of rabbits when a 4% solution was used. When tested neat using rabbits, it also was not considered an irritant. In a guinea pig maximization study, 2,4-Diaminophenoxyethanol HCl produced erythema in 3/10 guinea pigs at challenge. In a study using a Buehler test methodology, 2,4-Diaminophenoxyethanol HCl applied neat did not produce sensitization reactions. An LLNA study of 2,4-Diaminophenoxyethanol HCl at 0.5 to 10% in DMSO did show lymphoproliferative responses indicative of delayed contact hypersensitivity. Overall, 2,4-Diaminophenoxyethanol HCl could be considered a moderate sensitizer. No teratogenic effects were observed due to administration of 2,4-Diaminophenoxyethanol HCl in an oral study using rats or in a dermal study using mice. In another rat study, an oral dose of 125 mg/kg was maternally toxic and associated with fetal weight deficits and some delayed ossification; the NOEL was 20 mg/kg. Genotoxicity assays using bacterial, mammalian cells, drosophila, mice, and rats provided mixed results. In most bacterial assays, the results were negative, but an increase in mutation frequency was reported in two studies using *S. typhimurium* TA98 with metabolic activation. In mammalian cell assays, results were negative, except for one study which found an increase in micronucleated cells in human lymphocytes that were mitogen-stimulated for 48 h (but no increase with 24 h mitogen stimulation). In animal assays (dominant lethal, micronucleus, unscheduled DNA synthesis), no evidence of genotoxicity was reported. In 2 oral carcinogenic studies, treatment of mice and rats with 2,4-Diaminophenoxyethanol HCl produced no carcinogenic effects. No human studies were available that specifically addressed these two ingredients. Available epidemiology studies that consider the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other endpoints were described. These data were considered insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations and inconsistency of findings. Hair dyes containing these ingredients, as coal tar hair dye products, should have labeling which includes a caution statement and patch test instructions for determining whether the product causes skin irritation. Following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposure. While no toxicity studies were identified specifically for the sulfate salt, the toxicities of the two salts are expected to be the same, and their maximum use concentrations are the same, so exposures as used in hair dyes would be the same. Therefore, these two chemicals are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment.

INTRODUCTION

A safety assessment for 2,4-Diaminophenoxyethanol Dihydrochloride was published by the Cosmetic Ingredient Review (CIR) Expert Panel in 1991 with the conclusion that this ingredient is "safe as a cosmetic ingredient in the present practices of use and concentration" (Elder 1991). In the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol Dihydrochloride is now called 2,4-Diaminophenoxyethanol HCl (Gottschalck and McEwen 2006).

2,4-Diaminophenoxyethanol Sulfate has been added to the safety assessment. While no toxicity data were available for the sulfate salt, it is not considered likely that the toxicity of the sulfate is significantly different from that of the

hydrochloride salt or the free base. Both of these ingredients are used as couplers in permanent (oxidative) hair dyes.

CHEMISTRY

Definition and Structure

According to the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol HCl (CAS No. 66422-95-5) is the aromatic amine salt that conforms to the structure shown in Figure 1 (Gottschalck and McEwen 2006).

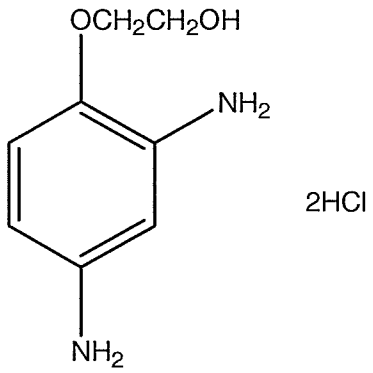

Figure 1. Chemical structure for 2,4-Diaminophenoxyethanol HCl

A technical name/synonym is Ethanol, 2-(2,4-Diaminophenoxy)-, Dihydrochloride (Gottschalck and McEwen 2006).

Trade names include:

- Colorex OAJ,
- Imexine OAJ,
- Jarocol DPE (2 HCl),
- Rodol 24 Dape, and
- Velsol Blue A42.

2,4-Diaminophenoxyethanol Sulfate (CAS No. 70643-20-8) is the substituted aromatic amine that conforms to the structure shown in Figure 2 (Gottschalck and McEwen 2006).

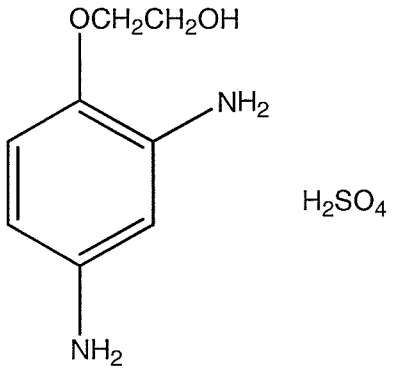

Figure 2. Chemical structure for 2,4-Diaminophenoxyethanol Sulfate

Technical names include:

- Ethanol, 2-(2,4-Diaminophenoxy)-, Sulfate (1:1) (Salt), and
- [4-(2-Hydroxyethoxy)-1,3-Phenylene]-Diammonium Phosphate.

A trade name is JAROCOL DPE (Gottschalck and McEwen 2006).

Properties

2,4-Diaminophenoxyethanol HCl is an odorless white, slightly gray powder that is soluble in water and insoluble in acetone and 95% ethanol. Physical and chemical properties of 2,4-Diaminophenoxyethanol HCl are summarized in Table 1. No physical or chemical properties were found for 2,4-Diaminophenoxyethanol Sulfate.

Analytical Methods

Groult (2004) described an analytical method for detection of 2,4-Diaminophenoxyethanol HCl using high performance liquid chromatography with UV radiation detection (300 nm). The accuracy of the method was >100 ± 10% and the imprecision was ≤10%. The author reported that this method can quantify 2,4-Diaminophenoxyethanol HCl over a concentration range of 5 - 100 μg/ml. The limit of detection was 0.005 mg/ml in purified water and 0.05 mg/ml in DMSO.

Impurities

1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene, and 2,4-diamino-1-ethoxybenzene are not detected as impurities in 2,4-Diaminophenoxyethanol HCl (Cosmair, Inc. 1989). No information regarding impurities that may be present in 2,4-Diaminophenoxyethanol Sulfate was found.

USE

Cosmetic

According to the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate function as hair colorants (Gottschalck and McEwen 2006), and the reported product categories are hair dyes and colors (all types requiring caution statements and patch tests).

Industry reports to the Food and Drug Administration (FDA) under the FDA's Voluntary Product Registration Program (VCRP) for 2,4-Diaminophenoxyethanol HCl in 2006 indicated 115 total cosmetic uses, all as hair dyes and colors (FDA 2006). 2,4-Diamino-phenoxyethanol Sulfate was reported to have a total of 5 uses, all in hair dyes and colors (FDA 2006).

An industry survey conducted by the Cosmetic, Toiletry, and Fragrance Association (CTFA) reported current use concentration ranges for 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate to be 0.05 - 2% and 0.4 - 2%, respectively (CTFA 2007).

Table 1. Chemical and Physical Properties of 2,4-Diaminophenoxyethanol HCl

Property	Description	Reference
Physical appearance	white powder, slightly gray	Cosmair, Inc. 1989
	lavender-gray powder	Brusick et al. 1982
Odor	None	Cosmair, Inc. 1989
Molecular weight	241	COLIPA 1988
Empirical formula	$C_8H_{12}N_2O_2 \cdot 2ClH$	Gottschalck and McEwen 2006
Melting point	198 - 216°C	Shahin et al. 1983
Free base melting point	84°C	Kalopissis 1981
Solubility	water - soluble at 25°C	Cosmair, Inc. 1989
	95% ethanol - insoluble at 25°C	
	acetone - insoluble at 25°C	
	acetone/olive oil - insoluble	Groult 2004
	dimethylformamide - insoluble	
	methyl ethyl ketone - insoluble	
	propylene glycol - insoluble	
	DMSO - 10% after 30 min stirring	
	10% in water	Toner 2005
Spectrum absorbance (λ_m - absorbancy)	238 ± 5 - 0.630 ± 0.050 286 ± 5 - 0.260 ± 0.020	Cosmair, Inc. 1989
Decomposition point (thermopan miscroscope)	198 - 217°C	COLIPA 1988
Titer, potentiometry	$\geq$ 99.5%	COLIPA 1988
Assay, % acid function	99 ± 1	Cosmair, Inc. 1989
Chloride concentration	28.0 - 31.0	Cosmair, Inc. 1989

Table 2 presents the current usage and use concentrations data for 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as a function of product category.

According to COLIPA (1988) the amount of 2,4-Diaminophenoxyethanol HCl added to an oxidative hair dye formulation is based on a desired shade, with concentrations generally as follows: less than 0.025% in light tones, between 0.025 and 0.1% in medium tones, and between 0.1% and 4% in darker tones (which represents a smaller part of the market). 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from the principal adulteration provision and from the color additive provisions in §601 and §706 of the Federal Food, Drug, and Cosmetic Act of 1938 (FDA 1979).

Product labels shall bear a caution statement and patch test instructions for determining whether the product causes skin irritation. In order to be exempt, the following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

> **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 eyelashes or eyebrows; to do so may cause blindness.

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

> The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.
>
> Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.

Table 2. Cosmetic product uses and concentrations for 2,4-Diaminophenoxyethanol salts.

Product Category	2006 uses (total number of products in a category; FDA 2006)	2007 use concentrations (CTFA 2007) (%)
	2,4-Diaminophenoxyethanol HCl	
Hair coloring products		
Dyes and colors	115 (1600)	0.05 - 2
Total uses/ranges for 2,4-Diaminophenoxyethanol HCl	**115**	**0.05 - 2***
	2,4-Diaminophenoxyethanol Sulfate	
Hair coloring products		
Dyes and colors	5 (1600)	0.4 - 2
Total uses/ranges for 2,4-Diaminophenoxyethanol Sulfate	**5**	**0.4 - 2***

*The maximum 4% concentration is reduced to a final on-head concentration of 2% when combined with hydrogen peroxide on application.

There is a consensus among dermatologists that screening patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group 1980; Eiermann et al. 1982; Adams et al. 1985). Basically, these procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder 1985).

During the August 26-27, 1991 public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 hours to 48 hours after application of the test material.

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

In the European Union (2005), 2,4-Diaminophenoxyethanol and its salts have been listed in Annex III (part 2) as provisionally allowed substances with use as oxidizing coloring agents for hair dyeing. The maximum authorized concentration in finished cosmetic products is 4.0%, with the understanding that these dyes are used in combination with hydrogen peroxide, so that the maximum use concentration of the dye upon application is 2.0%.

According to the Ministry of Health, Labor, and Welfare (MHLW) of Japan, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are not included on the list of ingredients that must not be used in cosmetic products that are marketed in Japan. However, in Japan, hair dyes are regulated as quasi-drugs and all ingredients, both active and inactive, must be specifically approved. 2,4-Diaminophenoxyethanol HCl is an approved hair dye active while the sulfate salt is not specifically approved (MHLW 2005).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Animal

Female hairless Wistar rats were used to determine the penetration of ^{14}C-2,4-Diamino-phenoxyethanol HCl (^{14}C uniformly on the ring-specific activity: 0.8 μCi/mg) (Tsomi and Kalopissis 1982a). The penetration of ^{14}C-2,4-Diaminophenoxyethanol HCl was determined as pure compound and in a complete commercial formulation consisting of ^{14}C-2,4-Diaminophenoxyethanol HCl (0.40%), ^{14}C-2,4-diaminoanisole (0.33%), p-phenyldiamine hydrochloride (1.8%), resorcinol (0.05%), and *m*-aminophenol (0.1%). Both the individual compound and the formulation were dissolved in a vehicle containing nonionic and amphoteric surfactants, alcohols, glycols, oleic acid, copra diethanoamide, antioxidants and complexing agents, water, and 10% aqueous ammonia. Immediately before use, the solution was mixed with an equal volume of 20% hydrogen peroxide solution. The animals were anesthetized, and 20 mg/cm^2 of compound was applied to a 25 cm^2 area of the dorsal region. The length of time the compound remained in contact with the skin was 40 min. Following the exposure period, excess test material was removed, and a stripping process was carried out on the site of application to avoid contamination of the excrement. For 4 days, the feces and total amount of urine excreted were collected at 24-h intervals

and analyzed. The animals were then killed and necropsied in order to determine the quantity of compound that had been absorbed and not excreted. In the majority of cases, the visceral organs, carcasses, skin (except for the site of application), and, in certain cases, an additional number of selected organs were examined for residual radioactivity.

The quantity of compound that penetrated was 5.05 ± 0.79 nM (0.84 ± 0.13 µg) of pure compound per cm^2 of skin and 2.83 ± 0.49 nM (0.47 ± 0.08 µg) of compound in the commercial formulation per cm^2 of skin. Penetration of 2,4-Diaminophenoxyethanol HCl in the commercial formulation was 40% of the penetration of the pure compound alone.

The study was then carried out applying the hair dye solutions every 30 to 40 days (duration of testing not given) to simulate human hair-dying frequency. The livers and thyroids of the treated rats were examined for accumulation of test article. At the highest doses, with the animals being killed 4 days after treatment, no radioactivity appeared in the thyroid, and only trace amounts appeared in the liver (Tsomi and Kalopissis 1982a).

Tsomi and Kalopissis (1982b) used female hairless Wistar rats to measure the absorption of [^{14}C]2,4-Diaminophenoxyethanol HCl dissolved in a commercial vehicle at concentrations of 0.40% (23.65 nM), 0.80% (47.30 nM), and 1.20% (70.95 nM).

The solution was mixed with an equal volume of 20% H_2O_2 before use, and 20 mg/cm^2 was applied to a 25 cm^2 area on the back of each rat, 6 rats per group, for a period of 40 min. The animals were then killed and necropsied to determine the amount of compound absorbed and not yet excreted.

The penetration per cm^2 was between 5.03 ± 0.79 nM (0.84 ± 0.13 µg) for the lowest concentration and 9.42 ± 0.84 nM (1.58 ± 0.14 µg) for the highest concentration (Tsomi and Kalopissis 1982b).

Human

Toner (2005) determined the percutaneous absorption of 2,4-Diaminophenoxyethanol (dihydrochloride salt) using human dermatomed skin. Skin samples were obtained from breast and abdominal skin, transferred on ice, and frozen until used. Skin was dermatomed to a thickness of 370-400 microns and mounted in diffusion cells.

2-(2,4-diamino[ring-U-^{14}C]phenoxy)ethanol hydrochloride as the radioactive tracer added to 98.7% pure 2,4-Diaminophenoxyethanol (dihydrochloride salt) comprised the test material. Table 3 describes the composition of three formulations (oxidative, non-oxidative, and "placebo") used in this study. A 4th formulation was the developer only, H_2O_2 at 6%.

Table 3. Percent composition of formulations used in percutaneous absorption studies (Toner 2005).

Ingredient	Formulation 1 (oxidative)	Formulation 2 (non-oxidative)	Formulation 3 ("placebo")
Oleyl Alcohol	5.00	5.00	5.00
p-Phenylenediamine	1.79	-	-
Hexylene Glycol	9.00	9.00	9.00
Trideceth-2 Carboxamide MEA	10.00	10.00	10.00
Oleic Acid	3.00	3.00	3.00
Ammonium Hydroxide	4.12	4.12	4.12
Pentasodium Pentatate	0.96	0.96	0.96
PEG-2 Oleamine	7.00	7.00	7.00
Propylene Glycol	4.00	4.00	4.00
Alcohol Denat.	6.52	6.52	6.52
2,4-Diaminophenoxyethanol HCl	3.59	3.59	-
[^{14}C]2,4-Diaminophenoxyethanol HCl	0.41	0.41	-
Polyglyceryl-2 Oleyl Ether	4.00	4.00	4.00
Polyglyceryl-4 Oleyl Ether	5.46	5.46	5.46
Sodium Metabisulfite	0.46	0.46	0.46
Water	32.05	33.84	37.43
Erythorbic Acid	0.31	0.31	0.31
Sodium Diethylaminopropyl Cocoaspartamide	2.75	2.75	2.75

For the oxidative hair dye simulation, immediately prior to dosing, equal parts of formulation 1 and formulation 4 were combined. For the non-oxidative hair dye simulation, immediately prior to dosing, equal parts of formulation 2 and degassed water were combined.

Absorption was assessed by collecting the phosphate buffered saline receptor fluid (calcium and magnesium free) hourly from 0 to 24 h. At 30 minutes after sample application, the skin was washed with water, 2% sodium dodecyl sulphate (w/v in water), and water again. At 24 h, the underside of the skin was rinsed with receptor fluid. The skin was removed from its mounting, dried, and tape-stripped. Samples were analyzed using liquid scintillation counting. No use of formulation 3 ("placebo") was described. A total of 12 skin samples were tested using the oxidative hair dye simulation, but data from 4 of these tests were rejected due to low mass balance (not further explained). All 12 samples in the non-oxidative hair dye simulation were used.

The author defined the dislodgeable dose as the mass of test item removable from the application site at the 30 minute wash. Unabsorbed dose is the mass of test item in the dislodgeable dose + unexposed skin (under the flange of the diffusion cell) + stratum corneum. The absorbed dose is the mass of test item reaching the receptor fluid within the specified time. Dermal delivery is the sum of the absorbed dose + the mass of test material in the dermis/epidermis.

Table 4 presents the data for oxidative and non-oxidative hair dye simulations. The author summarized the data into a single dermal absorption value of 1.74 µg equiv./cm^2 for the oxidative test preparation and 6.55 µg equiv./cm^2 for the non-oxidative test preparation (Toner 2005).

ANIMAL TOXICOLOGY

Acute Toxicity

Dossou (1977) orally administered a 1 ml/100 g volume of solution containing 2,4-Diaminophenoxyethanol HCl in the chlorhydrate form by gavage to 20 albino Swiss mice (10 males and 10 females), approximate weight 20 g each, and 20 albino Wistar rats (10 males and 10 females), approximate weight 200 g each. The animals were fasted for 12 h and then allowed to eat and drink normally for 2 h before dosing. Dose concentrations were calculated in geometric progression. The animals were observed for 3 h following administration and during the following week.

Tearing, agitation then calm, piloerection, vasoconstriction, ptosis, discolored urine, difficult breathing, salivation, and convulsions were observed in some mice. Salivation, tearing, piloerection, loss of grip reflexes, difficult breathing, shaking, bloody snouts, vasodilation, diarrhea, discolored urine, and convulsions were observed in some rats.

The oral LD_{50} calculated was 1760 mg/kg (confidence limits of 1595-1950 mg/kg) for male mice, 1739 mg/kg (confidence limits of 1356-2232 mg/kg) for female mice, 1745 mg/kg (confidence limits of 1539-1980 mg/kg) for male and female mice, 1191 mg/kg (confidence limits of 1075-1321 mg/kg) for male rats, 1040 mg/kg (confidence limits of 883-1225 mg/kg) for female rats, and 1113 mg/kg (confidence limits of 1037-1194 mg/kg) for male and female rats (Dossou 1977).

Six groups of 10 albino male Swiss mice, 25 to 30 g, were administered 2,4-Diamino-phenoxyethanol HCl by gavage (Segre 1976). The animals received 10 ml/kg of compound, either dissolved in water or suspended in methocel at 0.5% (pH nonmodified), at doses of 630, 790, 1000, 1580, 2000, or 2500 mg/kg. The mice were then observed for 14 days. The oral LD_{50} was calculated and determined, as an average, to be 1160 mg/kg (95% confidence limits of 850-2100 mg/kg).

Manciaux (1998a) reported a study using Sprague-Dawley rats (5 of each sex), fasted overnight, and given 2,4-Diaminophenoxyethanol (as the dihydrochloride salt) at a dose of 1000 mg/kg in water by oral gavage (10 ml/kg). During the 14-day observation period, 1 male and 3 female rats were found dead on day 2. Death was preceded by hypoactivity, piloerection, lateral decubitus and tonic-clonic convulsions; all of which were observed in surviving animals. All surviving animals recovered fully by day 5. No findings on necropsy were reported in either the animals that died or those killed at 14 days.

Table 4. Summary of percutaneous absorption data (Toner 2005).

	Oxidative		Non-oxidative	
Site[a]	µg equiv./cm^2	% applied dose	µg equiv./cm^2	% applied dose
dislodgeable dose	379.97 ± 24.54	89.68 ± 4.06	369.63 ± 21.37	94.11 ± 4.54
unabsorbed dose	399.60 ± 26.01	94.31 ± 4.23	390.47 ± 13.49	99.44 ± 2.79
absorbed dose	0.11 ± 0.12	0.03 ± 0.03	2.94 ± 3.30	0.75 ± 0.84
dermal delivery	1.74 ± 1.08	0.41 ± 0.26	6.55 ± 4.72	1.68 ± 1.23

[a] dislodgeable dose is the mass of test item removable from the application site at the 30 minute wash; unabsorbed dose is the mass of test item in the dislodgeable dose + unexposed skin (under the flange of the diffusion cell) + stratum corneum; the absorbed dose is the mass of test item reaching the receptor fluid within the specified time; and the dermal delivery is the sum of the absorbed dose + the mass of test material in the dermis/epidermis.

Subchronic Toxicity

Kuwabara et al. (1983) administered 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 0.01, 0.03, 0.05, 0.1, and 0.2% in tap water, *ad libitum*, to 6 groups of BDF_1 mice and 6 groups of F344 rats, 10 males and 10 females per group, for a period of 12 weeks.

No clinical signs were observed during treatment. The survival rate was 90% for the male mice in the 0.1 and 0.2% dose groups, 100% for all remaining mice and all rats. Two mice that had greatly decreased body weights died while on study. Their deaths were attributed to malnutrition due to inability to drink water. Both were necropsied, and atrophy of various organs was observed.

In the surviving mice, males in the 0.1 and 0.2% dose groups had decreased body weight gains. Females had satisfactory growth throughout the course of the experiment. Feed consumption was decreased in these 2 dose groups for both male and female mice. For the rats, there was a dose-dependent decrease in mean body weight gain for all treated groups; feed consumption was decreased at the 0.2% concentration. Water intake was decreased for all treatment groups, both mice and rats, when compared to the control group values.

Tissue specimens from 3 males and 3 females from each group, both mice and rats, were evaluated microscopically. Abnormalities of the kidneys were found in 2 mice, lesions of pneumonia were found in 7 mice, and pigment deposits were observed in the epithelial cells of thyroid follicles in a mouse from the 0.2% group. All male and female rats in the 0.2% dose group had pigment deposits in the epithelial cells of the thyroid follicles (Kuwabara et al. 1983).

Fournier (1978a) administered 2,4-Diaminophenoxyethanol HCl in a 5% Tween suspension by oral intubation to 20 Sprague-Dawley rats (10 males and 10 females) for a period of 3 months. The dose was 56 mg/kg/day (1/20 LD_{50}) at a volume of 10 ml/kg/day. A control group of 20 rats (10 males and 10 females) received vehicle alone.

Clinical observations included a dull appearance of the pelage and light brown areolas, pelage being soiled with urine, and a brown discoloration of the urine. Body weight gain of the treated group was slightly decreased, but the difference, as compared to the controls, was not statistically significant. At necropsy, a brown discoloration of the thyroid gland and of the trachea at the level of the thyroid gland was due to the hair dye.

The results of histological and clinical examination of the treated animals were normal, with the exception of an increased serum glutamic-oxaloacetic transaminase (SGOT) activity and a slight increase in serum glutamic-pyruvic transaminase (SGPT) activity, alkaline phosphatase activity, and uric acid values. However, the SGPT, SGOT, alkaline phosphatase, and uric acid values were within the normal limits for rats. The only mortality reported was the accidental death of 1 animal while on study (Fournier 1978a).

Chevalier (2005) reported a 13-week oral toxicity study using Sprague-Dawley rats. Daily administration of 2,4-Diaminophenoxyethanol (dihydrochloride salt) by oral gavage at 0, 4, 20, or 100 mg/kg/day in 5 ml water occurred over the course of the study. Each group consisted of 10 animals of each sex. An additional 6 animals of each sex were added to the control and high dose groups and held for a 4-week recovery period after treatment. An additional 6 animals were added to each group receiving the test material and were used for toxicokinetic evaluation performed on day 1 and during week 13. Mortality determinations, clinical observations (daily and weekly), functional test battery (during week 13), body weight and food intake measurement (weekly), ocular examination, blood hematology and clinical chemistry, and urinalysis were all performed. At the end of the study, animals were killed and necropsied.

Plasma levels of 2,4-Diaminophenoxyethanol in the toxicokinetic study were not detectable in the low dose group. In the 20 mg/kg/day group, plasma levels of 2,4-Diaminophenoxyethanol were detectable at 30 minutes after dosing (no shorter determination made). In the 100 mg/kg/day group, plasma levels of 2,4-Diaminophenoxyethanol were maximal at 30 minutes. The authors cited low stability of the test material in frozen plasma as a confounding factor in interpreting the toxicokinetic data.

There were no treatment-related deaths, adverse clinical signs, or changes in food intake. In the high dose group, increased salivation was observed. Body weights were decreased in the high dose group during the dosing period, but returned to normal at the end of the recovery period. Urine discoloration, traces of glucose/nitrates, and bilirubin were seen in the high dose group, but disappeared at the end of the recovery period. No increases in plasma glucose or bilirubin were seen.

Isolated statistically significant organ weight differences were reported including: 10% increase for relative brain weight in high dose males (but not females); and 10% increase for relative kidney weight in high dose females (but not males). After the recovery period, absolute (13%) and relative (15%) kidney weights were increased in females (but not males) and absolute (43%) and relative (52%) thymus weights were increased in females (but not males). No associated pathology was found in any of these organs.

Brown discoloration of thyroid glands and brown pigment seen on tissue examination in the high dose group was reported and this persisted to the end of the recovery period. No inflammatory, degenerative, or proliferative changes were seen in the thyroid gland histology.

Splenic hemosiderosis in the high dose group that persisted to the end of the recovery period was reported, but there were no associated hematological changes. The author reported the NOEL to be 20 mg/kg/day (Chevalier 2005).

Ocular Irritation

Dossou (1979a) instilled 0.1 ml of a 4% aqueous solution (pH 2.5) of 2,4-Diaminophenoxyethanol HCl into the conjunctival sac of 1 eye of 6 albino Bouscat rabbits (3 males and 3 females) and was not rinsed after administration. The other eye was untreated and served as a control. This solution was considered "practically not irritating" to the eyes of rabbits, with the ocular irritation index estimated to be 1.66/110 after 24 h, 0.33/110 after 48 h, and 0/110 after 72 h, 4 days, and 7 days.

Besson (1991a) reported a study using 3 female New Zealand White rabbits in which a 100 mg sample of 2,4-Diaminophenoxyethanol (dihydrochloride salt) was instilled neat into the conjunctival sac of the left eye (eyes were not rinsed). The right eye served as the control. Ocular effects were determined at 1, 24, 48, and 72 h post exposure and at days 8 and 15. Marked chemosis, slight to moderate conjunctival redness, slight to moderate corneal opacification, and slight iridal lesions were observed. While lessened, these effects had not disappeared after 15 days. The material as tested was considered an ocular irritant.

Sire (2004) used 3 female New Zealand White rabbits in which a 0.1ml aliquot of a 4% dilution of 2,4-Diaminophenoxyethanol (dihydrochloride salt) in water was instilled into the conjunctival sac of the left eye (eyes were not rinsed). The right eye served as the control. Ocular effects were determined at 1, 24, 48, and 72 h post exposure. Slight chemosis and redness were observed in 2/3 animals in the treated eyes, but the effect did not persist beyond day 2. There were no corneal or iridal effects. The material, tested as a 4% dilution in water, was not considered an ocular irritant.

Dermal Irritation

According to Dossou (1979b), 2,4-Diaminophenoxyethanol HCl was applied to the shaved intact and abraded skin of 6 albino Bouscat rabbits (3 males and 3 females) as a 4% solution in distilled water (pH 8.5). Each animal received 5 ml of solution. This solution was determined to be "slightly irritating" to rabbit skin with a primary irritation index of 0.08/8.

Besson (1991b) used 3 female New Zealand white rabbits in a dermal irritation study. A 0.5 g sample of 2,4-Diaminophenoxyethanol (dihydrochloride salt) was applied to a clipped area of the right flank, that had been moistened with 0.5 ml of paraffin oil. A non-occlusive dressing was applied for 4 h and then removed. At 1, 24, 48, and 72 h after the dressing was removed, cutaneous reactions were assessed on the treated and control (untreated left flank). Slight erythema was observed in 1 animal at the treatment site at the 48 h observation. No other effects were seen and the material, as tested, was not considered a dermal irritant.

Dermal Sensitization

Fournier (1978b) used 10 female Hartley guinea pigs to determine the sensitizing potential of 2,4-Diaminophenoxyethanol HCl following a modified Magnusson and Kligman technique. Before administration of the compound, two 0.2 ml injections of 50% Freund's adjuvant were administered intradermally to the site of application. The compound was moistened with a few drops of distilled water for better adherence and applied epicutaneously to a 3 cm^2 area of deeply abraded skin. The test site was covered with an occlusive patch for 48 h. On day 7, a second epicutaneous application of 25% test article in petroleum jelly was administered, and an occlusive patch was applied for 48 h.

On day 21, a challenge was performed by applying 25% 2,4-Diaminophenoxyethanol HCl in petroleum jelly to a 5 cm^2 shaved, previously untreated area of skin. This area was covered with an occlusive patch for 24 h. Five female nonsensitized guinea pigs also were treated with a 25% application of 2,4-Diaminophenoxyethanol HCl in petroleum jelly, and the site was covered with an occlusive patch for 24 h. This group served as the control group. The excess test substance was removed after patch removal, and sensitization readings were taken 48 and 72 h after the challenge application.

Erythema was observed in 3 of the 10 animals, all of which recovered within 5 days. The authors suggested, based on the results of this study, that 2,4-Diaminophenoxyethanol HCl could have a low sensitizing potential in humans (Fournier 1978b).

Manciaux (1998b) used 15 Dunkin-Hartley guinea pigs in a sensitization study. Animals were clipped and/or shaved at the anterior left flank treatment site before each application. Three induction exposures, using 10 male and 10 female guinea pigs, were done using a gauze pad moistened with 500 mg (neat) 2,4-Diaminophenoxyethanol (dihydrochloride salt). Five control animals of each sex received gauze pads with 0.5 ml water (control) on days 1, 8, and 15. The pads were held in place for 6 h using an occlusive dressing and sites were observed at 24 h after dressing removal. On day 29, control and treated animals received a topical challenge of 0.5 ml water applied to the left flank and 500 mg of the test material to the previously unexposed right flank. Sites were observed at 24, 48, and 72 h post-challenge.

The test material colored the skin purple. The author reported finding slight erythema in 2/20 (one of each sex) test animals at the 48 h time only. The authors concluded that the test material did not produce sensitization reactions (Manciaux 1998b).

Local Lymph Node Assay (LLNA)

Sire (2005) reported an LLNA in which 28 female CBA/J mice were divided into 5 treatment, 1 negative and 1 positive control groups. Treatment groups received 2,4-Diaminophenoxyethanol (dihydrochloride salt) at 0.5, 1.0, 2.5, 5, and 10% (w/v) in DMSO. The negative control received DMSO only and the positive control received α-hexylcinnamaldehyde at 25% (w/v) in DMSO. All exposures

were made to each ear at 25 µl of material per ear for 3 consecutive days. Ear thickness was measured on days 1, 2, 3, and 6. On day 6, a single injection of [^{3}H] methyl thymidine was given. Around 5 h after injection, animals were killed, the auricular lymph nodes were excised and pooled for each group, and radioactivity measured. The stimulation index (SI = treated animal/control animal radioactivity levels) and EC_3 theoretical concentration were calculated.

Treatment-related, but not dose-related ear swelling was noted. The SI for the 0.5% group was 0.92, the 1% group was 1.56, and the 2.5% group was 1.17. These were not considered positive. Positive lymphoproliferative responses were seen for the 5% group (SI = 4.21) and the 10% group (SI = 7.42). For comparison, the positive control SI was 8.51. The authors concluded that 2,4-Diaminophenoxyethanol (dihydrochloride salt) induces delayed contact hypersensitivity in the murine LLNA (Sire 2005) .

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

A dose range-finding study was conducted to determine the concentrations of 2,4-Diaminophenoxyethanol HCl to be used in a teratology study by Bottomley et al. (1981). 2,4-Diaminophenoxyethanol HCl was administered by intragastric intubation on days 1 through 10 of pregnancy to 4 groups (6 rats per group) of pregnant specific pathogen-free rats. The doses were 0, 125, 250, and 500 mg/kg/day, and the dose volumes were calculated on days 1, 4, and 7 to adjust for change in body weight. Control animals received vehicle, distilled water, only.

Clinical signs in the 500 mg/kg dose group included severely increased post-dose salivation, elevated gait, stained coats, fur loss in 1 animal, and discolored urine. Similar signs were observed in the 250 mg/kg dose group, with the exceptions that they had a later onset, were not as severe, less animals were affected, and more animals exhibited fur loss. At the 125 mg/kg dose, the only clinical signs observed were slight or moderate post-dose salivation and discolored urine.

Feed consumption was decreased on days 1 to 3 of dosing in the 250 and 500 mg/kg dose groups and continued throughout dosing in the 500 mg/kg group. On days 1 to 4 of dosing, there was a weight loss in the high-dose group, retarded weight gain in the mid-dose group, and minimal retardation of weight gain in the low-dose group. During days 4 to 11, weight gains were reduced in the 250 and 500 mg/kg dose groups, and, despite some recovery, weight gain was reduced in the post-dose period when compared to controls.

Animals were killed on day 15, and necropsied. Except for discolored fur on 4 animals, no compound-related changes were observed.

Based on the results of the preliminary study, doses of 0, 50, 100, and 200 mg/kg/day were chosen for a complete teratology study. 2,4-Diaminophenoxyethanol HCl was administered by gavage to 4 groups (20 per group) of pregnant specific pathogen-free rats on days 6 through 15 of pregnancy. The controls received vehicle, distilled water, only. Each rat received 10 ml/kg of solution, and the dose volume administered was calculated on days 6, 10, and 14 to adjust for changes in body weight.

Clinical signs included increased post-dose salivation and discolored urine in all dose groups and fur loss in the later stages of dosing and in the post-dosing period in the 200 mg/kg dose group. Body weight gain was decreased at the 100 mg/kg dose and even more at the 200 mg/kg dose.

On day 20 of pregnancy, the dams were killed, and the litters were examined. With the exception of discoloring or loss of fur for some animals, no dose-related changes were observed at necropsy.

No statistically significant changes in litter size were observed. Marginal decreases in litter size were evident in the 100 and 200 mg/kg dose groups, but were considered to be unrelated to treatment. No statistically significant differences were observed in litter and fetal mean weight values. However, lower litter and fetal mean weight values in the 200 mg/kg dose group were considered to be treatment-related due to maternal effect.

In the 200 mg/kg dose groups, there was a significant dose-related increase in the incidence of skeletal anomalies and skeletal variants. The authors stated that this increase was likely related to a nonspecific retardation of embryo/fetal development during gestation. The incidence of major malformations and minor visceral anomalies was comparable among all groups (Bottomley et al. 1981).

Pregnant C57B1/6 mice (crossed with T stock males) were used to determine the teratogenic potential of 2,4-Diaminophenoxyethanol HCl dissolved in corn oil (Beliles et al. 1978). There were 10 animals in the low and mid-dose groups and 18 animals in the high-dose group. The solution was topically administered to a shaved area on the back of each mouse. The volume applied was 0.2 ml at doses of 15, 150, or 1500 mg/kg. Negative and positive control tests were performed. Sixteen negative control animals received dermal applications of corn oil only, and 19 positive control animals were administered benzo[a]pyrene on day 10.5 of pregnancy by intraperitoneal injection. Following fetal evaluation, no teratogenic effect nor any significant difference in skeletal development, when comparing the dose groups to the negative controls, was reported.

Gaoua (2005) examined embryo/fetal developmental toxicity in female Sprague-Dawley rats exposed to 2,4-Diaminophenoxyethanol (dihydrochloride salt) daily by oral gavage from GD 6 - 19. Doses were 0, 4, 20, and 125 mg/kg/day in water (given at 5 ml/kg). Maternal clinical signs, body weight, and food intake were monitored. Dams were killed on GD 20. Gravid uterus weights were determined and fetuses were removed, sexed, weighed, and examined

externally. Implantation sites, preimplantation loss, and live and dead fetuses were recorded. Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

Maternal results included excessive salivation and significantly decreased body weights in the high dose group. There was no difference in the number of fetuses or in implantation sites between control and treatment groups. Only one dead fetus was reported in any group and that was in the high dose group (total fetuses 311). Fetal body weights were significantly decreased in male and female fetuses in the high dose group.

Short supernumerary 14th ribs, incomplete ossification of the centrum of the thoracic vertebrae, and incomplete ossification of the 5th sternebra were reported in the high dose group. External abnormalities were not seen in any fetus, except for one in the control group. No soft tissue malformations were seen in any group. The author reported that the NOEL for maternal and fetal developmental effects was 20 mg/kg (Gaoua 2005).

GENOTOXICITY

Genotoxicity study results are summarized in Table 5.

In Vitro Assays

Bacterial and Yeast Cell Assays

Hastwell and McGregor (1982) used *Escherichia coli* strains WP2, WP2uvrA, and WP2uvrA/recA to test for the genotoxic potential of 2,4-Diaminophenoxyethanol HCl. Concentrations of 30, 75, 189, 754, and 2000 µg/plate were tested both with and without metabolic activation by S9 mix. An *E. coli* reversion test was performed using strains WP2 and WP2uvrA.

A modified Ames test was performed in which the soft top agar contained 0.25 µg/ml of L-tryptophan instead of the histidine/biotin mixture. No increase in revertants was found with or without metabolic activation. Strains WP2, WP2uvrA, and WP2uvrA/recA were used in a DNA damage/repair test.

Another modified Ames test was performed, with the exception that the L-tryptophan concentration was increased to 1 µg/ml, and the test solution (0.1 ml) was pipetted into a hole (1 cm in diameter) cut into the center of each plate rather than being incorporated into the soft agar. A positive and negative control, 0.5 µg of 2-aminoanthracene and 200 µl of phosphate buffer, respectively, were included.

Salmonella typhimurium strain TA1535 was included in the test because the *E. coli* strain WP2uvrA did not significantly react with such a low dose of 2-amino-anthracene. There was no indication of differential damage (Hastwell and McGregor 1982).

S. typhimurium strains TA1538 and TA98 were used to test for mutagenic activity of 2,4-Diaminophenoxyethanol HCl both with and without metabolic activation (Mohn et al. 1982). Positive controls, used successfully, were 4-nitro-*o*-phenylenediamine (NOPD) and 2,4-DAA. 2,4-Diaminophenoxyethanol HCl was tested at concentrations of 0, 60, 120, 300, 600, and 1200 µg/plate. Results were negative with and without metabolic activation for 2,4-Diaminophenoxyethanol HCl.

An Ames test was performed by Shahin et al. (1980) using 5 *S. typhimurium* strains. The results are an average of 2 independent experiments, each using 3 plates/dose. The tests were run with and without metabolic activation by S9 mix. Two positive controls, 1,2-diamino-4-nitrobenzene and 2-aminoanthracene, also were used. *S. typhimurium* strains TA1537, TA1538, TA98, TA1535, and TA100 were administered compound at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate. No mutagenic activity due to 2,4-Diaminophenoxyethanol HCl was observed.

Shahin et al. (1982) determined mutagenic activity of 2,4-Diaminophenoxyethanol HCl using *S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 and the yeast *S. cerevisiae* strains D4 and XV185-14C. The tests were carried out with and without metabolic activation by S9 mix. The test was performed using all 5 *S. typhimurium* strains at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate, 3 plates per dose. No mutagenic activity was observed.

S. cerevisiae strain D4 was treated with compound at concentrations of 100 (only without metabolic activation), 250, 500, 1000, 1500, 2000, and 4000 (only with metabolic activation) µg/ml. *S. cerevisiae* strain XV185-14C was treated with 2,4-Diaminophenoxyethanol HCl at concentrations of 1000, 2000, 3000, 4000, 5000, and 6000 µg/ml.

No mutagenic activity was detected in either yeast strain. Positive controls were run with all tests using 1,2-diamino-4-nitrobenzene and 2-aminoanthracene (Shahin et al. 1982).

Venitt et al.(1983) used *S. typhimurium* strains TA1538, TA97, TA98, and TA100 and *E. coli* strain WP2uvrA (pKM101) to test for mutagenic potential of aqueous 2,4-Diaminophenoxyethanol HCl. Three plates were run per dose using 0, 4, 10, and 30% Aroclor 1254-induced S9 mix. A positive control, 2,4-DAA, was used. The test was performed on all strains at a concentration range of 5 to 100 µg/plate.

Negative results were obtained with *S. typhimurium* TA100 and *E. coli* WP2uvrA (pKM101) at all dose concentrations and with all S9 mixes.

A statistically significant increase in the number of *his*$^{+}$ revertants was obtained with *S. typhimurium* strains TA1538, TA97, and TA98 when in the presence of 10% S9 mix. The number of 2,4-Diaminophenoxy-ethanol HCl-induced revertants was greater than 3.5 times the background concentration using 20 to 40% S9 mix.

The number of induced revertants also was affected by the amount of NADP in the S9 mix. The number of *his*$^+$ revertants corresponded to the amount of NADP per plate. In the second set of tests using 30% S9 mix with strains TA1538 and TA98, 10-fold and 14-fold increases, respectively, were observed at 80 µg/plate.

Fluctuation tests, using S. *typhimurium* strains TA1538 and TA98, were performed by adding 20 µg of an overnight Lab-M nutrient broth No. 2 shake culture of the appropriate organism, a known amount of 2,4-Diaminophenoxyethanol HCl, and 3 ml of 2% S9 mix to 12 ml of Vogel-Bonner salts medium containing 1% glucose, 10 µg/ml biotin, and 1.5 µg/ml histidine. The tests also were performed by substituting the S9 mix with 3 ml of Vogel-Bonner medium. After 18 h of incubation, 1 ml of Vogel-Bonner medium supplemented with 1% glucose and 10 µg/ml bromcresol purple was added. The wells were then incubated for another 3 days.

In the presence of S9 mix, statistically significant dose-related increases in the number of positive wells were observed for both strains. Negative results were obtained in the absence of S9 mix (Venitt et al. 1983).

Williams (2005) reported the results of 2,4-Diaminophenoxyethanol HCl in an Ames assay with *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102, with and without S9 metabolic activation. Using the direct plating method, concentrations of 1.6, 8, 40, 200, 1000, and 5000 µg/plate were tested and using the pre-incubation approach, concentrations of 1.3 (TA102 only), 3.3 (TA102 only), 8.2 (TA98 and TA102 only), 20.5, 51.2, 128, 320, 800, 2000, and 5000 µg/plate were tested.

The authors noted that TA98 and TA1537 are frame-shift mutations and TA100, TA1535, and TA102 are base-pair substitution mutations. Positive controls were used as follows: 2-nitrofluorene, TA98; sodium azide, TA100 and TA1535; 9-aminoacridine, TA1537; glutaraldehyde, TA102; benzo[*a*]pyrene, TA98; and 2-aminoanthracene, TA100, TA1535, TA1537, and TA102. In the plate-incorporation study, a small increase in reverse mutations was seen for strains TA98 (maximum of 90 colonies/plate) and TA102 (maximum of 292 colonies/plate) with metabolic activation.

A small increase in reverse mutations in strain TA98 (maximum of 73 colonies/plate) was seen in the pre-incubation study, in the presence of S9 metabolic activation. Positive controls yielded expected results (with S9, mean colonies/plate >400 for TA98 and >800 for TA102). The authors concluded that 2,4-Diaminophenoxyethanol HCl was a mutagen in TA98 in the presence of metabolic activation, but that the mutagenic effect was not large (Williams 2005).

Mammalian Cell Assays

Chinese hamster ovary (CHO) cells were used to evaluate the mutagenic potential of 2,4-Diaminophenoxyethanol HCl at concentrations of 0.6 and 1.2 mg/ml (Darroudi et al. 1982). The tests were performed with and without metabolic activation. A positive control using 2,4-diaminoanisole dihydrochloride (2,4-DAA) was included in the study. The authors stated that there was no evidence of increase in chromosomal aberration due to 2,4-Diaminophenoxyethanol HCl either with or without metabolic activation.

Lloyd (2005) used L5178Y mouse lymphoma cells to evaluate the mutagenic effect of 2,4-Diaminophenoxyethanol HCl with and without S9 metabolic activation in two independent experiments. In one experiment, concentrations of 400, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, and 2410 µg/ml were used in the absence of S9 and a concentration of 200 µg/ml was added to the above concentrations in the presence of S9. Positive controls were 4-nitroquinoline-1-oxide and benzo[*a*]pyrene.

In the absence of S9, cell killing precluded using the high exposure. In the presence of S9, the top two concentrations could not be used for the same reason, and the 800 µg/ml was excluded because of excessive heterogeneity. In the second experiment, concentrations of 400, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, and 2410 µg/ml were used, with or without S9.

No increase in mutations was seen at any concentration in the absence of metabolic activation in either experiment. With metabolic activation, small increases in mutation frequency was seen at 1200 and 1800 µg/ml, but not at other concentrations in the first experiment. In the second experiment, with S9 activation, small increases in mutation frequency were seen at 400, 1600, and 2410 µg/ml, but not at other concentrations.

In all cases, the small increases in mutation frequency, while higher than the controls in this study, were within the range of historical control values. This, combined with the absence of a clear dose-response, led the author to conclude that 2,4-Diaminophenoxyethanol HCl was not mutagenic in this assay, with or without metabolic activation (Lloyd 2005).

Human Lymphocytes

Kalopissis (1981) reported a chromosomal aberration test using human lymphocytes. 2,4-Diaminophenoxyethanol HCl was administered at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M in the medium. The fixation time was 24 h, and the results obtained were negative.

Kumaravel (2005) performed a chromosome aberration test in human lymphocytes in culture using 2,4-Diaminophenoxyethanol HCl. In one experiment, 987, 1234, and 1542 µg/ml of the test material was used, with and without S9 metabolic activation. In the second experiment, concentrations of 50, 77, 97, and 121 µg/ml were used in the absence of S9, and concentrations of 1408, 1760, and 2200 µg/ml were used with S9. The positive control with S9 was 4-nitroquinoline and without S9 was cyclophosphamide.

In the first experiment, pulse exposure for 3 h was used, with

and without S9. In the second experiment, a 3 h pulsed exposure was used with S9, but the cultures without S9 were exposed up until harvesting 17 h later.

Positive controls yielded expected results. Pulse-treated cells in the second experiment had frequencies of cells with structural chromosome aberrations that were significantly elevated with metabolic activation compared to controls (and were high compared to historical controls), but not without metabolic activation. Continuous exposure in the second experiment, done without metabolic activation, also yielded frequencies of cells with structural chromosome aberrations that were significantly elevated compared to controls (and were high compared to historical controls). The first experiment yielded no increases. The authors concluded that 2,4-Diaminophenoxyethanol HCl induced chromosome aberrations in cultured human lymphocytes (Kumaravel 2005).

Whitwell (2005) performed an in vitro micronucleus test in cultured human lymphocytes exposed to 2,4-Diaminophenoxyethanol HCl. Two experiments were performed, with and without S9 metabolic activation. In the first experiment, with S9, concentrations of 85, 207, 324, and 1542 µg/ml were used; and, without S9, concentrations of 106, 133, and 166 µg/ml were used. In the second experiment, with S9, concentrations of 1542, 1928, and 2410 µg/ml were used; and, without S9, concentrations of 160, 222, and 361 µg/ml were used. The positive controls, with S9, were 4-nitroquinoline and vinblastine, and, without S9, was cyclophosphamide.

Prior to incubation with the test material, cells were incubated for 24 h (experiment 1) or 48 h (experiment 2) with phytohemaglutinin. In the absence of S9, treatment was 20 h. With S9, treatment was 3 h. Cells were harvested 72 hours after initiation of treatment with test material, with the last 27 h in the presence of cytochalasin B.

Positive controls yielded expected results. No increases in micronuclei were observed in cells in experiment 1. In experiment 2, statistically significant increases in micronuclei were seen, with and without metabolic activation. Only at the highest concentrations tested were the increases outside of historical control ranges. The author concluded that, with 48h mitogen stimulation, 2,4-Diaminophenoxyethanol HCl did induce micronuclei formation in cultured human lymphocytes (Whitwell 2005).

Bacterial, Yeast, and Mammalian Cell Assays

Loprieno et al. (1982) described several assays using 2,4-Diaminophenoxyethanol HCl to determine its metabolic activity including: reverse mutation assays using *S. typhimurium* strains TA1538 and TA98; forward mutation assays using the *Schizosaccharomyces pombe* strain SP ade6-60/rad10-198h- and using the V79 cell line of Chinese hamsters; mitotic gene conversion assays using the yeast *Saccharomyces cerevisiae* strain D4, genotype α/a, gal2/+, ade2-2/ade2-1, trp5-12/trp5-27, leu1/+; and an unscheduled DNA synthesis (UDS) test using the HeLa human cell line from a cervical carcinoma.

Methyl methanesulfonate, ethyl methanesulfonate, cyclophosphamide, hycanthone, N-nitrosodimethyl-amine, and 2,4-DAA were used as positive controls. All tests were performed with and without metabolic activation provided by Aroclor-1254-treated or phenobarbital + β-naphthoflavone-treated S9 mix for the reverse mutation assays and using phenobarbital + β-naphthoflavone-treated S9 mix for all remaining assays.

The Ames test was performed, with and without Aroclor-treated rat liver S9 mix, using *S. typhimurium* strain TA1538 at concentrations of 0, 1, 10, 50, 100, 500, and 1000 µg/plate. Concentrations of 0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µg/plate were administered to strains TA1538 and TA98 in the presence of phenobarbital + β-naphthoflavone-treated S9 mix. No positive results were observed using 2,4-Diaminophenoxyethanol HCl.

In the forward mutation assay using *S. pombe*, the cells were treated with 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 10, 15, 20, 30, and 40 mM. The forward mutation assay using the Chinese hamster V79 cell line was done by scoring 6-thioguanine-resistant mutant colonies using concentrations of 0, 5, and 20 mM. Negative results were obtained for both forward mutation assays.

The *S. cerevisiae* cell suspensions were treated during growth in the same manner as *S. pombe* and were exposed to 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 10, 20, and 40 mM. The HeLa cells used in the UDS test were exposed to 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 0.02, 0.06, and 0.2 mM. Results were negative for both tests (Loprieno et al. 1982).

In Vivo

Drosophila

Mutagenic activity of 2,4-Diaminophenoxyethanol HCl was evaluated by performing a sex-linked recessive lethal test using *Drosophila melanogaster* (Blijleven 1982). At 25°C, 2,4-Diaminophenoxy-ethanol HCl was dissolved in m/30 phosphate buffer (pH 6.8) and fed to 1-to-2-day-old Berlin K males for 3 days. These males were then mated individually to 3- to-5-day-old virgin In(1) sc^{S1L} sc^{8R} + S, $sc^{S1}sc^{8}$ w^{a} B females (Basc). The mating scheme used was one 3-day brood followed by two 2-day brood periods. At the end of each period, the treated male was transferred to a new vial and mated with more 3-to-5-day-old virgin females. Six-linked recessive lethals were scored in the F_2 generation and all suspected lethals were retested. No increase in mutation frequency was obtained from brood-fractionating experiments.

Mice and Rats

A micronucleus test was performed using CD-1 mice (Richardson and Richold 1982). Two doses of 2,4-Diaminophenoxyethanol HCl were administered orally in

sterile distilled water at concentrations of 25, 50, and 100 µg/ml per administration. The doses were 24 h apart and at a volume of 0.1 ml/10 g body weight. A negative control, vehicle, was administered orally, and a positive control, mitomycin C, was administered by intraperitoneal injection. After administration, ptosis, hypopnea, and lethargy were observed in all dose groups, and all animals excreted brown-pigmented urine. The animals were killed 6 h after administration of the second dose, and direct bone marrow smears were made. These slides were examined to determine the presence of micronucleated cells in 2000 polychromatic erythrocytes per animal. The ratio of normochromatic to polychromatic erythrocytes also was determined. There was no increase in the incidence of micronucleated cells in any dose group, but the ratio of normochromatic to polychromatic erythrocytes was significantly increased, indicating a toxic effect.

Brusick et al. (1982) reported a mouse dominant-lethal assay. A suspension of 2,4-Diaminophenoxyethanol HCl in corn oil was administered to the skin of T-strain male mice. Doses ranging from 15 to 1500 mg/kg, at a volume no greater than 0.5 ml/day, were applied to a shaved patch on the dorsal surface of the mouse. There may have been ingestion because the application site was not covered. Two mice per group received dermal applications of either the test compound or the control, corn oil, for 5 consecutive days. Another 2 received an intraperitoneal injection of the positive control, triethylene melamine (TEM), 2 days before mating. Two days after being dosed, each male was housed with 2 virgin C57B1/6 female mice for 7 days. These females were then replaced with 2 new virgin females. This sequence was repeated for 7 weeks. Fourteen days after the midweek of mating, the females were killed, and their uteri were examined for viable and nonviable fetuses, resorption sites, and total embryos.

The dominant lethality results were negative. No significant results were observed with respect to the fertility index. After 6 weeks of mating, a fertility rate of only 30% was reported in the high dose. This was not considered compound-related because the indexes for weeks 5 and 7 were normal values. The average number of embryos per pregnant female also was not significantly different.

These authors also performed a mouse spot test for somatic mutation using C57B1/6 female and T-strain male mice. The animals were mated, and a minimum of 50 females with semen plugs were used per group. On days 8, 9, and 10 of gestation, the dose groups received dermal applications of 2,4-Diaminophenoxyethanol HCl in corn oil at doses ranging from 15 to 1500 mg/kg. The negative control group received vehicle only. The application site was an uncovered shaved patch on the dorsal surface of the mouse. The positive controls received a single 150 mg/kg intraperitoneal injection of benz[a]pyrene on gestation day 10. All animals were allowed to deliver, and the newborns were scored for nonwhite spots on days 12 and 24 of lactation.

The high-dose group reported a coat color spot frequency of 1.9%; while the negative control reported a frequency of 0%. The author stated that the historical control frequency was between 1 and 2%, and did not consider the test material to be genotoxic in this assay. There was no reduction in fertility, and there were no midventral white spots observed. The 2,4-Diaminophenoxyethanol HCl solution was not systemically toxic or irritating to the skin of the dosed female (Brusick et al. 1982).

Erexson (2005) conducted a bone marrow micronucleus test using Sprague-Dawley rats. Rats were given 2,4-Diaminophenoxyethanol (dihydrochloride salt) by oral gavage at 0, 375, 750, or 1500 mg/kg in water (10 ml/kg). Five animals per sex were used at each dose level and an additional 6 animals per sex were added to the high dose group and 5 per sex were added to the control group. Five animals per sex were given a single dose of cyclophosphamide at 60 mg/kg as a positive control. Animals were killed 24 h after dosing, except that 5 animals per sex in the control and high dose group were retained and killed at 48 h. Bone marrow was harvested and smears prepared, stained, and scored for the number of micronucleated polychromatic erythrocytes.

The positive control yielded the expected result. Clinical signs of eye squinting were noted in the 750 mg/kg group and closed eyes, hypoactivity, and irregular breathing were noted in the high dose group. One male in the high dose group was found dead the day after dosing. The frequency of micronucleated cells in bone marrow cells was not different between control and treated animals, nor was the ratio of polychromatic cells to normochromatic cells different. The authors concluded that 2,4-Diaminophenoxyethanol (dihydrochloride salt), in rats treated up to 1500 mg/kg, did not induce micronuclei or damage bone marrow erythrocytes (Erexson 2005).

In Vivo/In Vitro

Brusick et al. (1982) evaluated excretion products of 2,4-Diaminophenoxyethanol HCl for genetic toxicity in a plate microbial assay using urine collected from treated male CD-1 mice.

The test compound, suspended in corn oil, was applied to the skin for 3 days. Doses ranged from 15 to 1500 mg/kg. The volume was no greater than 0.5 ml/day. The application site was uncovered so there may have been ingestion of compound. A negative control, corn oil, and positive controls, tris(2,3 dibromopropyl)PO4 and 2-acetylaminofluorene, were used. Urine was collected for an approximately 16-h period, as it was excreted into containers that were being kept at 0 to 4°C.

The collected urine was divided into 3 portions for testing. *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed to 0.1, 0.2, or 0.3 ml of urine or to deconjugated urine. Nonactivation tests also were performed by adding urine to the appropriate tubes and pouring it over the surface of selected agar plates.

Slight increases were observed when the TA100 strain was exposed to treated urine, but these increases were not statistically significant. No positive results were obtained. A standard Ames test analyzing 2,4-Diaminophenoxyethanol HCl excretion products using *S. typhimurium* strains TA1538 and TA98 was negative (Brusick et al. 1982).

A *Salmonella*/microsome test was performed using the urine of rats that were administered 2,4-Diaminophenoxyethanol HCl in order to determine its mutagenicity potential (Shahin et al. 1980). *S. typhimurium* strains TA1538, TA98, and TA100 were used.

Male Wistar rats, 3 rats per group, were either topically, orally, or intraperitoneally administered 2,4-Diaminophenoxyethanol HCl. Topical administration was made by applying 4 ml of phosphate buffer containing 120 mg of 2,4-Diaminophenoxyethanol HCl to a 55.4 $\pm$ 8.7 cm^2 area of the back for 20 min. The compound was then removed by shampooing and thorough rinsing. One group of rats was treated orally with 10 ml of distilled water containing 100 mg/kg 2,4-Diaminophenoxyethanol HCl, and another group received 10 ml intraperitoneal injections of 0.9% NaCl containing 100 mg/kg 2,4-Diaminophenoxyethanol HCl.

Negative controls using no urine or urine from rats given oral doses of 10 ml distilled water/kg were run. Positive controls were treated with 2,4-diaminoanisole. Urine was collected at -40°C for 24 h. The volume of urine that was used for each group was 100, 200, and 300 µl/plate. No mutagenic activity was detected (Shahin et al. 1980).

Cifone (2005) conducted an in vivo/in vitro unscheduled DNA synthesis assay in Sprague-Dawley rat hepatocytes. Rats received 0, 375, 750, or 1500 mg/kg 2,4-Diaminophenoxyethanol HCl by oral gavage and were killed at 2-4 h or 14-16 h post-treatment (4 males/group/killing time). Positive controls (8) were given N-dimethylnitrosamine and killed at the early (4) or late (4) time. Hepatocytes were isolated, cultured with [^{3}H]methylthymidine for 4 h, washed, and returned to culture medium for an additional 16-20 h. Slides were prepared and autoradiographed. Net nuclear grain count and percentage of nuclei with five or more net nuclear grains were reported.

The positive control yielded the expected large increase in nuclear labeling. Clinical signs were noted at all dose levels, including hypoactivity, squinted eyes, and irregular breathing, but there were no deaths. No substantial differences were seen between hepatocytes from control and treated animals in either the mean net nuclear grain count or the mean percentage of nuclei with ≥5 grains, regardless of when the animals were killed after treatment (Cifone 2005).

Table 5. 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
Bacterial Cell Assays				
30, 75, 189, 754, and 2000 µg/plate	*E. coli* strains WP2, WP2uvrA	Modified Ames reversion test, presence and absence of metabolic activation	Negative	Hastwell & McGregor 1982
30, 75 189, 754, and 2000 µg/plate	*E. coli* strains WP2, WP2uvrA, WP2 uvrA/recA	Modified Ames reversion test, presence and absence of metabolic activation	Negative	Hastwel & McGregor 1982
0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µg/plate	*S. typhimurium* strains TA1538 and TA98	Ames test, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 1, 10, 50, 100, 500, and 1000 µg/plate	*S. typhimurium* strain TA1538	Ames test, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 10, 15, 20, 30, and 40 mM	*Schizosaccharomyces pombe* strain SP ade-60/rad10-198h$^-$	Forward mutation assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 10, 20, and 40 mM	*Saccharomyces cerevisiae* strains D4, genotype α/a; ga12/+; ade2-2/ade2-1; trp5-12/trp5-27; leu1/+	Mitotic gene conversion assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982

Table 5 (continued). 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
0, 60, 120, 300, 600, and 1200 µg/plate	*S. typhimurium* strains TA1538, TA98	Ames test, presence and absence of metabolic activation	Negative	Mohn et al. 1982
5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate	*S. typhimurium* strains TA1537, TA1538, TA98, TA1535, TA 100	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1980
5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate	*S. typhimurium* strains TA1535, TA100, TA1538, TA1537, TA98	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
1000, 2000, 3000, 4000, 5000, and 6000 µg/plate	*S. cerevisiae* strain XV185-14C	Gene reversion test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
100, 250, 500, 1000, 1500, 2000, and 4000 µg/plate	*S. cerevisiae* strain D4	Gene conversion test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
5 - 1000 µg/plate	*S. typhimurium* strains TA1535, TA1537, TA1538, TA100, TA98	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1983
5 - 100 µg/plate	*S. typhimurium* strain TA100 and *E. coli* strain WP2uvrA(pKM101)	Ames test, presence and absence of metabolic activation	Negative	Venitt et al. 1983
5 - 100 µg/plate	*S. typhimurium* strain TA1538, TA97, TA98	Ames test, presence and absence of metabolic activation	Positive in the presence of at least 10% S9 mix	Venitt et al. 1983
5 - 100 µg/plate	*S. typhimurium* strains TA1538, TA98	Ames test, presence and absence of metabolic activation	10-fold (TA1538) and 14-fold (TA98) increases at 80 µg/plate with 30% S9 mix	Venitt et al. 1983
Concentration not reported	*S. typhimurium* strains TA1538, TA98	Fluctuation test, presence and absence of metabolic activation	Positive for both strains in presence of S9 and negative for both in the absence of S9	Venitt et al. 1983
1.6 - 5000 µg/plate for direct-plating; 1.3 - 5000 µg/plate for pre-incubaction	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102	Ames test, presence and absence of metabolic activation	Positive for TA98 with S9	Williams 2005
Mammalian Cell Assays				
0.6 and 1.2 mg/ml	Chinese hamster ovary cells	Chromosomal aberration test, presence and absence of metabolic activation	Negative	Darroudi et al. 1982
10^{-3}, 10^{-4}, and 10^{-5} M	Human lymphocytes	Chromosomal aberration test	Negative	Kalopissis 1981
0, 5, and 20 mM	Chinese hamster ovary cells strain V79	Forward mutation assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 0.02, 0.06, and 0.2 mM	Human HeLa cells	Unscheduled DNA synthesis test	Negative	Loprieno et al. 1982
50 - 2200 µg/ml	human lymphocytes in culture	structural chromosome aberrations	one experiment positive, one negative; overall the author concluded the response was positive	Kumaravel 2005

Table 5 (continued). 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
200 - 2410 µg/ml	L5178Y mouse lymphoma cells	6-thioguanine resistance mutation frequency, with and without metabolic activation	isolated positive findings - not dose-dependent and within historic control values; not considered mutagenic	Lloyd 2005
85 - 2410 µg/ml	human lymphocytes in culture	micronucleated cells	with sufficient mitogen stimulation (48 h), 2,4-Diaminophenoxyethanol HCl did induce micronuclei formation in cultured human lymphocytes.; no effect with 24 h mitogen stimulation	Whitwell 2005
Drosophila				
Concentration not reported, oral	*Drosophila melanogaster* Berlin K males and ln(1)$sc^{SIL}sc^{8R}$ + S, $sc^{S1}sc^{8}w^{a}B$ females (Basc)	Sex-linked recessive test	Negative	Blijleven 1982
Animal Assays				
15 - 1500 mg/kg, dermal	Mouse, T-strain males and C57B1/6 females	Mouse dominant-lethal assay	Negative	Brusick et al. 1982
25, 50, and 100 µg/ml, oral	Mouse strain CD-1	Micronucleus test	No increase in micronucleated cells. Ratio of normochromatic to polychromatic erythrocytes was significantly reduced	Richardson & Richold 1982
15 - 1500 mg/kg, dermal	Mouse, T-strain males and C57B1/6 females	Moue spot test for somatic mutation	Negative	Brusick et al. 1982
375 - 1500 mg/kg	Sprague-Dawley rats	Unscheduled DNA synthesis	Negative	Cifone 2005
375 - 1500 mg/kg	Sprague-Dawley rats	Micronucleus test	No increase in micronucleated cells, no difference in ratio of normochromatic to polychromatic erythrocytes	Erexson 2005
Animal and Bacterial Assays				
15 - 1500 mg/kg, dermal	Mouse strain CD-1 and *S. typhimurium* strains TA1535 TA1537, TA98, TA100	Plate microbial assay	Negative	Brusick et al. 1982
100 mg/kg, dermal, oral, and intraperitoneal injection	Wistar rats and *S. typhimurium* strains TA1538, TA98, TA100	*Salmonella*/microsome test	Negative	Shahin et a. 1980

CARCINOGENICITY

Kuwabara et al. (1983) used 3 groups of BDF_1 mice, 50 males and 50 females per group, to determine the carcinogenic effect of 2,4-Diaminophenoxyethanol HCl. 2,4-Diaminophenoxyethanol HCl was administered in tap water, *ad libitum*, at concentrations of 0, 0.04, and 6.07% for a period of 104 weeks. These doses were chosen by having first performed the subacute toxicity test on mice described earlier in this report.

There were no significant differences observed in body weight, organ weight, or survival rate between treated and control mice. At the termination of the study, gross and histopathological examinations were performed.

No significant difference was observed in target organs or

tumor incidence when comparing the treated and control groups. Pigment deposits in epithelial cells of thyroid follicles, which were histochemically negative for silver and iron and unrelated to tumor incidence, were observed in both treated groups. The authors concluded that chronic administration of 2,4-Diamino-phenoxyethanol HCl produced no carcinogenic effect in mice.

These authors also reported a study in which 3 groups of F344 rats, 50 males and 50 females per group, received 2,4-Diaminophenoxyethanol HCl in tap water, *ad libitum*, at concentrations of 0, 0.05, and 0.1% for a period of 104 weeks. The concentrations were determined by having first performed the subacute toxicity test on rats that was described earlier in this report. No dose was administered to the males in the 0.1% dose group during weeks 12 to 16 and to neither males nor females in that same dose group during weeks 32 to 36 due to a marked decrease in weight gain when compared to the controls.

Mean body weight gains for both treated groups, males and females, were reduced when compared to the controls. There was no significant difference in survival rate between treated and control groups.

After termination of treatment, necropsy and microscopic evaluation was performed on organs of all rats. There were no differences observed in the incidence or type of neoplasms between treated and control rats. Rats in the 0.1% dose group had pigment deposits in epithelial cells of thyroid follicles. These deposits were histochemically negative for silver and iron and were not related to the incidence of neoplasms. The authors concluded that chronic administration of 2,4-Diaminophenoxyethanol HCl produced no carcinogenic effect in rats (Kuwabara et al. 1983).

CLINICAL ASSESSMENT OF SAFETY

No human studies were available that specifically addressed these two ingredients.

HAIR DYE 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, while direct hair dyes are a preformed color. 2,4-Diamino-phenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate function as couplers in oxidative (permanent) hair dyes.

While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colourants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic" (IARC, 1993). The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

Rollison et al. (2006) reviewed the available epidemiology literature published from1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review. The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin's lymphoma. These findings, however, were not consistently observed across studies. Several studies published since this review are described below.

Bladder Cancer - A study by Kelsey et al. (2005) was a follow-up to the previously published case-control study in New Hampshire (Andrew et al. 2004) and examined the links between those bladder cancer cases with an inactivated tumor suppressor gene (TP53) and various exposures. Huncharek and Kupelnick (2005) performed a meta-analysis of 6 case-control and 1 cohort study. Takkouche et al. (2005) performed a meta-analysis of 9 personal use case-control studies and 1 cohort study. Ji et al. (2005) reported a cohort occupational study that included hairdressers not included in the above meta-analyses. Kogevinas et al. (2006) presented evidence from a case-control study in Spain. Lin et al. (2006) presented a case-control study of personal permanent hair dye use. Serretta et al. (2006) reported preliminary results from a multicentric study of risk factors in Ta-T1 transitional cell carcinoma of the bladder, including hair dye use. Pelucchi et al. (2006) reviewed data on bladder cancer mortality rates and the recognized or potential environmental (including hair dye exposures) and genetic risk factors. Bolt and Golka (2007) reviewed the published literature on bladder cancer risk and personal use of hair dyes (17 publications) or occupation as a hairdresser and/or barber (23 publications).

Lymphoma and Leukemia - Takkouche et al. (2005) reported a meta-analysis of reports of hematopoietic cancers (19 publications). Mester et al. (2005) reviewed ten 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. (2005) examined the association between lifetime hair dye exposure with various lymphomas, including chronic lymphocytic leukaemia. de Sanjosé et al. (2006) reported on the association between personal use of hair dyes and lymphoid neoplasm using data from a European multicenter case-control study. Chiu et al. (2007) evaluated non-Hodgkin's lymphoma subtypes defined according to the presence or absence of t(14:18) translocation as a function of smoking, familial hematopoietic cancer, and hair dye use,. Morton et al. (2007) examined the risk of non-Hodgkin's lymphoma as a function of hair dye use and genetic variation in N-acetyltransferase 1 (*NAT1*) and N-acetyltransferase 2 (*NAT2*).

Other Cancers - Takkouche et al. (2005) included breast cancer and childhood cancers in their meta-analysis. Efird et al. (2005) 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. (2005) studied 112 women in Nebraska newly diagnosed with brain cancer (glioma). McCall et al. (2005) reported on the relationship between childhood neuroblastomas and maternal hair dye use in 538 children born between 1992 and 1994 in the U.S. and Canada. Bluhm et al. (2006) reported on personal hair dye use and risks of glioma, meningioma, and acoustic neuroma. Chen et al. (2006) reported a case-control study of childhood germ cell tumors and exposure to residential chemicals, including prenatal and postnatal maternal hair dye use.

Reproductive and Developmental Outcomes - Axmon et al. (2006) compared fertility parameters in a cohort of Swedish hairdressers with matched controls. Hougaard et al. (2006) examined the risk of infertility among hairdressers in a 5-year follow-up of female hairdressers in Denmark. Zhu et al. (2006) reported on pregnancy outcomes among female hairdressers in Denmark. Thulstrup and Bonde (2006) conducted an in-depth review of 26 human studies of neural tube defects, cleft lip and cleft palate, congenital heart defects, urinary tract defects, and limb defects in which work and exposure status was known.

Other Endpoints - Park et al. (2005) reported an occupational case-control study of neurodegenerative diseases, including Alzheimer's disease, presenile dementia and motor neuron disease. Cooper et al. (2006) determined antinuclear antibody titer in individuals in the general population as a function of occupational history and ever/never use of hair dyes. Hueber-Becker et al. (2007) reported exposures of hairdressers to oxidative hair dyes (p-Phenylenediamine Hydrochloride) under controlled conditions, including estimates of systemic exposure. The authors discussed the adequacy of current safety precautions for handling hair dyes by hairdressers and the risk to health posed by the exposures found.

A presentation of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

SUMMARY

2,4-Diaminophenoxyethanol HCl is an aromatic amine that is an odorless white, slightly gray, or lavender gray powder. 2,4-Diaminophenoxyethanol Sulfate also is an aromatic amine salt. No chemical, physical, or toxicological data are available on the sulfate salt. 2,4-Diaminophenoxyethanol HCl is soluble in water and DMSO up to 10% (w/w), but is insoluble in solvents such as acetone and propylene glycol.

1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene and 2,4-diamino-1- ethoxybenzene are not detected as impurities in 2,4-Diaminophenoxyethanol HCl.

2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are used as couplers mixed with primary intermediates in permanent (oxidative) hair dyes. Both ingredients are used in concentrations up to 2%.

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.

In the European Union, 2,4-Diaminophenoxyethanol and its salts are listed in Annex III (part 2) with qualifications. In Japan, hair dyes are regulated as quasi drugs and all ingredients, both active and inactive, must be specifically approved: 2,4-Diaminophenoxyethanol HCl is an approved

hair dye active.

Dermal absorption of radioactive 2,4-Diaminophenoxyethanol HCl, as a pure compound and in an oxidative hair dye formulation, was determined using rats to be 5.05 ± 0.79 nM/cm^2 of pure compound and 2.83 ± 0.49 nM/cm^2 from the oxidative hair dye formulation (20 mg/cm^2 of compound was applied). In another dermal absorption study using rats, penetration ranged from 5.03 ± 0.79 nM/cm^2 (23.65 nM applied) to 9.42 ± 0.84 nM/cm^2 (70.95 nM applied). In an in vitro study using dermatomed human skin, the total dermal delivery from an oxidative hair dye preparation was 1.74 ± 1.08 μg equiv./cm^2, and 6.55 ± 4.72 μg equiv./cm^2 from a non-oxidative hair dye preparation.

The results of 3 acute toxicity studies found 2,4-Diaminophenoxyethanol HCl to have an LD_{50} ranging from 1160 to 1760 mg/kg in mice and 1000 to 1191 mg/kg in rats.

No clinical signs were observed in one subchronic toxicity test using mice and rats given concentrations of 2,4-Diaminophenoxyethanol HCl ranging from 0.01 to 0.2% in tap water. In a subchronic study in which 56 mg/kg/day were administered in solution to rats at a volume of 10 ml/kg/day, a dull appearance of the pelage and light brown areolas, pelage being soiled with urine, and a brown discoloration of urine were observed. In another rat study, a NOEL of 20 mg/kg/day was reported for 2,4-Diaminophenoxyethanol HCl; with brownish pigment reported in thyroid glands and a higher incidence of hemosiderosis (without associated hematological changes), increased salivation, lower weight gain in males, colored urine, traces of nitrites and bilirubin in urine at 100 mg/kg/day.

2,4-Diaminophenoxyethanol HCl was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits in two studies. When tested neat using rabbits, it was found to be an ocular irritant.

2,4-Diaminophenoxyethanol HCl was slightly irritating to the skin of rabbits when a 4% solution was used. When tested neat using rabbits, it also was not considered an irritant.

When evaluating the sensitizing potential of 2,4-Diaminophenoxyethanol HCl using a Magnusson/Kligman study design, erythema was observed in 3/10 guinea pigs at challenge with a 25% solution of test material in petrolatum. In a study using a Buehler test methodology, 2,4-Diaminophenoxyethanol HCl applied neat did not produce sensitization reactions. An LLNA study of 2,4-Diaminophenoxyethanol HCl at 0.5 to 10% in DMSO did show lymphoproliferative responses indicative of delayed contact hypersensitivity and the ingredient was considered a moderate skin sensitizer.

No teratogenic effects were observed due to administration of 2,4-Diaminophenoxyethanol HCl in an oral study using rats or in a dermal study using mice. In another rat study, an oral dose of 125 mg/kg was maternally toxic and associated with fetal weight deficits and some delayed ossification; the NOEL was 20 mg/kg.

Genotoxicity assays using bacterial, mammalian cells, drosophila, mice, and rats provided mixed results. In most bacterial assays, the results were negative, but an increase in mutation frequency was reported in two studies using *S. typhimurium* TA98 with metabolic activation. In mammalian cell assays, results were negative, except for one study which found an increase in micronucleated cells in human lymphocytes that were mitogen-stimulated for 48 h (but no increase with 24 h mitogen stimulation). In animal assays (dominant lethal, micronucleus, unscheduled DNA synthesis) no evidence of genotoxicity was reported.

The majority of the genotoxicity studies for 2,4-Diaminophenoxyethanol HCl did not observe any genotoxicity.

In 2 oral carcinogenic studies, mice and rats received 0.004 and 0.007% 2,4-Diaminophenoxy-ethanol HCl and 0.05 and 1.0% 2,4-Diaminophenoxyethanol HCl, respectively, in tap water for 104 weeks. No carcinogenic effects were reported.

Available epidemiology studies that consider the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other endpoints were described.

DISCUSSION

The CIR Expert Panel considered that the available acute and subchronic, oral, ocular, and dermal toxicity data are adequate to support the safety of 2,4-Diaminophenoxyethanol HCl with respect to systemic toxicity endpoints. This ingredient did not produce significant toxicity to the reproductive system or affect development of fetuses in animal studies at levels that were not maternally toxic. Based on the low dermal absorption of 2,4-Diaminophenoxyethanol HCl from oxidative hair dye formulations, such maternally toxic levels are highly unlikely. The Expert Panel noted that there were mixed results in the available genotoxicity data; however, 2,4-Diaminophenoxyethanol was not carcinogenic in mouse and rat studies.

2,4-Diaminophenoxyethanol HCl was slightly to non-irritating to the skin of rabbits. Dermal sensitization study results are mixed, but a maximization study and a local lymph node assay were positive, so this ingredient could be considered a moderate skin sensitizer. It is relevant that 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 exposure.

No toxicity studies were identified specifically for the sulfate

salt, 2,4-Diaminophenoxyethanol Sulfate, in the published literature. The toxicities of the two salts are expected to be the same, and their maximum use concentrations are the same, so exposures as used in hair dyes would be the same. Therefore, the Expert Panel determined that the toxicity data on 2,4-Diaminophenoxyethanol HCl could be extrapolated to 2,4-Diaminophenoxyethanol Sulfate.

While there were no human studies that specifically addressed these two ingredients, the CIR Expert Panel did review the available human epidemiology data. In considering these data, the CIR Expert Panel concluded that the available hair dye epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment.

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