Final Report on the Safety Assessment of Pyrogallol

Pyrogallol, a benzenetriol, is used in oxidative hair dyes at concentrations ranging from ≤0.1 to 5.0%. The oral LD₅₀'s in rats ranged from 800 to 1270 mg/kg. Pyrogallol was not an ocular irritant when tested at a concentration of 1%. It was slightly irritating and induced sensitization reaction in the skin of guinea pigs. Sensitization reactions were noted in 3 of 25 patients patch tested with Pyrogallol.

Significant teratogenic effects were not observed in the offspring of female rats dosed with Pyrogallol. No treatment-related effects were observed in a multigeneration reproductive toxicity study in which rats received dermal applications of a hair dye containing 0.4% Pyrogallol.

Pyrogallol was mutagenic in almost all systems tested. However, in two carcinogenicity studies, the number of neoplasms in mice dermally treated with 50% Pyrogallol in acetone was not significantly different from that of controls. Similar results were reported in a carcinogenicity study in which a hair dye containing 0.49% Pyrogallol and H₂O₂ in aqueous solution was applied to the skins of mice.

On the basis of the available animal and clinical data presented in this report, it is concluded that Pyrogallol is safe as a cosmetic ingredient in the present practices of use and concentration.

INTRODUCTION

The toxicity of Pyrogallol, a benzenetriol, is reviewed in this report. The Cosmetic Ingredient Review Expert Panel has evaluated the safety of the following benzenediols: 2-Methyl Resorcinol, Resorcinol, Hydroquinone, and Pyrocatechol.¹,²

CHEMISTRY

Chemical and Physical Properties

Pyrogallol: Pyrogallol (CAS No. 87-66-1) is an aromatic alcohol with the following structure:³
As a cosmetic ingredient, Pyrogallol consists of a minimum of 99% Pyrogallol.\(^{[4]}\) Other names for this ingredient are: 1,2,3-Benzene-triol, 1,2,3-Trihydroxybenzene, and Pyrogalllic acid.\(^{[3,5]}\) Technical synthetic and technical natural grades of Pyrogallol are available. Technical synthetic Pyrogallol contains 90 to 96% w/w Pyrogallol, and technical natural Pyrogallol contains not less than 98% Pyrogallol.\(^{[6]}\) Pyrogallol is stable in the dark and in the absence of alkali,\(^{[4]}\) and sublimes when heated slowly.\(^{[7]}\) It is oxidized easily when in alkaline solutions, and such solutions of Pyrogallol are potent reducing agents.\(^{[8]}\) A UV spectral analysis of chemically pure (99%) Pyrogallol, 0.1% w/v in methanol, showed a single absorbance maximum at 267.5 nm.\(^{[9]}\) Additional properties of Pyrogallol are listed in Table 1.

**Methods of Production**

Pyrogallol is prepared via the chlorination of cyclohexanol to tetrachlorocyclohexanone, followed by hydrolysis.\(^{[4]}\)

**Analytical Methods**

Pyrogallol has been assayed via the following methods: thin layer chromatography,\(^{[12-15]}\) gas chromatography,\(^{[16]}\) gas-liquid chromatography, high performance liquid chromatography, ultraviolet spectrophotometry, and mass spectrometry.\(^{[13]}\)
Impurities
Iron (0.001%) and heavy metals (5 ppm max) are impurities that have been detected in Pyrogallol. Data on possible organic impurities in cosmetic grade Pyrogallol, such as chlorinated aromatic hydrocarbons, are not available.

USE

Purpose in Cosmetics
Pyrogallol was the first synthetic organic dye to be used on human hair. It is being used at present as a modifier in oxidation dyes. Typical use concentrations of Pyrogallol in oxidative hair dyes range between 0.25 and 0.383% by weight.

Scope and Extent of Use in Cosmetics
The FDA cosmetic product formulation computer printout is compiled through voluntary filing of such data in accordance with Title 21 Part 720.4 of the Code of Federal Regulations. Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product. The actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration. Pyrogallol is present in 42 hair dyes and colors (all types requiring caution statement and patch test) at concentrations ranging from ≤0.1 to 5.0% (Table 2).

In countries of the European Economic Community, the maximum concentration of Pyrogallol allowed in hair dyes (for professional or general use) is 5.0%. Pyrogallol (Quasi Drug Use Only) has also been approved for use in cosmetic formulations marketed in Japan.

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

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total no. of formulations in category</th>
<th>Total no. containing ingredient</th>
<th>No. of product formulations within each concentration range (%)</th>
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<td>Pyrogallol</td>
<td></td>
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<tr>
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<td>42</td>
<td>&gt;1-5                            &gt;0.1-1                            ≤0.1</td>
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<tr>
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<td></td>
<td>42</td>
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</table>
The oxidative or permanent hair dyes containing Pyrogallol, as “coal tar” hair dye products\(^{23}\) are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and “patch test” instructions for determining whether the product causes skin irritation.\(^{24}\) In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

**Caution—**This product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eye-brows; to do so may cause blindness.

Patch test instructions call for a 24-h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.\(^{25}\)

### Noncosmetic Use

Pyrogallol may be used safely in combination with ferric ammonium citrate for coloring plain and chromic catgut sutures used in general and ophthalmic surgery. The concentration of the ferric ammonium citrate—pyrogallol complex shall not exceed 3.0% of the total weight of the suture material.\(^{26}\) Other uses of Pyrogallol are: developer in photography, making colloidal solutions of metals, mordant for wool, staining of leather, process engraving, manufacture of various dyes, manufacture of pesticides, dying furs, reagent for antimony and bismuth (in analytical chemistry), and active reducer for gold, silver, and mercury salts.\(^{7,26}\)

### BIOLOGICAL PROPERTIES

Pyrogallol inhibited rat thyroid peroxidase activity and the uptake and incorporation (into tyrosine) of \(^{131}\)I in rat thyroid slices in vitro.\(^{27}\) Other effects of Pyrogallol are summarized as follows: negative chronotropic effect on perfused rabbit and frog hearts and blood pressure elevation in dogs,\(^{28}\) increased cardiac output and alveolar-ventilation in normal sheep and sheep suffering from respiratory distress,\(^{29}\) uncoupling of oxidative phosphorylation in rat kidney and beef heart mitochondria in vitro,\(^{30}\) decreased ATP concentrations in mouse brain,\(^{31}\) inhibition of catechol-O-methyl transferase activity,\(^{32}\) inhibition of rat liver mitochondrial aldehyde dehydrogenase in vitro,\(^{33}\) and inhibition of beef heart mitochondrial succinoxidase and NADH-oxidase enzyme systems.\(^{34}\)

### Absorption, Distribution, Metabolism, and Excretion

Pyrogallol (100 mg/kg) was administered to 4 adult albino rats (weight 250–350 g) via either stomach tube or intraperitoneal injection. Urine samples were collected 24 h after administration, extracted with ether, and analyzed via thin layer chromatography. Some of the urine samples were subjected to acid hydrolysis before extraction. Pyrogallol was not detected in extracts of nonhydrolyzed urine. However, prominent spots, corresponding to Pyrogallol and 2-O-methylpyrogallol, were observed on
chromatograms of hydrolyzed urine extracts. Additionally, traces of resorcinol were detected in these extracts. Resorcinol also was detected in rat fecal extracts that had been incubated with Pyrogallol, indicating that Pyrogallol could have been metabolized to resorcinol.\(^{(35)}\) Results from guinea pig liver perfusion experiments indicated that Pyrogallol was conjugated with glucuronic acid. Glucuronic acid conjugates were detected in blood and urine via thin layer chromatography.\(^{(36)}\) Pyrogallol in urine from humans\(^{(37)}\) probably is derived from the decarboxylation of gallic acid, an ingredient in tea, in the alimentary tract.

Female mice (weights 20–25 g, number not stated) of an inbred strain were injected intraperitoneally with Pyrogallol (60 mg/kg). Concentrations of Pyrogallol in the brain were determined according to a modification of the procedure by Swain and Hillis.\(^{(38)}\) The maximum concentration of Pyrogallol in the brain, 28.4 μg/wet weight of brain, was noted 10 min after injection. At 15 min postinjection, the concentration of Pyrogallol approached zero.\(^{(31)}\)

**TOXICOLOGY**

**Acute Oral Toxicity**

The oral toxicities of technical synthetic Pyrogallol (92.2% w/w Pyrogallol) and technical natural Pyrogallol (98.8% w/w Pyrogallol) were evaluated using 54 male rats (weight 249–305 g) and 60 female rats (weight 191–240 g) of the Sprague-Dawley strain. Both test substances were diluted to a concentration of 500 mg/ml of distilled water, and the following doses were administered via gavage: 800 to 2261 mg/kg (natural Pyrogallol, 24 male rats), 566 to 1600 mg/kg (natural Pyrogallol, 24 female rats), 566 to 1600 mg/kg (synthetic Pyrogallol, 24 male rats), and 283 to 1131 mg/kg (synthetic Pyrogallol, 30 female rats). Six male and 6 female control animals were dosed with 4.5 ml of distilled water/kg of body weight. The oral LD\(_{50}\)’s for male and female rats dosed with technical synthetic Pyrogallol were 1270 mg/kg (95% confidence limits = 1054–1330 mg/kg) and 800 mg/kg (95% confidence limits = 664–964 mg/kg), respectively. Oral LD\(_{50}\)’s for male and female rats dosed with technical natural Pyrogallol were 1270 mg/kg (95% confidence limits = 839–1923 mg/kg) and 848 mg/kg (95% confidence limits = 733–982 mg/kg), respectively.\(^{(6)}\)

In another study, the acute oral toxicity of a 50% solution of Pyrogallol (in DMSO) was evaluated using 10 male Sprague-Dawley rats. The LD\(_{50}\) was 1800 mg/kg (95% confidence limits: 1420–2290 mg/kg).\(^{(39)}\)

The oral toxicity of Pyrogallol was evaluated using 5 deer mice (average weight 20 g). Twenty-five wheat seeds, treated with 2.0% (w/w) Pyrogallol, were placed in the cage of each mouse daily for 3 days. The number of wheat seeds consumed daily was recorded, and the total number of treated seeds consumed by all mice during the 3-day period was subtracted from the total number of seeds available. The difference was converted into what was termed the feed reduction (FR), defined as the percentage of seeds refused. The FR, average weight of individual wheat seeds (50 mg), and average weight of each mouse (20 g) were used to calculate the LD\(_{fr}\). The LD\(_{fr}\) represented the average amount of Pyrogallol (mg/kg/day) ingested without inducing > 50.0% mortality. The LD\(_{fr}\) for Pyrogallol was 1240 mg/kg/day.\(^{(40)}\)

Pyrogallol also was administered, in the diet, to three groups of 1-day-old chicks (10/group) for 4 weeks. The three groups were fed basal diets containing 0.1, 1.0, and
2.0% Pyrogallol, respectively. Group mortality rates for animals fed 0.1%, 1.0%, and 2.0% Pyrogallol were 0.0%, 10.0%, and 95.0%, respectively. Deaths in the 2.0% group occurred within 10 days. \(^{41}\)

**Acute Dermal Toxicity**

The dermal toxicity of technical synthetic Pyrogallol (92.2% w/w Pyrogallol) and technical natural Pyrogallol (98.8% w/w Pyrogallol) was evaluated using 18 male (weight 244–309 g) and 18 female (weight 200–238 g) Sprague-Dawley rats. Each test substance was diluted with distilled water to a concentration of 500 mg/ml and applied (dose = 2100 mg/kg) via occlusive patches to the backs of 6 male and 6 female animals. Patches remained for 24 h. A control group of 12 rats was treated with distilled water according to the same procedure. An LD\(_{50}\) could not be determined for either test substance at the administered dose. \(^{42}\)

**Subchronic Percutaneous Toxicity**

The percutaneous toxicity of a hair dye formulation containing 0.4% Pyrogallol was evaluated using 12 (6 males, 6 females) adult New Zealand white rabbits. The hair dye was mixed with an equal volume of 6.0% hydrogen peroxide and applied (1 ml/kg) to the dorsolateral aspects of the thoracic–lumbar area twice per week for 13 weeks. Hair was clipped from application sites throughout the study. The application sites on 6 animals were abraded on the first day of each week of treatment. Application sites on all animals were shampooed, rinsed, and dried 1 h after application of the dye. Three groups of untreated rats (12/group) served as controls. Analyses of blood and urine were done during weeks 0, 3, 7, and 13. Animals were killed after the 13th week, and both gross and microscopic examinations were performed. Slight thickening of the skin was observed only at sites where the dye had been applied. There were statistically significant differences in clinical chemistry and hematological values between experimental and control groups. The results of the urinalyses were unremarkable. Neither gross nor microscopic changes related to administration of the dye were observed. \(^{43}\)

**Immunotoxicity**

The immunosuppressive potential of Pyrogallol was evaluated using the Mishell-Dutton system. In this *in vitro* system, B lymphocyte cultures from dissociated mouse splenic cells were incubated with sheep red blood cells (antigens) for 5 days. The B lymphocytes mature into cells that produce antibodies directed against sheep red blood cells. These antibodies (along with complement) cause lysis of erythrocytes, indicated by a zone of lysis (or plaque) around the antibody-forming cells. The addition of Pyrogallol (5 \(\mu\)g/culture) resulted in \(\geq 90\%\) suppression of plaque formation. Toxicity, determined by trypan blue dye exclusion, was expressed as the test substance dose (\(\mu\)g/culture) resulting in a 50% reduction in viability. Pyrogallol induced toxicity at a dose of 5 \(\mu\)g/culture. In control cultures, the number of plaque-forming cells per culture ranged from 10,000 to 25,000. \(^{44}\)
Cytotoxicity

The effect of Pyrogallol on plasma membrane integrity was evaluated using human diploid embryonic lung fibroblasts (cell line MRC-5). Plasma membrane damage was quantified by the leakage of a cytoplasmic nucleotide marker from radioactive cells. Fibroblasts containing \(^3\)H-uridine were rinsed with salt solution and then treated with Pyrogallol for 30 min. Pyrogallol was added to cell cultures as a 25 mM solution, made from ethanol or dimethyl sulfoxide stock solutions by dilution with Tris-buffered saline. Cultures were then centrifuged, and the released radioactivity was measured. Results were expressed as percentages of the maximal amount of radioactivity released. There was no evidence of plasma membrane damage.\(^{45}\)

The effect of Pyrogallol on ciliary activity was evaluated using tracheal organ cultures prepared from 16 to 17-day-old chicken embryos. One tracheal ring was placed in a Plexiglas chamber that contained culture medium admixed with either ethanol or dimethyl sulfoxide solutions of Pyrogallol. The concentration of Pyrogallol was 5 mM. Ciliary activity was recorded (60 min period) using an inverted microscope connected to a TV camera, TV monitor, and videotape recorder. This procedure was repeated on at least three different occasions, using rings from different tracheal preparations. Ciliostasis was noted after 15 min of observation.\(^{46}\)

Ocular Irritation

The ocular irritation potential of Pyrogallol was evaluated using two groups of 6 male New Zealand white rabbits (weight 2.5–3.0 kg). In one group of animals, the test substance (100 mg, powder form) was instilled into the conjunctival sac of the left eye. In the other group, 0.1 ml of a 1.0% solution of Pyrogallol (in propylene glycol) was instilled into the left eye. Eyes (both groups) were not rinsed after instillation. Untreated eyes served as controls. Pyrogallol (powder form) induced ocular irritation, although the 1.0% solution of Pyrogallol was not an ocular irritant.\(^{47}\)

Skin Irritation

The primary skin irritation potential of Pyrogallol was evaluated using 6 albino rabbits. The test substance (500 mg, powder form) was applied to abraded and intact skin sites on each animal. Each site was covered for 24 h with a patch (type not stated) secured with adhesive tape. Reactions were scored 24 and 72 h after patch application. A primary irritation index of 0.5 was reported.\(^{48}\)

A skin irritation study of technical synthetic Pyrogallol (92.2% w/w Pyrogallol) and technical natural Pyrogallol (98.8% w/w Pyrogallol) was conducted using 6 Dunkin Hartley female guinea pigs (weight not < 350 g). Each test substance was diluted with distilled water to a concentration of 500 mg/ml and applied to two sites (0.05 ml/site) on the back of each animal via patches made of lint. The four patches (two per test substance) were covered with aluminum foil and held in place with waterproof plaster for 24 h. Sites were then washed with soap and water, rinsed, and dried. Each site was graded 1, 4, 24, 48, and 72 h after patch removal according to the scales: 0 (no erythema) to 4 (severe erythema to slight eschar formation) and 0 (no edema) to 4 (severe edema, raised more than 1 mm and extending beyond area of exposure). Very slight erythema was observed at one site treated with technical natural Pyrogallol (3 guinea pigs), and at one site treated with technical synthetic Pyrogallol (2 guinea pigs).
Additionally, dryness and thickening (leading to flaking) of the skin were observed at all treated sites (between 4 and 8 days after patch removal), except for one site treated with technical natural Pyrogallol and one site treated with technical synthetic Pyrogallol (same guinea pig). Both test substances were classified as slightly irritating to guinea pig skin. (45)

**Skin Sensitization**

The skin sensitization potential of unrefined Pyrogallol was evaluated using 29 female Hartley guinea pigs (average body weight 350 g). On three consecutive days, 0.1 ml volumes of 0.01 M Pyrogallol and 0.05 M Pyrogallol (solutions contained NaCl and a complete adjuvant) were injected subcutaneously into the feet of 21 guinea pigs. During the same week, a fourth injection was made at a site near the neck. Test solutions also were injected subcutaneously 4 weeks after the first injection. The remaining 8 guinea pigs were injected subcutaneously with 0.01 and 0.1 M Pyrogallol according to the same induction procedure. However, the challenge phase consisted of sealed cloth applications of test solutions. Injection sites on each animal were examined macroscopically and microscopically. Of the 21 guinea pigs tested, 7 and 14 animals had sensitization reactions to 0.01 M Pyrogallol and 0.05 M Pyrogallol, respectively. Of the remaining 8 guinea pigs, 3 and 6 animals had sensitization reactions to 0.01 M and 0.1 M Pyrogallol, respectively. (50)

In another study, the skin sensitization potential of Pyrogallol was evaluated using groups of 10 female Hartley albino guinea pigs. During induction, 0.05 ml of a 1.0% solution of Pyrogallol (in water) was injected intradermally. A 25.0% solution of the test substance (in propylene glycol) was applied topically 1 week later. Each site was covered with an occlusive patch for 48 h. After a 2-week nontreatment period, the animals were challenged with a single topical application of the 25.0% solution. There was no evidence of sensitization in any of the animals tested. (51)

**Reproductive Effects**

A multigeneration reproduction study was conducted using Charles River CD rats. A total of 40 males and 40 females were tested with a hair dye formulation that contained 0.4% Pyrogallol. The dye was mixed with an equal volume of 6% hydrogen peroxide and applied (0.5 ml) to the skin twice per week throughout mating, gestation, and during the period of lactation to weaning of the F1b, F2b, and F3c litters of the respective generations. There were no treatment-related changes in general behavior and appearance, body weight, or survival in parents or offspring. However, mild skin reactions, in treated animals, were noted intermittently throughout the study. Fertility, gestation, and viability indices were comparable between control and experimental groups. Additionally, there were no treatment-related gross or microscopic lesions observed in F1b parental rats or F3b weaning rats. (52)

**Teratogenicity**

Pyrogallol (in propylene glycol) was administered via gavage to 17 female Sprague-Dawley rats (weight 225–250 g) on days 6 to 15 of gestation. The following doses were administered: 100 mg/kg (5 rats), 200 mg/kg (6 rats), and 300 mg/kg (6 rats). Solutions
of Pyrogallol were prepared daily and dosed at a rate of 10 ml/kg. Animals in the vehicle control group (22 rats) were dosed with propylene glycol at a rate of 10 ml/kg. Vitamin A and aspirin were administered to positive control groups on day 9 of gestation and days 6 to 15 of gestation, respectively. Vitamin A was administered at a dose of 100,000 IU per animal, and aspirin was administered at a dose of 350 mg/kg. All dams were killed on day 20 of gestation via carbon dioxide inhalation. There were no mortalities in experimental or vehicle control groups during the gestational period. However, a significant decrease in the mean maternal weight gain occurred (days 6–16 of gestation) in rats that received 300 mg/kg doses of Pyrogallol. Smaller fetuses and a significant increase in the total number of fetal resorptions also were noted in this group. The numbers of fetal implantations and fetal anomalies in all experimental groups were not significantly different from those in the vehicle control group. A statistically significant increase in the number of abnormal fetuses with gross, soft tissue, and skeletal anomalies (p = 0.001) was observed in groups dosed with vitamin A or aspirin.53

The teratogenicity of a hair dye formulation containing 0.4% Pyrogallol was evaluated using 20 Charles River CD female rats. The hair dye (2 ml/kg) was applied to the dorsoscapular area (shaved skin) of each animal on days 1, 4, 7, 10, 13, 16, and 19 of gestation. Three groups of untreated rats (unshaved) served as controls. Animals in the positive control group were given acetylsalicylic acid (250 mg/kg) via gavage on days 6 to 16 of gestation. The dams were killed on day 20 of gestation via chloroform anesthesia, and fetuses were removed via cesarean section. One third of the fetuses from each litter were examined for visceral anomalies. The remaining fetuses were examined for skeletal anomalies. Toxic effects were not observed in experimental or control dams throughout the study. The mean numbers of corpora lutea, implantation sites, and live fetuses in experimental groups were not significantly different from those in control groups. There were also no significant differences in the number of females with resorption sites and the mean number of resorptions per pregnancy. The incidence of fetal soft tissue and skeletal anomalies in experimental groups was not significantly different from that of negative control groups. A significant increase in the number of fetuses with skeletal and soft tissue anomalies and in the number of dead or resorbed fetuses was observed in the positive control group.43

MUTAGENICITY

In Vitro Tests

The mutagenic potentials of technical natural Pyrogallol (not < 98% w/w Pyrogallol) and technical synthetic Pyrogallol (90–96% w/w Pyrogallol) were evaluated using strains TA1535, TA1537, TA1538, TA98, and TA100 of Salmonella typhimurium. Both test substances were diluted with water and tested at concentrations that ranged from 50 to 5,000 µg/plate (technical natural Pyrogallol) and 15 to 5000 µg/plate (technical synthetic Pyrogallol) according to the procedure by Ames et al.54 Both grades of Pyrogallol were mutagenic to strains TA1537 and TA100 in both the presence and absence of metabolic activation (Table 3).55

In another study, the mutagenicity of Pyrogallol was evaluated using the strains of S. typhimurium stated above. Tests were conducted with and without metabolic activation.54 Pyrogallol was tested at concentrations up to 3600 µg/plate. In the absence of metabolic activation, Pyrogallol was mutagenic to strain TA1537. Pyro-
gallol was mutagenic to strains TA98 and TA100 with and without metabolic activation (Table 3).\(^\text{56}\)

The mutagenicity of Pyrogallol (in DMSO) was evaluated using strains TA1538 and TA98 of *S. typhimurium*. Concentrations ranging from 20 to 1000 \(\mu\)g/plate were tested with and without metabolic activation.\(^\text{54}\) With metabolic activation, Pyrogallol was not mutagenic to strain TA98. Without metabolic activation, a weak mutagenic response to Pyrogallol (500 \(\mu\)g/plate) was observed in strain TA98. However, there was no linear correlation between mutagenicity and doses tested. Pyrogallol was not mutagenic to strain TA1538 with or without metabolic activation (Table 3).\(^\text{53}\)

In another study, the mutagenicity of Pyrogallol (in water) was evaluated using strains TA98, TA100, and TA1537 of *S. typhimurium*. Concentrations ranging from 5 to 200 \(\mu\)g/plate were tested.\(^\text{54}\) The 200 \(\mu\)g/plate concentration was tested with and without metabolic activation. Concentrations less than 200 \(\mu\)g/plate were tested without metabolic activation. Pyrogallol was mutagenic to strains TA100 and TA1537 with and without metabolic activation but was not mutagenic to strain TA98 (Table 3).\(^\text{57}\)

The mutagenicity of Pyrogallol (in water) was evaluated using strain TA100 of *S. typhimurium*. Pyrogallol was tested at a concentration of 100 \(\mu\)g/plate according to a modification of the procedure by Ames et al.\(^\text{54}\) Without metabolic activation, Pyrogallol was described as being moderately mutagenic to strain TA100. With metabolic activation, Pyrogallol was considerably mutagenic to strain TA100 (Table 3).\(^\text{58}\)

The Ames test was used to evaluate the mutagenic potential of Pyrogallol in strains TA98, TA100, and TA1537 of *S. typhimurium*. With and without metabolic activation, Pyrogallol was mutagenic to strains TA98 and TA100 within the range of concentrations tested (0.1–15.0 \(\mu\)mol/plate). In the spot test, Pyrogallol was mutagenic to strain TA1537 (Table 3).\(^\text{59}\)

The mutagenicity of Pyrogallol (in ethanol) was evaluated using strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium*. Tests were conducted with and without metabolic activation.\(^\text{54}\) In spot tests, Pyrogallol (3 \(\mu\)mol/plate) was not mutagenic to strains TA1535 and TA1537 with or without metabolic activation. The mutagenicity of Pyrogallol in strains TA98 and TA100 was questionable. In quantitative plate tests involving strain TA98, Pyrogallol was tested at concentrations ranging from 0.3 to 3.0 \(\mu\)mol/plate. In some of these tests, Pyrogallol was described as being weakly mutagenic to strain TA98 with and without metabolic activation (Table 3).\(^\text{60}\)

In the L5178Y mouse lymphoma cell assay, technical synthetic Pyrogallol (in distilled water) was tested at concentrations of 4 to 80 \(\mu\)g/ml. Compared to vehicle control values, Pyrogallol (between 17 and 80 \(\mu\)g/ml) increased the mutation frequencies and absolute mutant numbers in the presence of metabolic activation. Without metabolic activation, results with Pyrogallol (between 19 and 34 \(\mu\)g/ml) were the same. It was concluded that technical synthetic Pyrogallol was mutagenic (Table 3).\(^\text{61}\)

Technical synthetic Pyrogallol (in distilled water) was tested for induction of chromosomal aberrations in human lymphocytes cultured *in vitro*. Pyrogallol was tested without metabolic activation at concentrations of 10, 50, 75, and 100 \(\mu\)g/ml and with metabolic activation at concentrations of 100, 500, and 1000 \(\mu\)g/ml. Compared to the solvent control, a significantly higher proportion of cells with chromosomal aberrations was noted in cultures incubated with 50, 75, and 100 \(\mu\)g/ml concentrations (without metabolic activation). The same was true for cultures incubated with concentrations of 500 and 1000 \(\mu\)g/ml (with metabolic activation). It was concluded that
technical synthetic Pyrogallol was clastogenic with and without metabolic activation (Table 3).\(^{62}\)

Pyrogallol induced chromatid breaks and exchanges in cultures of Chinese hamster ovary cells (without metabolic activation). With metabolic activation, the chromosome damaging activity of Pyrogallol was suppressed.

Pyrogallol was tested at concentrations of 0.1 mg/ml and 3.0 mg/ml of culture medium, respectively, with and without metabolic activation. Results were based on the analysis of 200 metaphase plates per sample (Table 3).\(^{63}\)

The mutagenic activity of Pyrogallol in strain D7 of Saccharomyces cerevisiae was evaluated using the mitotic gene conversion assay. Pyrogallol was tested at a concentration of 0.3 mg/ml of culture medium. Significant \((P < 0.01)\) mitotic gene conversion was noted when the pH of the culture medium was alkaline (\(pH \geq 10\)). At \(pH 7\), significant mutagenic activity was not noted (Table 3).\(^{64}\)

**In Vivo Tests**

The mutagenicity of Pyrogallol was evaluated using the recessive lethal mutations test. One dose of Pyrogallol (in 5% saccharose) was fed to Berlin K (wild-type) and Basc strains of Drosophila melanogaster. The dose administered was close to the LD\(_{50}\). Approximately 1200 X-chromosomes were tested per experiment in each of three successive broods. F\(_2\) progeny cultures with two, or fewer, wild-type males were routinely retested in the F\(_3\) generation to confirm X-linked recessive lethal mutations. Pyrogallol significantly increased \((P = 0.05)\) the frequency of sex-linked recessive lethal mutations.\(^{56}\)

In the micronucleus test,\(^{65}\) Pyrogallol (252 mg/kg) was administered intraperitoneally to 4 mice at 0 and 24 h. An untreated group of 4 mice served as the control. Bone marrow smears were prepared at 30 h, and 1000 polychromatic erythrocytes were scored per mouse. Compared to the control, Pyrogallol significantly increased \((p < 0.01)\) the percentage of micronucleated polychromatic erythrocytes.\(^{56}\)

In another in vivo test, mice (3–4 months old) were injected intraperitoneally with 0.01 M, 0.02 M, and 0.03 M solutions of Pyrogallol. Bone marrow tissue was removed 24 h after administration and prepared for microscopic examination. One-hundred fifty metaphases were counted per slide. Chromatid breaks were observed only in bone marrow cells from mice dosed with 0.02 M and 0.03 M concentrations of Pyrogallol.\(^{39}\)

**CARCINOGENICITY**

The carcinogenicity of Pyrogallol was evaluated using 150 female Swiss mice (7 weeks old). Three groups of mice (50/group) were treated with 5%, 25%, and 50% solutions of Pyrogallol (in acetone), respectively. Each solution (0.02 ml) was applied to dorsal shaved skin, between the flanks, twice per week. A total of 135 mice served as the untreated control group. Mice treated with acetone and 7,12-dimethylbenzanthracene served as vehicle and positive controls, respectively. Gross and microscopic examinations were performed. In all treatment groups, the number of neoplasms induced was not significantly different from that of the untreated control group. Lymphomas, pulmonary adenomas, and hepatic hemangiomas predominated. There were no skin neoplasms. At week 100, 13 of the 150 mice of the Pyrogallol groups were
<table>
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<td>Pyrogallol</td>
<td>50–5000 μg/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, TA1535, TA1537, and TA1538</td>
<td>Ames et al.(^{(54)})</td>
<td>Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)</td>
<td>55</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>15–5000 μg/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, TA1535, TA1537, and TA1538</td>
<td>Ames et al.(^{(54)})</td>
<td>Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)</td>
<td>55</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>Up to 3600 μg/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, TA1535, TA1537, and TA1538</td>
<td>Ames et al.(^{(54)})</td>
<td>Mutagenic to strains TA98 and TA100 (presence and absence of metabolic activation) and strain TA1537 (absence of metabolic activation)</td>
<td>56</td>
</tr>
<tr>
<td>Pyrogallol (in DMSO)</td>
<td>20–1000 μg/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98 and TA1538</td>
<td>Ames et al.(^{(54)})</td>
<td>Not mutagenic to strain TA98 (presence of metabolic activation) and weakly mutagenic to strain TA98 (absence of metabolic activation)</td>
<td>53</td>
</tr>
<tr>
<td>Pyrogallol (in water)</td>
<td>5–200 μg/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, and TA1537</td>
<td>Ames et al.(^{(54)})</td>
<td>Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)</td>
<td>57</td>
</tr>
<tr>
<td>Pyrogallol (in water)</td>
<td>100 μg/plate</td>
<td><em>Salmonella typhimurium</em> strain TA100</td>
<td>Ames et al.(^{(54)})</td>
<td>Moderately mutagenic (absence of metabolic activation) and considerably mutagenic (presence of metabolic activation)</td>
<td>58</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.1–15.0 μmol/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, and TA1537</td>
<td>Ames et al.(^{(54)})</td>
<td>Mutagenic to strains TA98 and TA100 (presence and absence of metabolic activation)</td>
<td>59</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.1–15.0 μmol/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, and TA1537</td>
<td>Spot test (Ames et al.(^{(54)}))</td>
<td>Mutagenic to strain TA1537</td>
<td>59</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Test System</td>
<td>Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------</td>
<td>-------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (in ethanol)</td>
<td>3.0 µmol/plate</td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, TA1535, and TA1537</td>
<td>Mutagenicity to strains TA98 and TA100 was questionable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (in ethanol)</td>
<td>0.3-3.0 µmol/plate</td>
<td><em>Salmonella typhimurium</em> strain TA98</td>
<td>Weakly mutagenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (in distilled water)</td>
<td>4.0-80.0 µg/ml</td>
<td>LS17BY mouse lymphoma cell assay</td>
<td>Mutagenic (presence and absence of metabolic activation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (in distilled water)</td>
<td>50, 75 and 100 µg/ml</td>
<td>Chromosome aberrations assay involving human lymphocytes (absence of metabolic activation)</td>
<td>Clastogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (in distilled water)</td>
<td>100, 500, and 1000 µg/ml</td>
<td>Chromosome aberrations assay involving human lymphocytes (presence of metabolic activation)</td>
<td>Clastogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.1 mg/ml</td>
<td>Saccharomyces cerevisiae strain D7</td>
<td>Chromatid breaks and exchanges assay involving Chinese hamster ovary cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.3 mg/ml</td>
<td>Mitotic gene conversion assay</td>
<td>Significant mitotic gene conversion at pH 10 but not at pH 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
alive. None of the mice were alive at week 110. Survivors were noted in the control group during the 120th week. In another study (same procedure), the carcinogenicity of Pyrogallol in New Zealand rabbits (8 weeks old) was evaluated. Three groups of 5 rabbits were treated with solutions of 5%, 25%, and 50% Pyrogallol (in acetone or methanol), respectively. Fourteen rabbits served as untreated controls. Positive controls (15 rabbits) were treated with 9,10-dimethylbenz[a]anthracene. After 160 weeks of treatment, the only evidence of tumor formation in experimental groups was a uterine tumor in 1 animal treated with 50% Pyrogallol. A significant number of skin neoplasms (papillomas, squamous cell carcinomas, and keratoacanthomas) was observed in the positive control group. Pyrogallol was not carcinogenic at any of the concentrations tested.

Pyrogallol (in 50% DMSO) was administered subcutaneously (0.1 mg/kg body weight) to 9 male and 10 female 2-week-old, Fischer rats for 8 weeks. During the next 50 weeks of treatment, the dose was changed to 14 mg/rat. Rats in the control group were dosed with 50% DMSO. In the experimental group, histiocytomas were observed at the injection sites of 3 male rats and 1 female rat. Neoplasms were not observed in controls.

The carcinogenicity of an oxidative hair dye formulation containing 0.49% Pyrogallol was evaluated using random-bred Swiss Webster mice (6 weeks old). The experimental group and the two untreated control groups each contained 60 male and 60 female mice. Treatment was initiated when the mice were 8 weeks old. The dye was mixed with an equal volume of 6% H$_2$O$_2$ and applied (0.5 ml per application) once per week for a period of 20 months. Applications were made via a calibrated syringe to an area of skin, clipped free of hair, in the interscapular region. After 9 months of treatment, 10 males and 10 females were selected randomly from each group for clinical tests, hematology, and necropsy. Urine samples were analyzed for color, pH, occult blood, albumin, and glucose. Blood samples were obtained via cardiac puncture, and complete blood counts and differential white cell counts were determined. At 20 months posttreatment, the remaining animals were killed for necropsy. At the time of necropsy, complete and differential cell counts were performed on blood samples from 10 mice (5 males, 5 females) per group. Results from analyses of the blood and urine indicated no treatment-related effects. Pulmonary adenomas, hepatic hemangiomas, and malignant lymphomas were observed in experimental and control groups. Statistical analyses, chi-square and Fisher exact tests, of the incidence of hepatic hemangiomas, pulmonary adenomas, and malignant lymphomas indicated no significant differences between experimental and control groups.

**Cocarcinogenicity**

The cocarcinogenicity of Pyrogallol was evaluated using 50 female ICR/Ha Swiss mice (6–8 weeks old). Pyrogallol (5 mg in acetone) and benzo[a]pyrene (5 µg/0.1 ml acetone) were applied simultaneously to clipped dorsal skin three times weekly for 440 days. The control group (50 mice) was treated with benzo[a]pyrene according to the same procedure. Tumors (> 1 mm in diameter) persisting for 30 days or more were recorded. Animals with carcinomas were killed when moribund or approximately 2 months after tumors were clinically classified as malignant. All animals were necropsied, and specimens of neoplasms were examined microscopically. Ten of the 50 mice treated with benzo[a]pyrene developed squamous carcinomas, whereas 33 of
the 50 mice treated with benzo[a]pyrene and Pyrogallol developed squamous carcinomas. No neoplasms were observed in the mice treated with Pyrogallol alone.\(^{(69)}\)

**CLINICAL ASSESSMENT OF SAFETY**

**Skin Sensitization**

Twenty-five patients (average age 65 years) with leg ulcers were patch tested (Finn chambers) with Pyrogallol. Patch tests were evaluated according to the procedure of Wilkinson et al.\(^{(70)}\) The distribution of leg ulcers was as follows: varicose ulcers (12 patients), postphlebitic ulcers (6 patients), and both varicose and postphlebitic ulcers (7 patients). Patients who had lesions for less than 12 months were excluded. Positive reactions to Pyrogallol were observed in 3 patients.\(^{(71)}\)

A total of 8230 patients with allergic contact dermatitis were patch tested with cosmetic ingredients over a period of 15 years (1968–1983). Patch tests were conducted according to the method of Fregert et al.\(^{(72)}\) Positive reactions to Pyrogallol (1% in petrolatum) were not reported.\(^{(73)}\)

**SUMMARY**

Pyrogallol, a benzenetriol, is used in 82 hair dyes and colors at concentrations ranging from \(\leq 0.1\) to 5.0%. Typical use concentrations of Pyrogallol in oxidative hair dyes range between 0.25 and 0.383% by weight.

Noncosmetics uses of Pyrogallol include: developer in photography, mordant for wool, and the dying of furs.

Following the intraperitoneal injection of Pyrogallol (60 mg/kg) into female mice, the maximum concentration in the brain (28.4 \(\mu\)g/wet weight) was found at 10 min. At 15 min postinjection, the concentration of Pyrogallol approached zero.

Pyrogallol and resorcinol were detected (via TLC) in hydrolyzed urine extracts from adult albino rats 24 h after intraperitoneal injection (100 mg of Pyrogallol/kg) but were not detected in nonhydrolyzed urine extracts. Resorcinol was detected also in rat fecal extracts that had been incubated with Pyrogallol.

The oral LD\(_{50}\)’s for male and female rats dosed with technical synthetic Pyrogallol were 1270 mg/kg and 800 mg/kg, respectively. Oral LD\(_{50}\)’s for male and female rats dosed with technical natural Pyrogallol were 1270 mg/kg and 848 mg/kg, respectively. In another study, the oral LD\(_{50}\) of a 50% solution of Pyrogallol in DMSO was 1800 mg/kg (male rats).

Twenty-four hour applications of technical synthetic Pyrogallol and technical natural Pyrogallol in distilled water (doses = 2100 mg/kg) to the backs of Sprague-Dawley rats did not result in 50% mortality.

A hair dye containing 0.4% Pyrogallol did not induce gross or microscopic changes, except for slight thickening of the skin, in New Zealand white rabbits when applied (in hydrogen peroxide) to the skin twice weekly for 13 weeks.

In a study involving male New Zealand white rabbits, Pyrogallol (powder form) induced ocular irritation. Pyrogallol was not an ocular irritant when tested at a concentration of 1% in propylene glycol.
A 50% reduction in viability was noted in B lymphocyte cultures treated with Pyrogallol (5 μg/culture). Pyrogallol (in ethanol or DMSO) did not cause plasma membrane damage when added to cultures of lung fibroblasts from human embryos.

Both technical synthetic and technical natural Pyrogallol (in distilled water) were slightly irritating to the skin of Dunkin Hartley guinea pigs. Pyrogallol (unrefined) also induced sensitization reactions when applied to the skin of guinea pigs.

Significant teratogenic effects were not observed in the offspring of female Sprague-Dawley rats dosed (via gavage) with Pyrogallol (in propylene glycol) on days 6 to 15 of gestation. The same was true for Charles River CD rats dosed (dermal applications) with a hair dye containing 0.4% Pyrogallol on days 1 to 19 of gestation. No treatment-related effects were observed in a multigeneration reproductive toxicity study in which Charles River CD rats received dermal applications of a hair dye that contained 0.4% Pyrogallol. The dye was mixed with an equal volume of 6% H₂O₂ before application.

In the Ames test, Pyrogallol was mutagenic to TA98, TA100, and TA1537 tester strains of Salmonella typhimurium. Technical synthetic Pyrogallol was mutagenic to L5178Y mouse lymphoma cells (in vitro) with and without metabolic activation. Technical synthetic Pyrogallol also induced chromosomal aberrations in human lymphocytes (in vitro) with and without metabolic activation.

Pyrogallol induced chromatid breaks and exchanges in cultures of Chinese hamster ovary cells with and without metabolic activation. Pyrogallol (at pH 10) was also mutagenic to strain D7 of Saccharomyces cerevisiae (in vitro) in the mitotic gene conversion assay. However, significant mutagenic activity was not noted at pH 7.

Pyrogallol (in 5% saccharose) was mutagenic to Berlin K and Basc strains of Drosophila melanogaster in the recessive lethal mutations test (in vivo). In the micronucleus test (in vivo), Pyrogallol significantly increased the percentage of micronucleated polychromatic erythrocytes in mouse bone marrow smears over that of controls. Pyrogallol also induced chromatid breaks in mouse bone marrow cells (in vivo).

In two carcinogenicity studies, the number of neoplasms in mice treated (dermal applications) with 50% Pyrogallol in acetone was not significantly different from that of controls. Similar results were reported in a carcinogenicity study in which a hair dye containing 0.49% Pyrogallol and H₂O₂ in aqueous solution was applied to the skins of mice. In another study, histiocytomas was noted at the exposure sites of 4 of 19 Fischer rats injected subcutaneously with Pyrogallol (in 50% DMSO). On the skin of female ICR/HA mice, Pyrogallol was reported to be an active cocarcinogen when applied with benzo[a]pyrene.

Sensitization reactions were noted in 3 of 25 patients (with leg ulcers) patch tested with Pyrogallol. In another sensitization study, 8230 patients with allergic contact dermatitis were patch tested with cosmetic ingredients over a period of 15 years. Positive reactions to Pyrogallol (1% in petrolatum) were not reported.

**DISCUSSION**

In animals, Pyrogallol was not a skin irritant. Positive and negative results were reported in two animal skin sensitization studies. The results of provocative patch tests involving contact dermatitis patients were negative. Hair dyes containing Pyrogallol are exempt from the principal adulteration provision and from the color additive provisions.
ASSESSMENT: PYROGALLOL

in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when cautionary statements and patch test instructions are conspicuously displayed on the label. Therefore, additional predictive human skin irritation and sensitization studies were not requested.

The Expert Panel noted that Pyrogallol was mutagenic in three tester strains of Salmonella typhimurium but also recognizes that the compound was negative for carcinogenicity in three chronic skin painting studies.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the CIR Expert Panel concludes that Pyrogallol is safe as a cosmetic ingredient in the present practices of use and concentration.

ACKNOWLEDGMENT

The Scientific Literature Review and Technical Analysis were prepared by Wilbur Johnson, Jr., Scientific Analyst and Writer.

REFERENCES

4. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (No date). Submission of unpublished data by CTFA. Cosmetic ingredient chemical description of pyrogallol.*

*Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, DC 20036.
24. FEDERAL REGISTER. (Oct. 16, 1979). Cosmetic product warning statements: coal tar hair dyes containing 4-methoxy-m-phenylenediamine (2,4-diaminobenzene) or 4-methoxy-m-phenylenediamine sulfate (2,4-diaminobenzene sulfate). 44(90), 5959–10.
47. CLAIROL. (1979). Submission of unpublished data by CTFA. Rabbit eye irritation study.*
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51. CLAIROL. (1979c). Submission of unpublished data by CTFA. Studies in guinea pigs to determine the potential of hair dyes to induce allergic contact dermatitis.


