Safety Assessment of Pyrogallol as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	March 4, 2024
Panel Meeting Date:	March 28-29, 2024

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, MSES, Senior Scientific Writer/Analyst, CIR
Date: March 4, 2024
Subject: Re-Review of Pyrogallol

Enclosed is the re-review package of Pyrogallol. The original review of Pyrogallol was published in 1991 with the conclusion that "Pyrogallol is safe as a cosmetic ingredient in the present practices of use and concentration." (The original review is included in this report package as *originalreport_Pyrogallol_0320024*). Because it had been at least 15 years since this ingredient was reviewed, in accordance with CIR Procedures, a re-review was initiated in 2007 (*originalRR_Pyrogallol_032024*). The re-review was subsequently tabled at the June 2007 Panel meeting to await the findings of the National Toxicology Program (NTP) 2-year carcinogenicity study. Unfortunately, the re-review was never completed, and it has now been at least 15 years since the initial re-review was presented to the Panel.

The NTP study was finally published in 2013. There was no evidence of carcinogenic activity of Pyrogallol in male or female F344/N rats administered 5, 20, or 75 mg/kg. There was equivocal evidence of carcinogenic activity of Pyrogallol in male B6C3F1/N mice based on increased incidences of squamous cell papilloma of the skin at the site of application. There was some evidence of carcinogenic activity of pyrogallol in female B6C3F1/N mice based on increased incidences of squamous cell carcinoma of the skin at the site of application. The Panel should carefully review this data, along with synopses of additional data available from a search of the published literature dated 2004 forward, a historical overview, comparison of the original and new use data (*newdata_Pyrogallol_032024*) and use table (*usetable_Pyrogallol_032024*).

At the time the original report was written, the maximum concentration of Pyrogallol allowed in hair dyes in Europe was 5.0%; however, European regulations regarding cosmetic ingredients now categorize Pyrogallol in Annex II, the list of substances prohibited in cosmetic products in Europe. Use of Pyrogallol since the initial re-review was performed has decreased. In 2006, Pyrogallol was reported to be used in 11 hair dye formulations; however, no concentrations of use were reported in the Council's 2006 survey. According to 2023 FDA VCRP data, Pyrogallol was reported to be used in 1 "other" hair dye product. No concentrations of use were reported in the Council's 2022 survey.

If upon review of the new studies and updated use data the Panel determines that the Pyrogallol safety assessment should be re-opened for review, a draft amended report will be presented at an upcoming meeting.

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Re-Review - Pyrogallol - History and New Data

Christina Burnett – March 2024 meeting

Ingredients (# included)	Citation	Conclusion	Use - New Data	Use -Historical Data	Notes
Pyrogallol CAS# 87-66-1	JACT 10(1):67-85, 1991	safe as a cosmetic ingredient in the present practices of use and concentration	frequency of use (2023): 1 other hair coloring product conc of use (2023): no uses reported	frequency of use (1989): 42 hair dyes and colors conc of use (1989): ≤ 0.1 to 5%	Use has markedly declined since the initial review from 42 uses to 11 uses in hair dyes and colors to only1 use in "other hair coloring products". No use has been reported by the Council since the initial re-review. This ingredient was banned for use in cosmetics (Annex II) in the European Union in 1992.
	Re-review document represented to Panel in June 2007	Tabled to await the completion of NTP studies		frequency of use (2006): 11 hair dyes and colors conc of use (2006): no uses reported	

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
ECHA dossier on Pyrogallol (CAS No. 87-66-1). https://echa.europa.eu/registration-dossier/-/registered-dossier/25683	Acute dermal toxicity	LD_{50} > 2000 mg/kg bw in female rats; no skin reactions or clinical signs of toxicity; no abnormalities at necropsy	No LD_{50} determined in study reported in original assessment.
	Genotoxicity	Pyrogallol-containing hair gels were not mutagenic in an Ames test, with and without metabolic activation, at concentrations ranging from 648 to 5000 µg/plate	Pyrogallol was reported to be mutagenic in several in vitro and in vivo studies in the original assessment.
		Pyrogallol was mutagenic in *Salmonella typhimurium* strain TA100, with and without metabolic activation, in an Ames test at concentrations ranging from 3 to 333 µg/plate in an Ames test. No mutagenicity was observed in strain TA98	
		Pyrogallol was mutagenic in *S. typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 uvrA/pKM101 without metabolic activation. This ingredient was also mutagenic with metabolic activation in the *E. coli* strain but had equivocal results in the *S. typhimurium* strains.	
		Hair gels containing up to 5% Pyrogallol were not genotoxic in an in vivo mouse micronuclei assay	
		No genotoxicity observed in an in vivo micronuclei assay in which mice were injection intraperitoneally with 39 to 156 mg/kg Pyrogallol once daily for 3 d.	
	Dermal irritation – in vitro	Pyrogallol was considered corrosive to skin in an in vitro reconstructed human epidermis tissue assay	Slight skin irritation reported in animal studies in original assessment.
		Pyrogallol was considered an irritant to the skin in an in vitro reconstructed human epidermis tissue assay	
	Dermal irritation – animal	Pyrogallol was irritating at concentrations as low as 0.125% mouse ear swelling study	
	Dermal sensitization – animal	Pyrogallol was irritating and sensitizing in a local lymph node assay (LLNA) at concentrations as low as 0.5%	Equivocal results in animal sensitization studies in original assessment.
		Pyrogallol was irritating and sensitizing in a mouse ear swelling test at 5%	
		Pyrogallol was sensitizing in a study utilizing immunofluorescent staining of mouse lymph node cells at concentrations as low as 0.5%	
	Dermal sensitization – (Q)SAR	Pyrogallol was predicted to be able to cause allergic contact dermatitis.	

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Kim Y-J, Kim H-Y, Lee J-D, et al. 2022. Analytical method development and dermal absorption of pyrogallol, a hair dye ingredient. Toxics. 10(10):570.	Dermal absorption	Pyrogallol (2.0%) was applied to the mini pig skin in Franz diffusion cells (10 µl/cm^2) and wiped with a swab 30 min after application to replicate hair dye conditions. After 24 h, the skin was wiped again and the stratum corneum was collected using tape stripping. All samples were extracted with water and analyzed. Receptor fluid was recovered at 0, 1, 2, 4, 8, 12, and 4 h. The total dermal absorption rate of Pyrogallol was determined to be 26.0 $\pm$ 3.9%.	No dermal absorption data in original report or initial RR.
Hamed M, Martynuk CJ, Said REM, et al. 2023. Exposure to pyrogallol impacts the hematobiochemical endpoints in catfish (Clarias gariepinus). Environ Pollut. 333:122074	Acute and short-term toxicity in catfish	In the acute toxicity assay, it was determined that the 96-h median-lethal concentration of Pyrogallol for catfish was 40 mg/l. In short-term toxicity experiment, fish showed morphological changes such as erosion of the dorsal and caudal fins, skin ulcers, and discoloration following exposure to Pyrogallol for 96 h. Exposure to 1, 5, or 10 mg/l Pyrogallol caused a significant decrease in hematological indices, including red blood cells, hemoglobin, hematocrit, white blood cells, thrombocytes, and large and small lymphocytes in a dose-dependent manner. Several biochemical parameters (creatinine, uric acid, liver enzymes, lactate dehydrogenase, and glucose) were altered in a concentration dependent manner with short term exposures to Pyrogallol. Pyrogallol exposure also caused a significant concentration-dependent rise in the percentage of poikilocytosis and nuclear abnormalities of red blood cells in catfish.	Non-mammalian data not previously incorporated into CIR reports.
Hamed M, Said REM, Soliman HAM, et al. 2024. Immunotoxicological, histopathological, and ultrastructural effects of waterborne pyrogallol exposure on African catfish (Clarias gariepinus). Chemosphere. 349:140792.	Short-term toxicity in catfish	Short-term toxicity study (15-d) in catfish. Pyrogallol decreased immune parameters and increased pro-inflammatory cytokines. Histopathology demonstrated that Pyrogallol induced injury in the liver and spleen of the fish.	Non-mammalian studies were not previously included generally in earlier reviews.
Hamed M, Soliman HAM, Said REM, et al. 2024. Oxidative stress, antioxidant defense responses, and histopathology: Biomarkers for monitoring exposure to pyrogallol in Clarias gariepinus. J Environ Manage. 351:119845.		Related to the study described above.	
Hossain MZ, Gilbert SF, Patel K, et al. 2013. Biological clues to potent DNA-damaging activities in food and flavoring. Food Chem Toxicol. 55:557-67.	Genotoxicity; natural occurrence	Pyrogallol induced DNA breaks in a p53R assay and a comet assay; reference discussed natural occurrence of Pyrogallol in foods	Pyrogallol was genotoxic in several in vitro and in vivo studies, both in the original report and in the initial RR.
Takemura Y, Want DH, Sauriasari R, et al. 2010. Evaluation of pyrogallol-induced cytotoxicity in catalase-mutant *Escherichia coli* and mutagenicity in *Salmonella typhimurium*. Bull Environ Contam Toxicol. 84(3):347-50.	Cytotoxicity; mutagenicity	Pyrogallol was cytotoxic and mutagenic in *E. coli* strains. Effects seem to be attributable, at least in part, to reactive oxygen species formation.	Previous reviews contain cytotoxicity data and Pyrogallol was mutagenic in in vitro studies.

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
National Toxicology Program. 2013. Toxicology and carcinogenesis studies of pyrogallol (CAS No. 87-66-1) in F344/N rats and B6C3F1/N mice (dermal studies	Genotoxicity and carcinogenicity	In 2-yr dermal studies, *there was no evidence of carcinogenic activity of Pyrogallol in male or female F344/N rats* administered 5, 20, or 75 mg/kg. *There was equivocal evidence of carcinogenic activity of Pyrogallol in male B6C3F1/N mice based on increased incidences of squamous cell papilloma of the skin at the site of application. There was some evidence of carcinogenic activity of pyrogallol in female B6C3F1/N mice based on increased incidences of squamous cell carcinoma of the skin at the site of application*. Dermal administration of Pyrogallol caused increased incidences of nonneoplastic lesions of the skin at the site of application in male and female rats and mice, skin adjacent to the site of application in male and female mice, and mammary gland in female mice.	The Panel tabled the initial RR of Pyrogallol in 2007 to await the results of this study. The initial RR did capture the results of the dermal genotoxicity study.
Mercado-Feliciano M, Herbert RA, Wyde ME, et al. 2013. Pyrogallol-associated dermal toxicity and carcinogenicity in F344/N rats and B6C3F1/N mice. Cutan Ocul Toxicol. 32(3):234-240.	Carcinogenicity	Analysis of the above NTP study. During the 2-yr study, survival of dosed rats and male mice was comparable to controls; however, survival of 75 mg/kg female mice was significantly decreased compared to controls. The incidences of microscopic non-neoplastic lesions at the site of application were significantly higher in all dosed groups of rats and mice and in both the 3 mo and 2-yr studies. In the 2-yr study, hyperplasia, hyperkeratosis and inflammation tended to be more severe in mice than in rats, and in the mice they tended to be more severe in females than in males. The incidence of squamous cell carcinoma at the site of application (SOA) in 75 mg/kg female mice and SOA squamous cell papillomas in 75 mg/kg male mice were greater than controls. Pyrogallol was carcinogenic in female mice and may have caused tumors in male mice.	The RR was tabled for the completion of the NTP report.
Revathi S, Hakkim FL, Kumar NR, et al. 2019. In vivo anti-cancer potential of pyrogallol in murine model of colon cancer. Asian Pac J Cancer Prev. 20(9):2645-2651.	Anti-tumor	Mice were treated with 10, 20, or 40 mg/kg bw *Acacia nilotica* or Pyrogallol. Tumor size was considerably reduced in Pyrogallol-treated mice similar to doxorubicin. Tumor bearing mice treated with *A. nilotica* and Pyrogallol showed mild decline in body weight. It was concluded that Pyrogallol was found to be an effective anti-colon cancer agent with less toxicity.	One in vitro anti-tumor study was captured in the initial RR. Mechanism of anti-tumor activity not in previous reviews of Pyrogallol.
Revathi S, Hakkim FL, Kumar NR, et al. 2018. Induction of HT-29 colon cancer cells apoptosis by pyrogallol with growth inhibiting efficacy against drug-resistant *Helicobacter pylori*. Anticancer Agents Med hem. 18(13):1875-1884.		Related research to the study above. Pyrogallol repressed the growth of *Helicobacter pylori* and induced apoptosis in HT-29 cells.	
Zhou B, Wang L, Ren Z, et al. 2023. Pyrogallol promotes growth arrest by activating the p53-mediated up-regulation of p21 and p62/SQSTM1-dependent degradation of β-catenin in nonsmall cell lung cancer cells. Environ Toxicol. Online ahead of print.		Pyrogallol treatment induced cell cycle arrest at the G2/M phase and apoptosis in two different non-small cell lung cancer (NSCLC) cell lines. The induction of cell cycle arrest in NSCLC cells at the G2/M phase by Pyrogallol was due to the up-regulation of p21 in a p53-dependent manner. A blockade of p53 and p21 effectively abolished the cell cycle arrest at the G2/M phase. Meanwhile, p53 inhibition has been found to abrogate the Pyrogallol-induced apoptosis of the two NSCLC cells. In vivo experiments demonstrated that Pyrogallol exerted growth inhibition on NSCLC with low toxicity through the same molecular mechanism as observed in vitro. The findings contribute to the understanding of the mechanism by which Pyrogallol negatively regulates NSCLC growth, which could be effective in treating NSCLC.	

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Wass Thilakarathna WPD, Vasantha Rupasinghe HP. 2019. Microbial metabolites of proanthocyanidins reduce chemical carcinogen-induced DNA damage in human lung epithelial and fetal hepatic cells in vitro. Food Chem Toxicol. 125:479-493.	Anti-tumor; cytotoxicity	The ability of Pyrogallol to reduce chemical carcinogen-induced toxicity in human lung epithelial cells and human fetal hepatic cells was evaluated. Pre-incubation with Pyrogallol provided protection against induced DNA damage. Pyrogallol also mitigated induced cytotoxicity. Mechanism of protection discussed. Pyrogallol suppressed the activation of the extrinsic apoptotic pathway	Additional data on cytotoxicity; anti-tumor activity not in previous reviews.
Yang CJ, Wang CS, Hung JY, et al. 2009. Pyrogallol induces G2-M arrest in human lung cancer cells and inhibits tumor growth in an animal model. Lung Cancer. 66(2):162-8.		The effects of Pyrogallol on human lung cancer cell lines was investigated. The MTT (cytotoxic) data showed the inhibition of growth of lung cancer cells followed Pyrogallol treatment. The cell cycle of lung cancer cells was arrested in G2/M phase using flow cytometry. Using Western blot analysis, the cell cycle related proteins cyclin B1 and Cdc25c were decreased in a time-dependent manner and the phosphorylated Cdc2 (Thr14) was increased within 4 h Pyrogallol treatment. Moreover, the higher cleavage of poly (ADP)-ribose polymerase and the increase of Bax concurrent with the decreased of Bcl-2 indicated that Pyrogallol treatment resulted in apoptosis of lung cancer cells. Cell apoptosis was also directly demonstrated using Annexin V-FITC and TUNEL stain. Additionally, the tumoricidal effect of Pyrogallol was measured using a xenograft nude mouse model. After 5 wk of treatment, Pyrogallol could cause the regression of tumor. Taking in vitro and in vivo studies together, these results suggest that Pyrogallol can be developed as a promising anti-lung cancer drug particular for the NSCLC.	
Lim JY, Kim C-M, Rhee JH, et al. 2016. Effects of pyrogallol on growth and cytotoxicity of wild-type and katG mutant strains of *Vibrio vulnificus*. PLoS One. 11(12):e0167699.	Cytotoxicity; non-cosmetic use	Pyrogallol protected HeLa cells from *Vibrio vulnificus*-induced cytotoxicity. Pyrogallol also decreased the growth of *V. vulnificus*; this inhibitory effect was more significant during log phase than stationary phase. No growth was observed for the katG- mutant in the presence of Pyrogallol (50 µg/ml) even after 24 h, whereas the wild-type strain demonstrated growth recovery following a prolonged lag phase. Pyrogallol-mediated growth inhibition of the katG- mutant strain was partially rescued by exogenous catalase treatment. These results indicate that the mechanism by which Pyrogallol inhibits the growth and cytotoxicity of *V. vulnificus* likely involves polyphenol-induced prooxidant damage.	Previous CIR reports contained some cytotoxicity data.
Han YH and Park WH. 2010. Pyrogallol-induced As4.1 juxtaglomerular cell death is attenuated by MAPK inhibitors via preventing GSH depletion. Arch Toxicol. 84(8):631-40.	Cytotoxicity	Study focused on ways of mitigating cell death from Pyrogallol.	Previous reviews contained some cytotoxicity data.
Park WH, Han YH, Kim SH, et al. 2007. Pyrogallol, ROS generator inhibits As4.1 juxtaglomerular cells via cell cycle arrest of G2 phase and apoptosis. Toxicology. 235(1-2):130-9.		Related study to above.	

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Han YH, Kim SZ, Kim SH, et al. 2008. Apoptosis in pyrogallol-treated Calu-6 cells is correlated with the changes of intracellular GSH levels rather than ROS levels. Lung Cancer. 59(3):301-14.		Same research group as above studied mechanism of apoptosis in Pyrogallol-treated cells.	
Ahamad N, Anjum S, Ahmed S. 2021. Pyrogallol induces oxidative stress defects in the fission yeast *S. pombe*. MicroPub Biol. 2021:10.		Pyrogallol-induced oxidative stress and the production of reactive oxygen species in fission yeast cells.	
Ekozin A, Otuechere CA, Adewuyi A. 2022. Apocynin loaded silver nanoparticles displays potent in vitro biological activities and mitigates pyrogallol-induced hepatotoxicity. Chem Biol Interact. 365:110069.	Hepatotoxicity	Study focused on treatment to mitigate hepatotoxicity induced by Pyrogallol.	First re-review has a study on hepatotoxicity caused by Pyrogallol in rats.
Matic S, Stanic S, Bogojevic D, et al. 2013. Methanol extract from the stem of Cotinus coggygria Scop., and its major bioactive phytochemical constituent myricetin modulate pyrogallol-induced DNA damage and liver injury. Mutat Res. 755(2):81-9.		Study focused on treatment to mitigate hepatotoxicity induced by Pyrogallol.	
Matic S, Stanic S, Bogojevic D, et al. 2011. Extract of the plant Cotinus coggygria Scop. attenuates pyrogallol-induced hepatic oxidative stress in Wistar rats. Can J Physiol Pharmacol. 89(6):401-11.		Related to the research described above.	
Upadhyay G, Singh AK, Kumar A, et al. 2008. Resveratrol modulates pyrogallol-induced changes in hepatic toxicity markers, xenobiotic metabolizing enzymes and oxidative stress. Eur J Pharmacol. 596(1-3):146-52.		Study focused on treatment to mitigate hepatotoxicity induced by Pyrogallol in mice.	
Upadhyay G, Kumar A, Singh MP. 2007. Effect of silymarin on pyrogallol- and rifampicin-induced hepatotoxicity in mouse. 565(103):190-201.		Related to the research described above.	

NOTABLE NEW DATA [present data in same order as done in full reports]			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Mendes V, Vilaca R, de Freitas V, et al. 2015. Effect of myricetin, pyrogallol, and phloroglucinol on yeast resistance to oxidative stress. Oxid Med Cell Longev. 2015:782504.	Antioxidant activity	Study on the mechanism for antioxidant protection by Pyrogallol in yeast.	Not mentioned in the previous reviews.
Guo TL, Germolec DR, Zhang LX, et al. 2013. Contact sensitizing potential of pyrogallol and 5-amino-*o*-cresol in female BALB/c mice. Toxicology. 314(203):202-8.	Dermal sensitization	LLNA and mouse ear swelling test of Pyrogallol. Pyrogallol was both a sensitizer and an irritant in female BALB/c mice.	Same author did an earlier study that was in the first re-review. Hair dye ingredients may be dermal sensitizers.

Search (from 2004 forward)

PubMed

(pyrogallol) OR (1,2,3-trihydroxybenzene) OR (1,2,3-benzenetriol) OR (87-66-1[EC/RN Number]) – 1493 hits; searches further limited with the terms “carcinogenicity”, “genotoxicity”, “toxicity”, “dermal” – relevant studies are summarized above.

ECHA

Entry for CAS # 87-66-1 resulted in finding a dossier for Pyrogallol. Pertinent data not found in the original report is summarized in the above table.

Frequency (2023/2006) and concentration (2022/2006) of use of Pyrogallol according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[1]	**2006[2]**	**2022[3]**	**2006[2]**
Totals*	**1**	**11**	**NR**	**NR**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*1*	*11*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	1	11	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR
as reported by product category				
Hair Coloring Preparations				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	11	NR	NR
Other Hair Coloring Preparation	1	NR	NR	NR

NR – not reported
*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
**likely duration and exposure are derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD. 2023. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023.)

2. Cosmetic Ingredient Review (CIR) Expert Panel. Re-Review on Pyrogallol. 2007. Unpublished re-review for Pyrogallol presented at the June 4-5, 2007 CIR Expert Panel meeting. Washington, D.C. Washington, D.C.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Pyrogallol. Unpublished data submitted by the Personal Care Products Council on January 10, 2022.

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6
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 LD_{50}'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 H_2O_2 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.

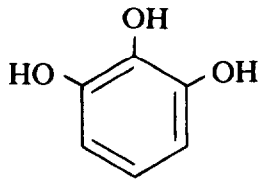
INTRODUCTION

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-Benzenetriol, 1,2,3-Trihydroxybenzene, and Pyrogallic 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.

TABLE 1. PROPERTIES OF PYROGALLOL

Property	*Description*	*Reference*
Molecular weight	126.11	10
Form	White crystals	7
Odor	None	7
Solubility	Readily soluble in water, ethanol, or ether. Slightly soluble in benezene or chloroform	4
Boiling point	309°C	7
Melting range	131–133°C	7
Specific Gravity (25°C)	1.45–1.50	4
Refractive index	1.561	10
Vapor pressure	10 mm at 167.7°C	11
Residue on ignition	0.1% max	4

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.[15]

Impurities

Iron (0.001%) and heavy metals (5 ppm max) are impurities that have been detected in Pyrogallol.[4] 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.[7] It is being used at present as a modifier in oxidation dyes.[4] Typical use concentrations of Pyrogallol in oxidative hair dyes range between 0.25 and 0.383% by weight.[8]

Scope and Extent of Use in Cosmetics

The FDA cosmetic product formulation computer printout[9] is compiled through voluntary filing of such data in accordance with Title 21 Part 720.4 of the Code of Federal Regulations.[20] 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).[19]

In countries of the European Economic Community, the maximum concentration of Pyrogallol allowed in hair dyes (for professional or general use) is 5.0%.[21] Pyrogallol (Quasi Drug Use Only) has also been approved for use in cosmetic formulations marketed in Japan.[22]

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 2. PRODUCT FORMULATION DATA[19]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*		
			>1–5	*>0.1–1*	*≤0.1*
Pyrogallol					
Hair dyes and colors	1164	42	1	22	19
1989 Totals		42	1	22	19

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 sutues 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.[20] 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 ^{125}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 substracted 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 μg/culture) resulted in ≥ 90% suppression of plaque formation. Toxicity, determined by trypan blue dye exclusion, was expressed as the test substance dose (μg/culture) resulting in a 50% reduction in viability. Pyrogallol induced toxicity at a dose of 5 μg/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.[49]

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 F_{1b}, F_{2b}, and F_{3c} 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 F_{1b} parental rats or F_{3b} 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).[56]

The mutagenicity of Pyrogallol (in DMSO) was evaluated using strains TA1538 and TA98 of *S. typhimurium*. Concentrations ranging from 20 to 1000 μg/plate were tested with and without metabolic activation.[54] With metabolic activation, Pyrogallol was not mutagenic to strain TA98. Without metabolic activation, a weak mutagenic response to Pyrogallol (500 μg/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).[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 μg/plate were tested.[54] The 200 μg/plate concentration was tested with and without metabolic activation. Concentrations less than 200 μg/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).[57]

The mutagenicity of Pyrogallol (in water) was evaluated using strain TA100 of *S. typhimurium*. Pyrogallol was tested at a concentration of 100 μg/plate according to a modification of the procedure by Ames et al.[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).[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 μmol/plate). In the spot test, Pyrogallol was mutagenic to strain TA1537 (Table 3).[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.[54] In spot tests, Pyrogallol (3 μmol/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 μmol/plate. In some of these tests, Pyrogallol was described as being weakly mutagenic to strain TA98 with and without metabolic activation (Table 3).[60]

In the L5178Y mouse lymphoma cell assay, technical synthetic Pyrogallol (in distilled water) was tested at concentrations of 4 to 80 μg/ml. Compared to vehicle control values, Pyrogallol (between 17 and 80 μg/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 μg/ml) were the same. It was concluded that technical synthetic Pyrogallol was mutagenic (Table 3).[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 μg/ml and with metabolic activation at concentrations of 100, 500, and 1000 μg/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 μg/ml concentrations (without metabolic activation). The same was true for cultures incubated with concentrations of 500 and 1000 μg/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 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 3. IN VITRO MUTAGENICITY TESTS

Test substance	*Concentrations tested*	*Strains tested*	*Procedure*	*Results*	*Reference*
Pyrogallol (≤ 98% w/w)	50–5000 μg/plate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames et al.[54]	Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)	55
Pyrogallol (90–96% w/w)	15–5000 μg/plate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames et al.[54]	Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)	55
Pyrogallol	Up to 3600 μg/plate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames et al.[54]	Mutagenic to strains TA98 and TA100 (presence and absence of metabolic activation) and strain TA1537 (absence of metabolic activation)	56
Pyrogallol (in DMSO)	20–1000 μg/plate	*Salmonella typhimurium* strains TA98 and TA1538	Ames et al.[54]	Not mutagenic to strain TA98 (presence of metabolic activation) and weakly mutagenic to strain TA98 (absence of metabolic activation)	53
Pyrogallol (in water)	5–200 μg/plate	*Salmonella typhimurium* strains TA98, TA100, and TA1537	Ames et al.[54]	Mutagenic to strains TA100 and TA1537 (presence and absence of metabolic activation)	57
Pyrogallol (in water)	100 μg/plate	*Salmonella typhimurium* strain TA100	Ames et al.[54]	Moderately mutagenic (absence of metabolic activation) and considerably mutagenic (presence of metabolic activation)	58
Pyrogallol	0.1–15.0 μmol/plate	*Salmonella typhimurium* strains TA98, TA100, and TA1537	Ames et al.[54]	Mutagenic to strains TA98 and TA100 (presence and absence of metabolic activation)	59
Pyrogallol	0.1–15.0 μmol/plate	*Salmonella typhimurium* strains TA98, TA100, and TA1537	Spot test (Ames et al.[54])	Mutagenic to strain TA1537	59

Pyrogallol (in ethanol)	3.0 μmol/plate	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537	Spot test (Ames et al.[54])	Mutagenicity to strains TA98 and TA100 was questionable	60
Pyrogallol (in ethanol)	0.3–3.0 μmol/plate	*Salmonella typhimurium* strain TA98	Ames et al.[54]	Weakly mutagenic	60
Pyrogallol (in distilled water)	4.0–80.0 μg/ml		L5178Y mouse lymphoma cell assay	Mutagenic (presence and absence of metabolic activation)	61
Pyrogallol (in distilled water)	50, 75 and 100 μg/ml		Chromosome aberrations assay involving human lymphocytes (absence of metabolic activation)	Clastogenic	62
Pyrogallol (in distilled water)	100, 500, and 1000 μg/ml		Chromosome aberrations assay involving human lymphocytes (presence of metabolic activation)	Clastogenic	62
Pyrogallol	0.1 mg/ml		Chromatid breaks and exchanges assay involving Chinese hamster ovary cells	Chromatid breads and exchanges (presence and absence of metabolic activation)	63
Pyrogallol	0.3 mg/ml	*Saccharomyces cerevisiae* strain D7	Mitotic gene conversion assay	Significant mitotic gene conversion at pH 10 but not at pH 7	64

alive. None of the mice were alive at week 110. Survivors were noted in the control group during the 120th week.[66] 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 keratocanthomas) was observed in the positive control group. Pyrogallol was not carcinogenic at any of the concentrations tested.[67]

Pyrogallol (in 50% DMSO) was administered subcutaneously (0.1 mg/g 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.[59]

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_2O_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.[68]

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 ≤ 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 μg/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% H_2O_2 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 H_2O_2 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

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.

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June 5, 2007

Memorandum

To: CIR Expert Panel

From: Wilbur Johnson, Jr.
Senior Scientific Analyst

Subject: Pyrogallol

In 1991, CIR published a Final Report with the following 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. A copy of this Final Report is included. Additionally, a search of the currently available scientific literature on Pyrogallol was performed, and the resulting data are summarized in the attached re-review background document. It is important to note that a 2-year dermal carcinogenicity study sanctioned by the National Toxicology Program was completed this year and that a draft report will be developed for peer review.

Pyrogallol was reported as being used in 42 hair dyes and colors (at concentrations of $\leq$0.1 % to 5%) in 1989. Data provided by FDA in 2006 indicated that Pyrogallol was being used in 11 hair dyes and colors. The results of an industry survey indicate that Pyrogallol is not being used in cosmetics, and, thus, the absence of use concentration data.

The task for the Panel at this meeting is to determine whether the conclusion on Pyrogallol is still valid. If it is not, an amendment should be initiated. If the conclusion is still valid, then the Panel should decide if there is a need for an addendum to add significant new safety or other data. If there is no such need, the Panel may simply describe the new information considered, reaffirm the original conclusion, and decide to not reopen.

RE-REVIEW DOCUMENT ON PYROGALLOL

In the *International Cosmetic Ingredient Dictionary and Handbook* **(Gottschalck and McEwen, 2006),** Pyrogallol is is defined as a phenol. The CIR Expert Panel has evaluated the safety of Pyrogallol in cosmetics, and a Final Report with the following conclusion was published in 1986: 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 **(Elder, 1991)**

An updated search of the literature was performed to identify studies on Pyrogallol that have been published since the Panel's Final Safety Assessment was issued or were omitted from that report. These studies are summarized in this re-review document.

CHEMISTRY

DEFINITION

Pyrogallol

According to **Gottschalck and McEwen (2006)**, Pyrogallol (CAS No. 87-66-1) is a phenol with three OH substituents on the benzene ring. Technical/other names for this chemical include: Benzene-1,2,3-Triol, 2,3-Dihydroxyphenol, and 1,2,3-Trihydroxybenzene **(Gottschalck and McEwen, 2006).**

CHEMICAL AND PHYSICAL PROPERTIES

Poitrast and Keller (1988) reported a log K_{ow} of 0.26 for 1,2,3-Trihydroxybenzene (Pyrogallol).

According to **Marklund and Marklund (1994)**, Pyrogallol is a potent generator of superoxide anion (O_2^-) and also generates hydroxyl radical (OH•) and hydrogen peroxide by the Haber-Weiss reaction ($O_2^- \pm H_2O_2/OH\bullet \pm O_2^- \pm O_2$).

Sottofattori et al. (1998) studied the effect of benzenediols and benzenetriols on the nitrosation of propranolol. The nitrosation of propranolol under the standard conditions recommended by the World Health Organization (10 mM propranolol hydrochloride, 40 mM sodium nitrite, PH 3.5) was carried out in the absence and in the presence of phenol, benzenediols, and benzenetriols added to the nitrosation mixture at concentrations ranging from 2 mM to 40 mM. The yield of N-nitrosopropranolol (NOP) was reduced, with potency decreasing in the following order: 1,2-benzenetriol > 1,2,3-benzenetriol (Pyrogallol)

> 1,4-benzenediol; the inhibitory effect was dose-dependent. The yield of NOP was increased by 1,3-benzenediol and 1,3,5-benzenetriol, but this effect was inversely related to the concentration.

USE

PURPOSE IN COSMETICS

Pyrogallol functions as a fragrance ingredient and hair colorant **(Gottschalck and McEwen, 2006).**

SCOPE AND EXTENT OF USE IN COSMETICS

Frequency of use data provided by **FDA in 2006** indicated the use of Pyrogallol in 11 cosmetic products **(FDA, 2006)**. Previously, product formulation data submitted to FDA in 1989 indicated that Pyrogallol was being used in 42 hair dyes and colors at concentrations up to 5% **(Elder, 1986)**. These data are included in Table 1. No uses/use concentrations of Pyrogallol were reported in a cosmetics industry survey **(CTFA, 2007).**

Table 1. Historical and current cosmetic product uses and concentrations for Pyrogallol

Product Category (FDA 2006)	1989 uses (Elder 1991)	Total products in category (Elder 1991)	2006 uses (FDA 2006)	Total products in category (FDA 2006)	1989 concentrations (Elder 1990) (%)	2007 concentrations (CTFA 2006) (%)
Hair coloring products	42	1164	11	1600	≤0.1 to 5	-*
Total uses/ranges for Pyrogallol	42		11		≤0.1 to 5	-

*In industry survey, no use concentrations reported for this category.

In the EEC (formerly the European Economic Community; currently known as the European Union) Cosmetics Directive (Annex II), Pyrogallol appears on the list of substances that must not form part of the composition of cosmetic products **(European Commission, 2007).**

For cosmetic products marketed in Japan, Pyrogallol is included on the list of ingredients that cosmetics shall not contain. However, it is listed as an ingredient of quasi drug products designated for use directly on the body. Quasi drugs, by definition, must have a mild effect on the body, but are neither intended for the diagnosis, prevention, or treatment of disease, nor to affect the structure or function of the body **(Ministry of Health, Labor, and Welfare, 2001).**

NONCOSMETIC USE

According to **21 CFR 73.1375**, the Food and Drug Administration classified Pyrogallol as exempt from certification as a color additive. Additionally, Pyrogallol may be safely used in combination with ferric ammonium citrate for coloring plain and chromic catgut sutures for use in general and ophthalmic surgery **(21 CFR 73.1375).**

BIOLOGICAL PROPERTIES

METABOLISM

Miyazaki et al. (2004) investigated the oxidoreductive reactions of human hemoglobin with Pyrogallol, and the metabolism of Pyrogallol by the protein , which contains a protoporphyrin IX-like cytochrome P-450. Pyrogallol, having three hydroxy groups, oxidized human oxyhemoglobin to methemoglobin and reduced human methemoglobin to oxyhemoglobin. Since superoxide dismutase and catalase inhibited these reactions extensively, active oxygens such as superoxide and hydrogen peroxide were considered to be involved in the oxido-reductive reaction of human hemoglobin by Pyrogallol. It was also found that the metabolism of Pyrogallol to purpurogallin occurred quickly in human erythrocytes, i.e., when Pyrogallol was added to human erythrocyte suspensions, it oxidized intracellular hemoglobin and produced purpurogallin. The metabolism of Pyrogallol to purpurogallin was explained by the Pyrogallol oxidation with the superoxide and hydrogen peroxide that was produced during the oxidoreductive reactions of human hemoglobin with Pyrogallol.

EFFECT ON MUSCLE CONTRACTION

Goldschmidt and Tallarida (1991) studied the effect of Pyrogallol on muscle contraction (endothelium-intact aortic rings) in an isolated muscle bath preparation. In preconstricted rings with an intact endothelium, Pyrogallol (1 μM) produced a rapid increase in contractile tension that reached a stable level within one minute. In aortic rings denuded of endothelium, Pyrogallol had no effect.

EFFECT ON PROTEIN SYNTHESIS

Adcock et al. (1994) examined the effect of Pyrogallol, an O_2- generator, on the ability of human epithelial cells to produce active DNA binding proteins and inducible nitric oxide synthase (iNOS) mRNA in cultured type II airway epithelial cells (A549 cells). Cells were incubated with a range of concentrations of Pyrogallol (10^{-7} to 10^{-4} M) for various time periods of up to two hours. NfkB binding in the nuclei of these

cells was determined by electrophoretic mobility shift assays. iNOS mRNA was measured using reverse transcription and the polymerase chain reaction (PCR) method. There was a time- and concentration-dependent induction of Nf*k*B binding, followed by a time- and dose-dependent increase in iNOS mRNA levels. These results suggest that, in the airways, the initial response to oxidative stress may be to induce Nf*k*B-responsive genes, such as iNOS, which may play an important role in defending the airway against oxidative stress.

EFFECT ON RESPIRATION

In a study by **Szabó et al. (1996)**, the exposure of J774 cells (mouse macrophage cell line) to Pyrogallol (100 µM) for 24 hours produced a statistically significant ($p < 0.01$, compared to control) decrease in mitochondrial respiration.

TOXICOLOGY

SUBCHRONIC DERMAL TOXICITY

Batelle Labs (2004) conducted a subchronic dermal toxicity study on Pyrogallol using groups of twenty F344 rats (10 males, 10 females per group). This study is actually the dose range-finding study for NTP's 2-year dermal carcinogenicity study on Pyrogallol. A status report on this study is included in the section on Carcinogenicity later in the report text. Pyrogallol was administered (in 95% ethanol) dermally at dosages of 0, 9.5, 18.75, 37.5, 75, and 150 mg/kg for up to 14 weeks (weekdays only). Treatment-related clinical signs were brown staining and irritation at the site of application. There was one early death (0 mg/kg female) that was unrelated to dosing with Pyrogallol. There were no treatment-related body weight effects in this study. In addition, no treatment-related hematology or clinical chemistry changes were noted. Treatment-related histopathologic findings were reported only at the site of application (epidermal hyperkeratosis, epidermal squamous hyperplasia, and chronic-active inflammation of the dermis), generally with an increase in incidence and severity, corresponding to increasing dosages throughout all of the dosages administered (except for the 9.5 mg/kg female). Based on the results of this study, it was recommended that dosages of 150, 75, and 37.5 mg/kg be considered for the chronic study using male and female F344 rats.

CYTOTOXICITY

Koo et al. (2004) studied the role of superoxide anion ($O2^{-}$) in neuronal cell injury induced by reactive oxygen species (ROS) in PC12 cells using Pyrogallol as a donor to release $O2^{-}$. Cell death was assessed by the measurement of released lactate dehydrogenase (LDH) into the culture medium. Pyrogallol at concentrations greater than 0.2 mM caused a time-dependent cell death. After exposure to 0.5 mM Pyrogallol, the released LDH rapidly increased up to approximately 72% at 5 hours. Mild cell death was observed at 0.2 mM Pyrogallol, when approximately 56% of the LDH was released at 24 hours. However, at 0.1 mM Pyrogallol, the released LDH level was comparable to that of control cells.

HEPATOTOXICITY

Gupta et al. (2002) studied the hepatotoxicity of Pyrogallol using groups of 8 adult Wistar albino rats (4 males, 4 females/group; weights = between 200 and 250 g). Pyrogallol was injected intraperitoneally at a dose of 100 mg/kg. At 1 hour after dosing, blood was drawn by cardiac puncture for the estimation of serum markers (aminotransaminase [AST], alanine aminotransaminase [ALT], and alkaline phosphatase). The rats were later killed and livers were removed and processed for MDA and GSH and tissue histology. Pyrogallol (dose = 100 mg/kg) produced significant liver damage, as indicated by a marked increase in serum AST and ALT, compared to the control group ($p < 0.05$). The serum AST and ALT increased to 357.0 ± 30.7 IU/l and 147.8 ± 28.4 IU/l, respectively, compared to 208.4 ± 4.1 IU/l and 84.5 ± 19.5 IU/l, respectively, in control animals. However, there was an insignificant change in the levels of alkaline phosphatase in the Pyrogallol-treated group; values for Pyrogallol-treated animals and the control group were 216.6 ± 44.1 IU/l and 240 ± 16.3 IU/l, respectively.

Pyrogallol (dose = 100 mg/kg) produced significant oxidative stress in liver tissue, as indicated by the marked increase in malondialdehyde (MDA) and glutathione (GSH) levels (markers of oxidative stress), compared to the control group. The MDA levels increased significantly to 311 ± 18.29 nmol/g wet tissue, compared to 170 ± 16.8 nmol/g wet tissue, respectively ($p < 0.05$). Glutathione levels also increased significantly to 37 ± 2.25 µg/g wet tissue, compared to a control value of 24.4 ± 3.6 µg/g wet tissue ($p < 0.05$).

The Pyrogallol-treated rats had mild inflammatory changes in the liver. The changes included

cellular infiltration of leukocytes and sinusoidal dilation, even as early as 1 hour after Pyrogallol administration **(Gupta et al. 2002)**.

ANTITHYROID ACTIVITY

Lindsay et al. (1992) evaluated the antithyroid activity of Pyrogallol. Porcine thyroid peroxidase (TPO)-catalyzed iodination of bovine serum albumin was assayed. The concentration at which 50% inhibition of porcine thyroid peroxidase activity (ID50) occurred was determined. The mean ID50 for Pyrogallol (based on 5 experiments) was 3.8 ± 0.12 nmol/ml). The known TPO inhibitor and antithyroid drug 6-N-propyl-2-thiouracil (PTU) served as the reference standard. The mean ID50 for PTU was 7.2 ± 0.16 nmol/ml.

INDUCTION OF HYPERPLASIA

In a study by **Hirose et al. (1986),** Pyrogallol (1% in the diet) was fed by continuous oral administration to groups of 15 male Syrian golden hamsters for 20 weeks. The control group (15 hamsters) was fed basal diet. Mild hyperplasia of the forestomach was noted in 15 hamsters ($p < 0.001$, compared to control group). Four hamsters had moderate hyperplasia of the forestomach. Oral dosing induced neither severe hyperplasia nor papillomas. Seven hamsters fed basal diet only had mild hyperplasia of the forestomach, and 1 hamster fed basal diet only had moderate hyperplasia of the forestomach.

EFFECT ON GENE EXPRESSION

Choi and Moore (1993) studied the induction of c-fos and c-jun gene expression by phenolic antioxidants. Following the treatment of quiescent human hepatoma HepG2 cells with Pyrogallol (200 μM) , the levels of c-fos and c-jun mRNAs were substantially increased. Phenolic antioxidants specifically induce expression of the c-fos and c-jun mRNAs. The antioxidant-specific induction of c-fos/CAT (chloramphenicol acetyltransferase) promoter constructs in transient transfections indicates that at least a portion of this response is transcriptional.

GENOTOXICITY/MUTAGENICITY

Sakagami et al. (1986) conducted a study to evaluate the mutagenicity of Pyrogallol, using the Ames test and the liquid rec assay. The Ames test was performed with *Salmonella typhimurium* strains TA98 and TA100, and the liquid rec-assay (for DNA damage) was performed with *Bacillus subtilis* strains

H-17 (Rec^+) and M-45 (Rec^-). Both assays were conducted with metabolic activation. Pyrogallol was tested at a dose of 125 µg/ml in strains TA98 and TA100 (Ames test). In the rec assay , Pyrogallol was tested at doses of 375 µg/ml (strain M-45) and 500 µg/ml. Results for Pyrogallol were positive in both assays.

Sakagami et al. (1988) evaluated the genotoxicity of Pyrogallol in the *umu* test, with and without metabolic activation. This genotoxicity assay is based on the ability of toxic chemicals to induce *umu* gene expression in *Salmonella typhimurium* TA1535/pSWK 1002 in which a plasmid pSK 1002 carrying a fused gene *umu*C' - '*lac*Z has been introduced. Pyrogallol was tested at concentrations up to 3200 µg/ml, and results were positive without, but not with, metabolic activation.

Kawanishi et al. (1989) studied the reactivity of Pyrogallol with DNA using a DNA sequencing technique that involved ^{32}P 5'-end-labeled DNA fragments that were obtained from human c-Ha-ras-1 protooncogene. The reaction mixture contained ^{32}P-labeled 351 base pair DNA fragment and 5 mM of benzene or Pyrogallol in 200 µl of 20 mM Tris-HCl buffer at pH 8.0. The mixture was incubated for 30 or 90 minutes. Little or no cleavage was observed with Pyrogallol, even with alkali treatment.

Glatt et al. (1989) evaluated the genotoxicity of Pyrogallol in the micronucleus test (Chinese hamster V79 cells) and in the sister chromatid exchanges assay (Chinese hamster V79 cells). In the micronucleus test, an elevated frequency of micronucleated cells was noted. At the optimal/maximal concentration of 50 µM Pyrogallol, the frequency of micronucleated cells x 10^3 above background was 32. It was noted that higher test concentrations were used; however, the frequency of the micronucleated cells declined towards the value of the solvent controls. In control cultures, the frequency of micronucleated cells was usually approximately 10 x 10^{-3}.

In the sister chromatid exchanges assay, at the optimal/maximal concentration of 25 µM Pyrogallol, the mean number of SCEs per cell above background was 3.2. This value was statistically significant ($p > 0.05$). The number of SCEs (mean ± standard deviation) in control cultures varied from 6.2 ± 2.9 to 8.4 ± 2.6 Pyrogallol induced sister chromatid exchanges **(Glatt et al.1989).**

Watanabe et al. (1991) evaluated the mutagenicity of Pyrogallol using *Salmonella typhimurium* strain TA98 both with and without metabolic activation. Pyrogallol was tested at doses up to 30 µg/plate, and was not mutagenic either with or without metabolic activation.

Lin and Lee (1992) evaluated the mutagenicity of Pyrogallol in the Ames *Salmonella* mutagenicity test using *Salmonella typhimurium* strains TA97, TA98, and TA100. Pyrogallol was tested at does up to 500 µg/plate both with and without metabolic activation. Pyrogallol was highly mutagenic to strains TA97 and TA100. Mutagenicity was reduced slightly by the treatment of Pyrogallol with chlorine or nitrite.

Lee and Lin (1994) studied the mutagenicity of Pyrogallol (in DMSO; test concentration = 56 µg/plate) using the Ames test with and without metabolic activation. *Salmonella typhimurium* strains TA97, TA98, and TA100 were used. Pyrogallol was highly mutagenic to strain TA100 without, but not with, metabolic activation.

Watanabe et al. (1998) evaluated the mutagenicity of Pyrogallol in the plate incorporation assay (testing at two laboratories) using the following bacterial strains without metabolic activation: *Salmonella typhimurium* strains TA102 and TA2638 and *Escherichia coli strains* WP2/pkM101 and WP2 *uvr*A/pKM101. Because of toxicity induced in the strains tested, the highest concentration at which it was possible to conduct tests involving all of the strains was 625 µg/plate. Pyrogallol was mutagenic to all of the bacterial strains that were tested; results were positive at both laboratories.

Silva et al. (2003) studied the induction of chromosomal aberrations by Pyrogallol in V79 cells at different pH values (6.0, 7.4, and 8.0). Pyrogallol caused a clear clastogenic effect in a pH-dependent way. At pH 6.0, Pyrogallol at higher doses (60 and 80 µM) significantly increased the level of chromosomal aberrations in V79 cells. However, at pH values of 7.4 and 8.0, Pyrogallol also induced significant levels of chromosomal aberrations at lower doses (< 80 µM). Pyrogallol at higher doses, and in a pH-dependent manner, caused a significant induction of multi-aberrant cells (cells with more than 10 chromosomal aberrations). The authors noted that the results of this study (all experiments) suggest that the genotoxicity of Pyrogallol is almost exclusively mediated by reactive oxygen species.

In a micronucleus test **(National Toxicology Program [NTP] 2004)**, groups of B6C3F1 mice (5 males, 5 females/group) were dosed dermally with Pyrogallol (in 0.2% Methylcellulose) over a period of 90 days. The groups received doses of 0, 38, 75, 150, 300, and 600 mg/kg, respectively. Blood samples were collected at 24 hours. Results were equivocal for male mice and negative for female mice.

Mazzei et al. (2006) investigated the possible mutagenicity of three commercial Pyrogallol-

containing (concentration of Pyrogallol not stated) hair gels made in Brazil (pH range: 3.5 to 4.0) using the *Salmonella* mutagenicity assay and the mouse bone marrow micronucleus assay. In the *Salmonella* mutagenicity assay using 648 to 5000 µg/plate of cosmetic samples, none of the samples reached a 2-fold increase in revertants relative to controls. Both with and without metabolic activation, the dose-response relation in strains TA98, TA100, TA102, TA1535, and TA1537 was not significant ($p > 0.01$).

In the mouse bone marrow micronucleus assay, 10 Swiss male mice were dosed orally with 2000 mg/kg of sample per body weight/day. The animals were killed at 48 hours after administration of the first dose. Bone marrow was extracted from the femur and slides prepared. The ratio between polychromatic and normochromatic erythrocytes as well as the presence of micronuclei in bone marrow cells were determined. Equal numbers of micronucleated polychromatic erythrocytes were detected between the cells of each treated group and the negative control using ANOVA and χ-square analyses. Thus, none of the products induced mutagenesis in either assay **(Mazzei et al. 2006).**

CARCINOGENICITY

According to the **National Toxicology Program (NTP) (2007)**, the 2-year dermal carcinogenicity study on Pyrogallol has been completed and the histopathology review is ongoing. NTP projects that a draft report will be issued in approximately five months. The results of the dose range-finding study that was conducted prior to initiation of this 2-year dermal carcinogenicity are summarized in the section on Subchronic Dermal Toxicity earlier in the report text.

TUMOR PROMOTION

Bohrman et al. (1988) reported the results of an interlaboratory study to evaluate the usefulness of the Chinese hamster V79 cell metabolic cooperation assay to predict the tumor-promoting activity of selected chemicals. The biological basis for this assay is the inhibition of gap-junctional communication by test chemicals. Results were positive for Pyrogallol (test concentration range: 0.25 to 3.0 µg/ml) at one laboratory and negative at the other; overall, the results for Pyrogallol were classified as equivocal. Chemicals were scored as positive (at least two concentration levels statistically different from the control), equivocal (only one concentration statistically different), or negative. An overall evaluation of the V79 system for predicting in vivo promotion activity was said to be difficult because of the organ specificity of

certain chemicals and/or the limited number of adequately tested nonpromoting chemicals.

ANTITUMOR ACTIVITY

Khan et al. (2002) studied the antiproliferative activity of Pyrogallol on human tumor cell lines. The effects of this chemical on in vitro cell growth were presented; the inhibition of tumor cell proliferation was consistently observed. The IC50 of Pyrogallol on K562, Jurkat, HEL, and Raji cell lines was found to be in the range of 10 to 30 μM.

EFFECT ON ATPASE ACTIVITY AND ANTIGEN EXPRESSION

Gruner et al. (1992) studied the effect of Pyrogallol on ATPase activity and Ia antigen expression on murine epdermal Langerhans cells. Balb/c mice (8 mice per group) were smeared with ointments containing different concentrations of Pyrogallol and tested 3 days later for ATPase and IA positive Langerhans cells. A statistically significant reduction of ATPase positive Langerhans cells was observed at Pyrogallol concentrations of 5% or higher ($p < 0.05$).

SKIN SENSITIZATION

In a study conducted by **White and Guo (2006)**, the objective was to determine the potential for Pyrogallol to elicit an allergic response when applied dermally to groups of 6 female BALB/c mice (hepatitis and Sendai virus free). Measurement of the contact hypersensitivity response was initially accomplished using the local lymph node assay (LLNA). In the first LLNA, the mice were sensitized to 2.5%, 5%, 10%, 25%, and 50% Pyrogallol. Since Pyrogallol , at all five concentrations, produced a significant increase in the proliferatiion of lymph node cells, two additional LLNAs were performed. The concentrations of Pyrogallol in the second study were 0.5%, 1.0%, and 2.5%. In the third study, Pyrogallol was tested at concentrations of 0.25%, 0.5%, 1.0%, 2.5%, 5%, and 10%.

Based on the results of the LLNA, a primary irritancy assay study was conducted using exposure levels of 0.125%, 0.25%, 1.0%, 5%, and 10%. Data from the irritancy study showed that Pyrogallol was an irritant at a concentration as low as 0.125%. As noted in the LLNA, a positive response was observed at a concentration of 0.5% Pyrogallol. Thus, it seemed that the significantly increased proliferation observed in the LLNA might have been due to the irritant effect of Pyrogallol.

To further determine if Pyrogallol was primarily an irritant, two mouse ear swelling tests (MEST) were performed. In the first MEST, mice were sensitized with Pyrogallol at concentrations of 0.25%, 1%, and 5%, and challenged with 0.25% Pyrogallol. There was no significant difference when the naive, vehicle, and three dose groups were compared to background control at either the 24- or 48-hour time point. In the second MEST, mice were sensitized with Pyrogallol at concentrations of 1% and 5%, and challenged with 1% Pyrogallol. A significant increase in mouse ear thickness was observed at 66 hours after challenge in mice that were sensitized with 5% Pyrogallol. 1-fluoro-2,4-dinitrobenzene (2,4-dinitrofluorobenzene, DNFB) was used as the positive control at a concentration of 0.15% for the irritancy test, LLNA, and MEST.

Taken together, these results demonstrate that Pyrogallol is a weak sensitizer, but a strong irritant in female BALB/c mice. The positive response observed in the LLNAs is mainly due to the irritant effect of Pyrogallol **(White and Guo 2006).**

EFFECT ON CONTACT SENSITIZATION

Gruner et al. (1992) conducted an experiment (12 Balb/c mice) to determine whether contact sensitization to DNFB is altered in skin sites treated with Pyrogallol. A shaved abdominal skin site on each animal was treated with an ointment containing Progallol on two consecutive days, before and after contact sensitization with 1-fluoro-2,4-dinitrobenzene (DNFB), to affect the induction phase of contact hypersensitivity (antigen presentation by Langerhans cell). At 5 days later, challenge with DNFB was performed on the ear skin; the swelling response at this site was measured two days later. No reduction of contact sensitization was observed after treatment with Pyrogallol (in ointment).

CLINICAL ASSESSMENT OF SAFETY

SKIN SENSITIZATION

Guerra et al. (1992a) reported the frequency of sensitization to hairdressing allergens in a group of patients with contact dermatitis. From 1985 to June of 1990, 261 hairdressers' clients (5 males, 256 females; mean age = 43.3 years) were examined. Patch tests were conducted and reactions were read ant 2 and 3 days. The rate of sensitization to Pyrogallol was 2.3%.

Guerra et al. (1992b) presented the results of a multicenter study that was performed in 9 Italian centers by members of the Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali (GIRDCA). The purpose of this study (from January 1985 to June 1990) was to evaluate the frequency and source of contact sensitization in a group of 302 hairdressers (mean age: 24.6 years) with dermatitis. Patch tests were performed according to International Contact Dermatitis Research Group (ICDRG) recommendations, using Finn chambers on Scanpor tape. The reactions were read at 2 and 3 days. A low incidence of sensitization (1.3%) was reported for Pyrogallol (1% in petrolatum).

Frosch et al. (1993) presented patch test results for 809 hairdressers tested at a total of nine European Centers. With the exception of three centers, the majority of the patients were seen during the years 1988 to 1991. Most of the patients were hairdressers with hand eczema. Reactions were classified as allergic reactions based on their morphology and time course, according to the generally accepted criteria of the International Contact Dermatitis Research Group. Doubtful or irritant reactions were excluded. The sensitization frequency for Pyrogallol (1% in petrolatum) in hairdressers was 0.8%.

Leino et al. (1998) patch tested (Finn chambers) 54 hairdressers with suspected occupational eczema. The tests were scored when patches were removed (at 48 hours), and, also at 24 and 48 to 120 hours later. The tests were scored according to the recommendations of the International Contact Dermatitis Research Group. Cutaneous reactions that were scored as 1+, 2+, and 3+ were considered allergic. None of the 54 hairdressers had a positive reaction to Pyrogallol.

Goossens et al. (1999) reported the results of a retrospective European survey of allergic contact reactions to cosmetics. Data on 475 patients with contact allergy to cosmetic ingredients, observed during a 4-month period (January-April 1996), were collected in 5 European dermatology centers. One reaction to Pyrogallol (from center in Belgium) was reported.

Barbaud et al. (2001) evaluated the skin sensitization potential of Pyrogallol using 19 patients in a standard patch test conducted according to the European Contact Dermatitis Research Group patch test procedure. Pyrogallol was tested at a concentration of 1% in vaseline. Positive reactions were observed in nine of the 19 patients tested.

Hillen et al. (2007) patch tested 1320 patients (1030 females, 290 males) because of suspected

allergic contact dermatitis of the scalp. The patients were patch tested between 1993 and 2003. Of the 1320 patients, 628 were patch tested with 1% Pyrogallol according o the criteria of the International Contact Dermatitis Research Group. Of the 628 patients, 5.4% had positive reactions to 1% Pyrogallol.

EPIDEMIOLOGY

The CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints. A discussion of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml. **

The Expert Panel recognizes that Pyrogallol is used as a hair dye ingredient and may be a sensitizer. However, hair dyes containing these ingredients, as coal tar hair dye products are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that continuing to follow this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

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