Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics

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ABBREVIATIONS

ARE	antioxidant responsive element
BrdU	bromodeoxyuridine
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Cu^{2+}	copper (II)
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
DNCB	2,4-dinitrochlorobenzene
EC1.5	interpolated concentration resulting in a 1.5-fold luciferase induction
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC_3	estimated concentrations of an SI of 3
ED_{50}	median effective dose
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
Fe^{3+}	iron (III)
FISH	fluorescence in situ hybridization
FOX	ferrous oxidation-xylenol orange
GC	gas chromatography
HPLC	high-performance liquid chromatography
[^{3}H]TTP	tritiated thymidine triphosphate
IC_{50}	50% inhibitory concentration
IgE	immunoglobulin E
IU	international units
LLNA	local lymph node assay
LNC	lymph node cell
LOAEL	lowest-observed-adverse-effect-level
LPS	lipopolysaccharide
MOE	margin of exposure
MOS	margin of safety
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
NOAEL	no-observed-adverse-effect-level
Nrf2	nuclear factor erythroid 2-related factor 2
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PoD	point of departure
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SED	systemic exposure dose
SI	stimulation index
TBARS	thiobarbituric acid-reactive substances
TG	test guideline
TGx	toxicogenomics
TMA	trimellitic anhydride
UV	ultraviolet light
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 1,2,4-Trihydroxybenzene, which is an oxidative dye reported to function as a hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of 1,2,4-Trihydroxybenzene as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), this ingredient, also called 1,2,4-benzenetriol, 4-hydroxycatechol, or THB, is reported to function as a hair colorant.[1]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last performed April 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the published data included in this safety assessment was found in the opinions of the Scientific Committee on Consumer Products (SCCP)[2] and Scientific Committee on Consumer Safety (SCCS).[3,4] Please note that these opinions provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCP and SCCS are cited.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, 1,2,4-Trihydroxybenzene (CAS No. 533-73-3) is the phenol that conforms to the structure in Figure 1.[1, CIR staff]

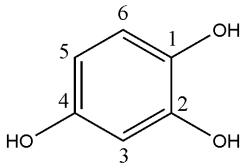
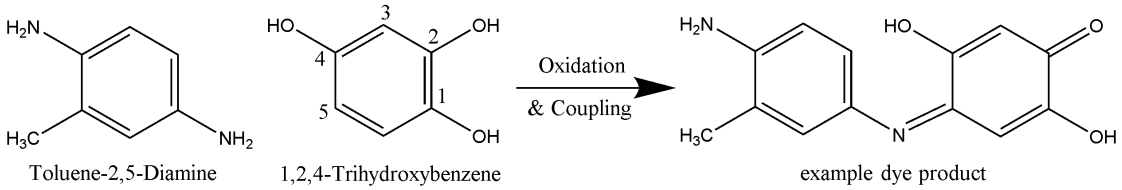


Figure 1. 1,2,4-Trihydroxybenzene

1,2,4-Trihydroxybenzene is an oxidative dye used in permanent hair dye formulations and gradual hair coloring shampoos and does not require hydrogen peroxide to activate oxidation and subsequent coupling reactions.[4] This ingredient is intended to be used in the presence of primary intermediates such as *p*-phenylenediamine, *p*-toluenediamine, *p*-aminophenol, etc. However, if 1,2,4-Trihydroxybenzene is partially oxidized prior to coupling, the rates of active hair dye formation may be different from traditional oxidative hair dyes. While this ingredient can be used alone, as an "auto-oxidative" hair dye, it is commonly used with reactants, much like traditional oxidative hair dyes.

Reaction Chemistry

The hydroxyl substituent pattern of 1,2,4-Trihydroxybenzene affects its reactivity.[4] The hydroxyl groups direct the substitution reaction on the benzene ring, making the C5 position on the ring (see Scheme 1) most likely to participate in coupling reactions. 1,2,4-Trihydroxybenzene reacts in the presence of oxygen and primary intermediates to form coupled products, without peroxide.[4,5] In formulation, once the reaction mixture is exposed to air, the oxidative coupling of 1,2,4-Trihydroxybenzene with an available primary intermediate proceeds rapidly, slowed only by temperature and pH adjustment.



Scheme 1. Example dye formation with 1,2,4-Trihydroxybenzene

1,2,4-Trihydroxybenzene, at physiological pH, can be oxidized spontaneously (auto-oxidation) or enzymatically (through the action of myeloperoxidase).[6] Auto-oxidation is the "uncatalyzed" oxidation of a substance exposed to oxygen in air; however, these reactions are usually metal catalyzed, as redox active metals can serve as free radical initiators. 1,2,4-

Trihydroxybenzene oxidation can be catalyzed by iron and copper salts, with copper (II) (Cu^{2+}) being a more active catalyst than iron (III) (Fe^{3+}). Of the metabolites of benzene, 1,2,4-Trihydroxybenzene is the most reactive toward molecular oxygen and rapidly auto-oxidizes to its corresponding quinone via semiquinone radical intermediates.

The auto-oxidation of 1,2,4-Trihydroxybenzene produces reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals.[6] In the process, 1,2,4-Trihydroxybenzene is oxidized to 2-hydroxy-1,4-benzoquinone. (See Figure 2.)

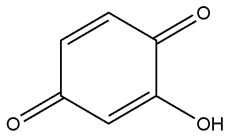


Figure 2. 2-hydroxy-1,4-benzoquinone

The quantitative determination of hydrogen peroxide formation from 50 or 100 µM 1,2,4-Trihydroxybenzene (98.1% pure) in various solvent media was performed using the ferrous oxidation−xylenol orange (FOX) assay.[4,5] The formation of hydrogen peroxide was measured as a function of concentration and time in phosphate buffer at pH 7.4. The quantitative generation of hydrogen peroxide from 1,2,4-Trihydroxybenzene was also evaluated in various solvents (deionized water, phosphate-buffered saline (PBS; which also acts as a buffer), serum-free Roswell Park Memorial Institute (RPMI) medium, and RPMI 1640 complete medium used for preparing dosing solutions commonly reported for evaluation of genotoxic effects in vitro and in vivo of 1,2,4-Trihydroxybenzene. The time point of 30 min was assumed as the time between preparation of dosing solution to actual dosing. The control was 30% (w/w) hydrogen peroxide solution.

PBS provided the most efficient matrix for the generation of hydrogen peroxide. In this solvent, 1 mole of 1,2,4-Trihydroxybenzene generated 0.9 moles of hydrogen peroxide. Deionized water was the least efficient solvent in generating hydrogen peroxide, likely due to the lack of a buffering capacity to maintain a pH. The authors of the study concluded that 1,2,4-Trihydroxybenzene is a spontaneous hydrogen peroxide releasing compound in "cell-free" solution on exposure to atmospheric oxygen. The release of hydrogen peroxide is facile, quantitative, and is time, solvent, pH and buffer, ionic salts, and transition metal dependent. The efficiency of the dosing solvents in catalyzing hydrogen peroxide generation from 1,2,4-Trihydroxybenzene was determined as follows: PBS > serum free RPMI > phosphate buffer > RPMI > water.[4,5]

In a time-dependent hair color-simulating consumer usage study, a representative gel-cream-based hair color formulation with 2% 1,2,4-Trihydroxybenzene (pH 9) was made in de-aerated water purged with nitrogen and tested on 90% virgin gray hair swatches.[4,5] A control formulation was made under the same conditions without 1,2,4-Trihydroxybenzene. A reverse phase high-performance liquid chromatography (HPLC) method was utilized to determine the amount of 1,2,4-Trihydroxybenzene reacted at time 0, 5, 15, and 30 min. This experiment was performed in duplicate. The resulting data showed that more than 70% of 1,2,4-Trihydroxybenzene reacted within the first 5 min and ~95% of the test material has reacted by the end of 15 min. Less than 3% of the original 2% formulation of 1,2,4-Trihydroxybenzene remained after 30 min. The study authors concluded that 1,2,4-Trihydroxybenzene in a representative formulation, when applied to hair, undergoes rapid oxidation when exposed to the atmosphere. The results of the study suggested that the consumer exposure to 1,2,4-Trihydroxybenzene is expectedly low, not only based on its limited skin permeability, but also based on the rapid decline in concentration in alkaline medium in the presence of hair under normal use conditions.[4,5] The SCCS was concerned about the proportion of unreacted 1,2,4-Trihydoxybenzene in the final hair dye formulation and that the transformation of 1,2,4-Trihydroxybenzene results in the generation of (semi)quinones.[4]

Chemical Properties

Chemical properties for 1,2,4-Trihydroxybenzene are summarized in Table 1. The molecular weight of 1,2,4-Trihydroxybenzene is 126.11 g/mol and the melting point ranges from 139 - 150°C.[2,3] 1,2,4-Trihydroxybenzene is a light-medium beige powder with an estimated log P_{ow} of 0.2 and an ultraviolet light (UV) spectrum peak of 291 nm.[2-5]

Method of Manufacture

1,2,4-Trihydroxybenzene may be produced by mixing *p*-quinone with acetic anhydride and concentrated sulfuric acid at below 40° C.[7] The resulting compound is precipitated by water or alcohol to yield 1,2,4-triacetoxybenzene. This acetate is then mixed with cold, absolute alcohol and concentrated hydrochloric acid before heating in an inert gas stream on a water bath at 80° C. The solvent and acid are removed in a vacuum prior to solidifying with chloroform and nucleation (glass scratching).

Impurities

Total impurity content of 1,2,4-Trihydroxybenzene is reported to be < 2% in a batch analyzed by HPLC and < 0.2% in a batch analyzed by potentiometry.[2] The four impurities detected by HPLC were tetrahydroxybenzene; 1,1'-biphenyl-2,2',4,4',5,5'-hexol; 2-hydroxybenzo-1,4-quinone; and 1,3,2-benzodioxathiole-5,6-diol 2,2-dioxide. Residual solvents were dichloromethane (300 µg/g) and n-propanol (1500 µg/g); isopropanol and ethyl acetate were below levels of detection. Heavy metal content was characterized as the following: aluminum (3 mg/kg), chromium (3 mg/kg), iron (11 mg/kg), nickel

(2 mg/kg), zinc (2 mg/kg), and mercury (< 0.1 mg/kg).[3] Silver, arsenic, barium, bismuth, cadmium, cobalt, copper, manganese, molybdenum, lead, palladium, platinum, antimony, selenium, tin, titanium, vanadium were each < 1 mg/kg.

In the 2019 opinion by the SCCS, the purity of 1,2,4-Trihydroxybenzene, determined by gas chromatography (GC) with UV detector, was reported to be 97.8%.[4] Impurities identified by nuclear magnetic resonance (NMR) spectroscopy and GC-mass spectrometry (MS) of 3 lots of 1,2,4-Trihydroxybenzene were: tetrahydroxybenzene; 4-mercaptophenol; hydroquinone; and 2-(2,3,4-trimethoxyphenyl)-5,6,7-trimethoxynaphthalene. The heavy metal content of the 3 lots of 1,2,4-Trihydroxybenzene was: arsenic (0.20 - 0.33 ppm), chromium (0.12 - 0.17 ppm), lead (0.03 - 0.05 ppm), mercury (0.02 - 0.03 ppm), and zinc (6.9 - 14 ppm). Cadmium was not detected.

Natural Occurrence

1,2,4-Trihydroxybenzene is a metabolite in biodegradation of many aromatic chemicals, including benzene.[8] Benzene is metabolized in the liver via benzene epoxide to phenol, which is then further hydroxylated to catechol, hydroquinone, and 1,2,4-Trihydroxybenzene.[6] Studies have detected 1,2,4-Trihydroxybenzene in the urine of humans and hamsters exposed to benzene, and in the urine of rats exposed to phenol, quinol, or catechol.[9,10] Metabolites of benzene, including 1,2,4-Trihydroxybenzene, may mediate the myelotoxicity and carcinogenicity of benzene.[11]

1,2,4-Trihydroxybenzene also occurs as a biodegradation product by fungi, yeast, and bacteria of catechin, resorcinol, and other aromatic chemicals.[12-14] Additionally, 1,2,4-Trihydroxybenzene is found in roasted coffee beans, with a typical cup of coffee containing 0.1-1.7 mg of the chemical. [15-18]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) in 2022 - 2023 (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, 1,2,4-Trihydroxybenzene is reported to be used in 18 hair dye formulations and 1 hair shampoo (coloring).[19] The results of the concentration of use survey submitted by the Council in 2023 indicate 1,2,4-Trihydroxybenzene is used at up to 2.5% in hair dyes and colors.[20]

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

These ingredients are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act (FD&C Act). 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 eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations and may even cause morbidity in consumers.[21,22] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the FD&C Act.

Hair dye formulations containing 1,2,4-Trihydroxybenzene are filled into specialized, oxygen barrier packaging for commercialization.[3] Stability tests are conducted under standard conditions (both room and elevated temperatures) appropriate for cosmetic products. Final product stability meets standard requirements until the package is opened by the consumer for use. Upon dispensing and application, the combination of dyes (including 1,2,4-Trihydroxybenzene and other precursors) in the formulation undergo oxidative coupling reactions as predicted by their chemical structure.

Under European regulations for cosmetic ingredients, 1,2,4-Trihydroxybenzene, when used as a substance in hair and eyelash dye products, is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.[23] The SCCS does not consider 1,2,4-Trihydroxybenzene safe due to potential genotoxicity when used as an auto-oxidative hair dye component in permanent hair dye formulations.[4] Recent available in vivo genotoxicity data cannot be considered by the SCCS due to the ban on animal testing of cosmetics and cosmetic ingredients in Europe.

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The dermal absorption/percutaneous penetration potential of 1,2,4-Trihydroxy [U-^{14}C]benzene (93.5% radiochemical purity) through dermatomed human skin (~ 400 µm) was determined for a formulation containing 2.78% of the radiolabeled active dye.[2,3] The formulation also contained 50% PEG-6 and approximately 47% water. The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. Using flow-through diffusion cells, 20 mg/cm^2 of the formulation, corresponding to 556 µg/cm^2, was applied for 30 min to 8 samples. The receptor fluid (PBS containing 0.01% sodium azide) was pumped at a rate of approximately 1.5 ml/h. Application of the test material was terminated by rinsing with water (10x), 2% sodium dodecylsulfate solution, and water (10x) again. The washing solutions were combined, and the amount of radioactivity was determined. Post-exposure time was 23.5 h. Tape stripping was then performed to determine the 24-h penetration profile. The recovery of radioactivity was 105%. Most of the test material was recovered in the wash after 30 min of exposure. Virtually no penetration of radioactivity into the receptor fluid after 24 h was observed (0.0019 µg$_{eq}$/cm^2 or 0.0003% of the applied dose).

In another dermal penetration study, [^{14}C]1,2,4-Trihydroxybenezene (98.3% pure; 2.00 MBq/mg) was incorporated at a final concentration of 2.5% (w/w) into two hair dye formulations, one with and one without 2.25% *p*-toluenediamine.[4,5] The study was performed in accordance with OECD TG 428. The formulations were applied to dermatomed human skin (400 µm thick) in static glass diffusion cells at a dose of 20 mg/cm^2 of the test article; the dose of 1,2,4-Trihydryoxybenezene was approximately ~ 500 µg/cm^2. After 30 min, the skin samples were washed with a mild soap solution. After washing, the diffusion cells were returned to the water bath for the remaining 23.5 h. At the end of the experiment, the 24-h penetration profile was determined using tape stripping and a heat separation technique. The mean recovery of the applied test material without and with *p*-toluenediamine was 101% and 99.2%, respectively. The total systemically available dose (epidermis, dermis, and receptor fluid (PBS)) from the test formulation without *p*-toluenediamine was 1.13 $\pm$ 0.58 µg$_{eq}$/cm^2 or 0.226%. The total systemically available dose from the test formulation with *p*-toluenediamine was 1.94 $\pm$ 1.76 µg$_{eq}$/cm^2 or 0.393%.

Absorption, Distribution, Metabolism, and Excretion

No absorption, distribution, metabolism, or excretion studies were reported for 1,2,4-Trihydroxybenzene in the published literature and unpublished data were not submitted. Many studies are available on the absorption, distribution, metabolism, and excretion of benzene as a starting material, but not on 1,2,4-Trihydroxybenzene.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

In an acute dermal toxicity study performed in accordance with OECD TG 402, 5 male and 5 female Sprague-Dawley rats received 2000 mg/kg bw 1% 1,2,4-Trihydroxybenzene (98.1% pure) in carboxymethylcellulose/water on skin (application site not described).[2,3] The test site was semi-occluded for 24 h. The rats were observed for a period of 14 d following the single application. No mortality was observed during the study period. Hypoactivity, piloerection, and dyspnea were observed in all female rats from days 2 through 8. One of the female rats had tremors. Overall body weight gain was comparable to historical control animals in all but 1 animal; one female had slightly reduced body weight gain during the second week of the study. A black coloration of the skin was noted in all animals from day 2 until study end. Erythema was observed in 2 males on day 2 and persisted in 1 animal until day 3. Edema was recorded between days 2 and 5 in 2 males and in all females between days 2 and 6. No apparent abnormalities were noted at necropsy in any animal.

Oral

In an acute oral toxicity study, groups of 5 male and 5 female OFA Sprague-Dawley-derived rats received 100, 250, 350, 500, or 1000 mg/kg bw 1% 1,2,4-Trihydroxybenzene in carboxymethylcellulose/water via gavage.[2,3,5] The animals were observed for 14 d following the single administration. No further details were provided. The LD_{50} for both sexes was between 350 and 500 mg/kg bw for a preparation containing 1% 1,2,4-Trihydroxybenzene.

Short-Term Toxicity Studies

Oral

In an oral study, male BALB/c mice (number not reported) received diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1, 2, 4-Trihydroxybenzene (1.3% w/w) for 1 wk.[24] A control group received the feed mixed with untreated water. No observable toxic effects were observed. (No further details provided.)

In a 4-wk study, groups of 8 male KKAy mice received purified water or water with 100 or 500 mg/l 1,2,4-Trihydroxybenzene 5 times/wk.[25] Individual body weights were recorded weekly, feed intake was measured every 2-3 d, and water consumption was measured each time the water was replaced throughout the study. Metabolic rate was measured after 3 wk by indirect calorimetry. Blood, urine, and tissue samples were collected after 4 wk. Body and liver weights of the mice were not affected. Feed intake and water consumption decreased with 1,2,4-Trihydroxybenzene, with consumption levels significantly lower ($p < 0.05$) in the high dose group than in controls. Blood glucose, serum triglyceride, and non-esterified fatty acid levels did not differ among the groups. Serum and urinary hydrogen peroxide levels increased with 1,2,4-Trihydroxybenzne, with the 500 mg/l dose group significantly higher ($p < 0.01$) than the control group. Reduced blood nitric oxide metabolites ($p < 0.05$) and liver *S*-nitrosylated protein levels (not significant) and decreased whole-body fat utilization ($p < 0.001$) were observed following treatment with 1,2,4-Trihydroxybenzene, with the latter occurring in a dose-dependent manner.

In an oral study, 5 male and 5 female F344 rats received 1.5% 1,2,4-Trihydroxybenzene in diet continuously for 4 wk.[26] A control group of the same composition of animals received regular diet. At week 4, the rats were injected intraperitoneally with 50 mg/kg bw bromodeoxyuridine (BrdU) and after 1 h, the rats were then sequentially killed. The stomachs were removed for histopathological and immunohistochemical examination. The number of cells incorporating BrdU into DNA per 2000 basal cells of the forestomach and the numbers of cells labeled with BrdU per 50 pyloric glands were counted. No deaths were observed during the treatment period and there were no clinical effects of toxicity observed, with the exception of a statistically significant reduction in weight gain in both sexes treated with 1,2,4-Trihydroxybenzene. This observation was associated with a decrease in feed consumption. No erosion or ulcer formation was observed in any of the treated animals or the controls. Significantly increased DNA synthesis ($p < 0.01$) was noted in both sexes of the treated animals when compared to the control animals. 1,2,4-Trihydroxybenzene was not associated with any hyperplasia changes in glandular stomach mucosa.

Subchronic Toxicity Studies

Oral

In a 90-d gavage study, groups of 15 male and 15 female Han Wistar rats received 0, 50, 100, or 200 mg/kg bw/d 1,2,4-Trihydroxybenzene (purity not reported) in sterile water.[2,3,5] The study was performed in accordance with OECD TG 408. Clinical signs of toxicity, mortality, and water consumption were monitored daily. Examinations of individual animals for signs of reaction to the test material were performed daily immediately after dosing and approximately 1 and 3 h after dosing during the first 3 wk of the study; after this, observations were performed at approximately 15 min and 1 and 2 h after dosing until the end of the study. Prior to the commencement of treatment and weekly thereafter, each animal was subjected to a detailed clinical examination, including an evaluation of neurotoxicity. Body weight and feed consumption were recorded weekly. An ophthalmological examination was performed prior to the start of the study and in week 12. "Motor activity of the first 5 males and 5 females was measured once during week 12 of treatment." (No further details of this methodology were provided.) Hematology, blood clinical chemistry, and urinalysis were performed in week 13 of treatment. At study end, all surviving animals were killed and underwent macroscopic examination. Select organs (not described) were weighed and microscopic examination was performed of specified tissues and organs (lungs from all animals, other organs not described) from all rats that died during the study, all control and high-dose rats killed at the end of the study, and in animals where gross anomalies were noted.

During the study, 12 animals died: 1 male in each the control, low- and intermediate-dose groups and 5 males and 4 females of the high-dose group. Microscopic examination indicated that mis-dosing was the cause of death for the first 3 groups while the main cause of death for the high-dose rats was stomach ulcerations. Piloerection and salivation were observed in the 100 and 200 mg/kg bw/d dose groups. An overall slight reduction in body weight gain was observed in treated males when compared with controls from approximately 1 mo of treatment. A 14% decrease in feed consumption was observed at week 13 in high dose males, but this result was not observed in treated females. A statistically significant increase in mean red blood cell volume, mean corpuscular hemoglobin, and platelets and a statistically significant decrease in hematocrit, red blood cell count, and hemoglobin were observed in animals treated with 100 and 200 mg/kg bw/d, when compared to the controls; however, values remained within the normal range for this strain of rats. A statistically significant increase in bilirubin was observed in rats of the high dose group of both sexes; however, the color of the test compound may have interfered with the methodology used. No toxicological significance was given to the statistically significant increase in urea observed in treated females only. Statistically significant increases in the absolute weight and/or organ-to-body weight ratios were observed in treated males for the spleen (all dose levels), liver and kidney (100 and 200 mg/kg bw/d), and testes and heart (200 mg/kg bw/d). In the females, statically significant increase in the absolute weight and/or organ-to-body weight ratios were observed for the liver, spleen, and kidneys at 200 mg/kg bw/d. Ulcerations in the non-glandular gastric region were observed in 1/10 males and 1/11 females of the high dose group and in 1/14 males in the intermediate dose group at study end. The histopathological evaluation of the stomach in the remaining animals of the intermediate dose group did not reveal any further treatment-related gastric lesions. Dark-brown, microgranular pigmentation was clearly evident in single cells or in the lumen of renal cortical tubes of 10/15 males and 10/15 females in the high dose group and in 2/15 males and 1/15 females of the intermediate dose group. The no-observed-adverse-effect-level (NOAEL) was determined by the study authors to be 50 mg/kg bw/d. However, the SCCP concluded that no NOAEL could be derived in this study as the

relative organ weight was increased significantly in the spleen of the 50 mg/kg bw/d male rats.[2,4] This increase continued in a dose-dependent manner in the male rats.[4] Absolute organ weight of the spleen was also increased in male rats, but the increase was not significant at 50 mg/kg bw/d. The value 50 mg/kg bw/d was considered to be the lowest-observed-adverse-effect-level (LOAEL) by the SCCP.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

The teratogenic potential of 1,2,4-Trihydroxybenzene (99.8% pure) was evaluated in groups of 25 mated female Sprague-Dawley (Crl CD (SD) BR) rats in accordance with OECD TG 414.[2,3,5] The rats received 0, 30, 100, or 300 mg/kg bw/d of the test material dissolved in water on gestation days 6 through 15. On day 20 of gestation, the rats were killed. The number of corpora lutea, resorptions, live and dead fetuses, and implantation sites were recorded. Live fetuses were weighed and examined externally. Half of the live fetuses per litter underwent skeletal examination and the remaining fetuses underwent soft tissue examination.

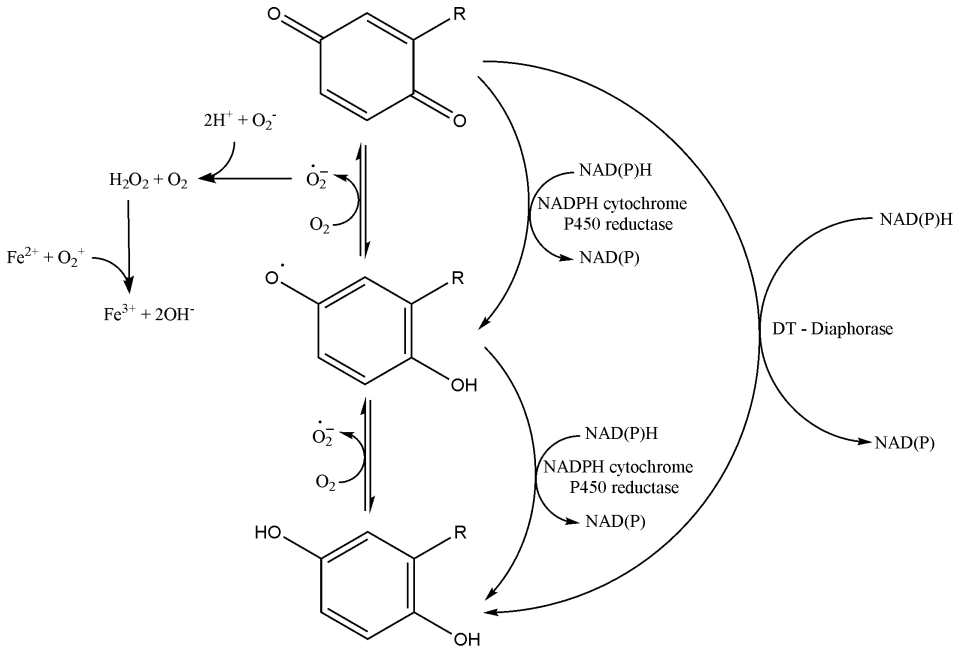
No clinical signs of toxicity or deaths occurred in the 0, 30, or 100 mg/kg bw/d groups. In the 300 mg/kg dose group, 3 females died or were killed in moribund conditions due to mis-dosing. Another female died without any clinical signs. At necropsy, gaseous dilatation of the stomach and intestine and congested lungs were noted. The mean body weight gain and feed consumption of females that survived to day 20 were similar to the control and other dose groups. In the 300 mg/kg dose group, the mean body weight gain was slightly lower than that of the controls between days 6 and 9 (not significantly) and the feed consumption was also slightly lower than that of the control females during the treatment period. No further details of these observations were provided. The litter parameters were comparable in the control and treated groups. No fetal external malformations were observed in the 0, 30, or 100 mg/kg bw/d groups; however, in the 300 mg/kg bw/d dose group, 4 fetuses from the same litter (out of 325 fetuses) had exencephaly associated with opened eyelids. Historical incidence of exencephaly in control fetuses from the test facility was noted (mean incidence: 0.06%; range of incidence per study: 0.0 - 1.0%). This incidence was slightly higher (1.2%) than that of the historical data, but it was considered a congenital malformation due to the fetuses coming from the same dam, and no other malformations were noted in any other litters. The dam showed no sign of any toxicity. No other treatment-related fetal skeletal variations, anomalies, malformations, and/or fetal soft tissue anomalies or malformations were observed. 1,2,4-Trihydroxybenzene was maternotoxic at 300 mg/kg bw/d, but not embryotoxic or teratogenic.[2,3,5]

GENOTOXICITY STUDIES

1,2,4-Trihydroxybenzene is a compound known to induce DNA damage and is considered genotoxic in vitro. This compound can result in oxidative DNA damage, which is linked to the generation of ROS.[27-32] Additionally, 1,2,4-Trihydroxybenzene has been studied for its effects on cellular structures, inducing DNA strand breaks and mutations as well as potential epigenetic modifications, potentially leading to various diseases, including cancer.[33,34] 1,2,4-Trihydroxybenzene may inhibit mitochondrial DNA replication.[35] Furthermore, several studies have elucidated the role of 1,2,4-Trihydroxybenzene in causing chromosomal damage and subsequent biological consequences.[36-38] Genotoxic effects may be modulated in vivo (Scheme 2). However, catalase, peroxidases, and other detoxification (antioxidant) enzymes in the epidermis, along with smaller antioxidant molecules, work to minimize ROS damage within skin cells.[39,40] Additional detoxification enzyme systems in skin may contribute to the detoxification of 1,2,4-Trihydroxybenzene, 1,2,4-Trihydroxybenzene-derived active oxygen species, and 1,2,4-Trihydroxybenzene-derived quinones, including superoxide dismutase, glutathione peroxidases, glutathione-*S*-transferases, and quinone reductases.[41-44]

In vitro and in vivo genotoxicity studies on 1,2,4-Trihydroxybenzene summarized here are detailed in Table 2. Genotoxicity was observed in a majority of the in vitro studies where auto-oxidation was not minimized or efforts to reduce auto-oxidization were not reported. Specifically, 1,2,4-Trihydroxybenzene was mutagenic in Ames tests when tested at up to 4000 µg/plate.[2,3,45] Genotoxicity of 1,2,4-Trihydroxybenzene was observed in a gene mutation assay (tested at up to a maximal concentration of 10 µM), DNA strand break tests (at up to 1000 µM), DNA synthetic activity inhibition assays (at up to 24 µM), and sister chromatid exchange (SCE) assays (at up to 500 µM).[3,33,45-49] 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml).[2,3] Increases in micronucleus induction were observed in Chinese hamster V79 cells (at up to 25 µM) and human TK6 lymphoblastoid cells (at up to 30 µg/ml).[45,48] 1,2,4-Trihydroxybenzene was clastogenic and aneugenic in a cytokinesis-block micronucleus test with human lymphocytes at up to 100 µM.[3,50] Chromosomal damage was observed in fluorescence in situ hybridization (FISH) procedures when 1,2,4-Trihydroxybenzene was tested at up to 100 µM [36-38]

In studies where degassed solutions were used, 1,2,4-Trihydroxybenzene was mutagenic in an Ames test when tested at up to 5000 µg/plate; however, the mutagenic effect of 1,2,4-Trihydroxybenzene was eliminated in another Ames test when evaluated in the presence of radical scavengers catalase (up to 20,000 IU) and L-glutathione (up to 10 µM).[4,5] Catalase also reduced the toxicity of 1,2,4-Trihydroxybenzene. In other studies with degassed solutions, 1,2,4-Trihydroxybenzene was not genotoxic in a 3D comet assay (at up to 1250 µg/ml) or in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml). In in vivo micronucleus tests in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at up to 50 mg/kg bw.[2,51]



Scheme 2. Potential pathways, relevant to genotoxicity, for benzoquinone and 1,2,4-Trihydroxybenzene via redox cycling between the quinone and its hydroxylated counterpart.[33] This also demonstrates the concomitant production of reactive oxygen species (benzoquinone: R = H; 1,2,4-Trihydroxybenzene: R = OH)

The Panel took into consideration outside expert opinions and analyses of the available genotoxicity data.[52,53] These experts noted that the effects produced by 1,2,4-Trihydroxybenzene observed in vitro (induction of sister chromatid exchanges, micronuclei, chromosomal aberration and gene mutations in mammalian cells, and DNA breaks) are all considered to be the result of the formation of ROS. DNA damage induced by 1,2,4-Trihydroxybenzene is inhibited by superoxide dismutase, catalase and benzoate and other hydroxyl radical scavengers. The addition of metabolic activation greatly reduced the observed genotoxic effects, suggesting that the ingredient is negative for in vivo genotoxicity due to rapid inactivation. This is supported by the negative in vivo micronucleus test in mice. The outside experts concluded that 1,2,4-Trihydroxybenzene does not pose a genotoxic risk.

Mechanism

Oxidant-mediated genotoxicity of 1,2,4-Trihydroxybenzene is a multifactorial process.[6] Redox reactions of 1,2,4-Trihydroxybenzene yield oxygen species, semiquinones, and quinones. Copper salts stimulate oxidation of 1,2,4-Trihydroxybenzene, leading to 1,2,4-Trihydroxybenzene-induced genotoxicity. Copper salts change the mechanism of reaction from superoxide-propagated 1-election transfer pathway to Cu^{2+}-mediated 2-electron transfer pathway during the oxidation of 1,2,4-Trihydroxybenzene.

CARCINOGENICITY STUDIES

Dermal

The dermal carcinogenicity potential of a semi-permanent hair dye formulation containing 0.5% 1,2,4-Trihydroxybenzene (purity not reported), 0.5% 2,4,5-toluenetriol, and 0.25% *p*-toluenediamine sulfate was evaluated in treatment and control groups of 50 male and 50 female Swiss Webster mice each.[2,3,54,55] The test material (0.05 ml) was applied once weekly for 23 mo on a 1 cm^2 area of clipped skin on the interscapular region. Test sites were not occluded. The mice were observed daily for mortality and clinical signs of toxicity and were weighed monthly. A weekly record was maintained for any skin lesions noted. After 9 mo of treatment, 10 males and 10 females per group were necropsied. Skin and internal organs were evaluated histologically. Four males and 4 females that received 1,2,4-Trihydroxybenzene survived until study termination, while 3 males and 8 females survived until study termination in the control groups (further information on survival rates not provided). There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 9 mo. There were no statistically significant differences in the distribution of tumors among treated and control groups. The SCCS determined that these data were insufficient to conclude on the carcinogenic potential of 1,2,4-Trihydroxybenzene.

OTHER RELEVANT STUDIES

Cytotoxicity

The cytotoxicity of 1,2,4-Trihydroxybenzene was investigated in K562 erythroleukemia cells.[56] Cultured K562 cells were exposed to 0, 0.1, 0.2, 0.3, 0.4, or 0.5 mM 1,2,4-Trihydroxybenzene for 24 h. The K562 cells showed significant inhibition of viability ($p < 0.05$) in a concentration-dependent manner.

In another study, K562 cells were treated with 0, 0.01, 0.02, 0.04, or 0.08 mM 1,2,4-Trihydroxybenzene for 24 h.[57] Eighty-five percent (85%) of the total cells were viable after treatment at concentrations less than 0.08 mM. At 0.08 mM, cell viability was slightly greater than 60%.

Cytotoxicity was also investigated using HL-60 human promyelocytic leukemic cells incubated with 10 - 100 µM 1,2,4-Trihydroxybenzene, hydroquinone, and *p*-benzoquinone for 1 - 4 h.[58] The rank order of cytotoxicity of these benzene metabolites to the cells were determined to be *p*-benzoquinone > hydroquinone > 1,2,4-Trihydroxybenzene at any given time period. In further in vitro testing, cells were exposed to 50 µM of each metabolite for 2 h. Cell viability was more than 80% (no further details provided).

Hematotoxicity

In vitro studies using mouse bone marrow adherent stromal cells and K562 cells have been used to study the mechanisms of benzene hematotoxicity.[57,59,60] 1,2,4-Trihydroxybenzene (3.1×10^{-6} to 500×10^{-6} M) was studied for its effect on the ability of stromal cells to influence granulocyte/monocyte colony growth after incubation with the test material for 3 d.[59] 1,2,4-Trihydroxybenzene inhibited colony growth at concentrations $\geq 100 \times 10^{-6}$ M. K562 cells were used to determine the effects of 1,2,4-Trihydroxybenzene on erythroid differentiation.[57,60] The results of the studies on K562 cells indicated that 1,2,4-Trihydroxybenzne inhibited hemin-induced erythroid differentiation in concentration-dependent manner (tested at up to 40 µM).

Oxidative Stress

In an investigation on oxidative stress, groups of 6 mice received diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1, 2, 4-Trihydroxybenzene (1.3% w/w) for 1 - 2 wk, with or without exposure to air containing 10 ppm nitrogen dioxide.[24] Control groups received normal feed mixed with untreated water, with or without exposure to nitrogen dioxide. At the end of the exposure period, blood was collected, and the heart, kidney, liver, and lungs were obtained from 1 mouse to measure thiobarbituric acid-reactive substances (TBARS) in assays with ethylenediaminetetraacetic acid (+ EDTA) and without EDTA (– EDTA). In the red blood cell membranes, there were no significant differences in the levels of TBARS in the 1,2,4-Trihydroyxbenzene or the control groups with the EDTA assay. In the organs, the level of TBARS with the - EDTA assay was significantly decreased by 1,2,4-Trihydroxybenzene in the kidney and liver. In the – and + EDTA assays of the lung, levels of TBARS from malonaldehyde derivatives plus alkadienal/alkenal derivatives were remarkably increased by 1,2,4-Trihydroxybenzene, but those from malonaldehyde derivatives alone were not. There were no effects of nitrogen dioxide inhalation on lung lipid peroxidation; lung lipid peroxidation was enhanced by 1,2,4-Trihydroxybenzene, but this effect was not greatly impacted in other tissues.

Neuroprotective Effects

1,2,4-Trihydroxybenzene (tested at 10 - 100 µM) was found to significantly inhibit lipopolysaccharide (LPS)-stimulated nitric oxide production in BV-2 microglia cells treated with 1 µg/ml LPS followed by the test material for 24 h.[61] 1,2,4-Trihydroxybenzene also inhibited inducible nitric oxide synthase mRNA and protein expression (cells treated with test material and LPS for 4 h). Additionally, 1,2,4-Trihydroxybenzene significantly reduced the generation of ROS in hydrogen-peroxide-induced BV-2 cells (treated with 1 mM hydrogen peroxide followed by test material) and in hydrogen peroxide-cell free conditions (scavenging effect of test material assessed with 1 mM hydrogen peroxide or with 0.1 mg/ml ferrous sulfate heptahydrate). The neuroprotective effect of 1,2,4-Trihydroxybenzene (30 mg/kg) was observed in the ischemic male Sprague-Dawley rat brain under middle cerebral artery occlusion in an in vivo infarction assay.

Melanogenesis Inhibition

1,2,4-Trihydroxybenzene is characterized kinetically as a tyrosinase substrate, and the action of tyrosinase on 1,2,4-Trihydroxybenzene may cause the inactivation of the enzyme through a suicide inactivation mechanism.[62] The activity of tyrosinase on 1,2,4-Trihydroxybenzene was assessed through the measurement of the formation of 2-hydroxy *p*-benzoquinone via spectrophotometric assay. The author suggested that 1,2,4-Trihydroxybenzene, being a potent suicide substrate of tyrosinase, could play a role in the depigmenting effect of hydroquinone. (Tyrosinase can catalyze hydroxylation of hydroquinone to 1,2,4-Trihydroxybenzene).[63]

Immunomodulatory Effects

The effect of 1,2,4-Trihydroxybenzene on type IV and type I allergy responses was studied using male BALB/c mice.[24] Type IV allergy responses were investigated through contact sensitization responses induced by 2,4-dinitrochlorobenzene (DNCB). Groups of 3 mice received a diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1,2,4-Trihydroxybenzene (1.3% w/w) for 1 - 2 wk, with or without exposure to air containing 10 ppm nitrogen dioxide. Control groups received normal feed mixed with untreated water, with or without exposure to nitrogen dioxide. The mice in each group were then treated on both ears with DNCB (25 µl of 1% (w/v) in acetone/olive oil (4:1 v/v)), daily for 3 consecutive

days, starting on the 11th day. On the 14th day, the draining auricular lymph nodes were excised. This experiment included measurements of lymph node weight, lymph node cell (LNC) number, and assessments in the local lymph node assay (LLNA) induced by DNCB sensitization. Lymph node weight was significantly lowered (p not reported) in mice fed a diet containing 1,2,4-Trihydroxybenzene, but total lymph node cell (LNC) number was unaffected. No difference in the weight and LNC number was noted between nitrogen dioxide exposed and non-exposed groups. The degree of DNCB-sensitized cell proliferation was increased approximately 2-fold by supplementation of 1,2,4-Trihydroxybenzene in both nitrogen dioxide exposed and non-exposed groups.

Type I allergy responses were measured in serum immunoglobulin E (IgE) levels in the mice treated with the test material as described above. The mice were then sensitized using 50 µl of 1% DNCB or 25% (w/v) trimellitic anhydride (TMA) solution in acetone/olive oil on both shaved flanks. Controls were the same as described above. After 7 d, the mice received 25 µl of the same inducing agent on both ears. Seven days after the challenge, serum IgE was measured in an enzyme-linked immunosorbent assay (ELISA). 1,2,4-Trihydroxybenzene enhanced the serum IgE levels induced by DNCB and TMA, with no significant differences noted in the IgE levels between the nitrogen dioxide exposed and non-exposed groups. TMA sensitization was enhanced to a greater extent by 1,2,4-Trihydroxybenzene. Effects produced by nitrogen dioxide inhalation were observed only in control mice with TMA sensitization, but not in mice that received 1,2,4-Trihydroxybenzene. The authors concluded that intake of a large amount of 1,2,4-Trihydroxybenzene may have an adverse effect on both type IV and type I allergy responses.[24]

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

The irritation potential of 3% 1,2,4-Trihydroxybenzene (98.1% pure) in water was assessed in 3 male New Zealand White rabbits in accordance with OECD TG 404.[2,3,5] The test material (0.5 ml) was applied to 1 rabbit for durations of 3 min, 1 h, and 4 h. In the remaining 2 rabbits, the test material was applied for durations of 1 h and 4 h, each. The test material was placed on a dry gauze pad that was then applied to the clipped flanks of the animals with a semi-occluded dressing. The sites were clipped thereafter on several days up to day 9. Untreated skin served as the control. After 3 min, very slight or well-defined erythema (grade 1 or 2) was noted from day 2 to day 6. After the 1-h exposure in the same animal, a very slight or well-defined erythema (grade 1 or 2) was noted from day 1 to day 8. In the other 2 animals, discrete erythema was noted on day 1 and 2 in 1 animal and no erythema was observed in the other animal. After the 4-h exposure, a brown coloration of the skin was noted in all animals from day 1 to day 2, 6, or 9; this could have masked very slight or well-defined erythema. No other cutaneous reactions were recorded during the study. Based on the 1-h exposure, 3% 1,2,4-Trihydroxybenzene in water was slightly irritating to rabbit skin.

Sensitization

In Vitro

In an antioxidant responsive element-nuclear factor erythroid 2-related factor 2 (ARE-Nrf2) luciferase KeratinoSens™ test, HaCaT keratinocytes were exposed to 1,2,4-Trihydroxybenzene (97.8% pure) at concentrations ranging from 0.977 - 2000 µM in 1% dimethyl sulfoxide (DMSO) in 1% Dulbecco's modified Eagle medium.[4,5] The test was performed in accordance with OECD TG 442D. 1,2,4-Trihydroxybenzene was tested in 3 definitive assays, and the positive control was cinnamic aldehyde at concentrations ranging from 4 - 64 µM. The interpolated concentration resulting in a 1.5-fold luciferase induction (EC1.5) value for the test material was 374.31 µM, and is below the threshold value of 1000 µM. For comparison, the EC1.5 value of cinnamic aldehyde was 10.37 µM. 1,2,4-Trihydroxybenzene has the potential to be a sensitizer, but is not equivalent to the potent sensitizer, cinnamic aldehyde. The SCCS noted that 1,2,4-Trihydroxybenzene was positive at a concentration of 500 µM, but the dose-response curve had a large variation in gene induction at 500 µM. According to test guidelines, the assay is positive when gene induction is statistically significant from the solvent control in at least 2 out of 3 replicates. Statistical analysis of the data for the results was not provided, and the SCCS determined the results of the assay inconclusive.

Animal

An LLNA was performed using 1,2,4-Trihydroxybenzene (98.1% pure) in accordance with OECD TG 429.[2,3] Female CBA mice were divided into groups of 4 and received 0.25, 0.5, 1, 2.5, or 5% (w/v) of the test material in dimethylformamide in experiment 1 and 0.01, 0.05, 0.1. 0.25, or 0.5% (solvent not stated) in experiment 2. The test material was applied to the ear surface (25 µl) once daily for 3 consecutive days. α-Hexylcinnamaldehyde (25% v/v) was used as the positive control. Five days after the first topical application, all animals were injected intravenously with [^{3}H]methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured.

No clinical signs or mortality related to treatment were observed. In experiment 1, dryness of the skin was noted on day 6 in 2/4 and 4/4 animals that received the test material at 1 and 2.5%, respectively. Additionally, a moderate increase in ear thickness (up to 45%) was observed at 2.5 and 5%, indicating irritation potential of the test material at these concentrations. No cutaneous reactions or noteworthy increases in ear thickness was observed in experiment 2.

In experiment 1, positive lymphoproliferative responses were observed at all tested concentrations, but without a clear dose-response relationship. Positive responses observed at concentrations of 0.25 and 0.5% were attributed to delayed contact hypersensitivity as there was no local irritation. The stimulation indices (SI) in experiment 1 ranged from 12.68 to 26.41 using concentrations from 0.25 to 5%. In experiment 2, a dose-related increase in SI (except for 0.1%) was noted and the threshold positive value of 3 was exceeded at 0.25%. The estimated concentration for an SI of 3 (EC_3) was calculated on the basis of the results in experiment 2 to be 0.08%. It was concluded that 1,2,4-Trihydroxybenzene induced delayed contact hypersensitivity, and based on the EC_3 value, should be categorized as an extreme sensitizer.[2,3]

OCULAR IRRITATION STUDIES

Animal

In an ocular irritation study performed in accordance with OECD TG 405, 3 male New Zealand rabbits received approximately 0.1 ml of a 3% dilution of 1,2,4-Trihydroxybenzene in water in the conjunctival sac of the left eye.[2,3] The right eyes served as the controls. The eyes were not rinsed after administration of the test material. Eyes were observed for reactions 1, 24, 48, and 72 h after instillation. Very slight chemosis and very slight redness of the conjunctiva were observed in all animals on day 1, which persisted in 2 of the 3 animals up to day 3. No other reactions were observed. It was concluded that 3% 1,2,4-Trhydroxybenzene was slightly irritating to rabbit eyes.

MARGIN OF EXPOSURE

Margin of exposure (MOE) is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and EU SCCS guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The basis for this MOE value of 100 comes from two multiplication factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intraspecies extrapolation). The MOE is sometimes referred to as the margin of safety (MOS), despite the parameters being definitionally different.

An MOE was calculated for 2.5% 1,2,4-Trihydroxybenzene in a hair dye to be 466 for a dermal exposure. This calculation is based on the LOAEL of 50 mg/kg bw/d (concentration at which increased spleen weights were observed) from a 90-d oral rat study[2,3] as a point of departure (PoD), an assessment factor of 3 for extrapolation from LOAEL to NOAEL, and an SED of 0.03577 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 3.70 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg).

The parameters that are used for the MOE calculation are listed below:

Systemically available dose: 3.70 $\mu g_{eq}/cm^2$ (derived from an in vitro study using frozen human dermatomed skin[4]
Skin surface area for application: 580 cm^2 (½ area head)[64]
Dermal absorption per treatment: 3.70 μg_{eq} /cm^2 × 580 cm^2 = 2146 µg = 2.146 mg
Human body weight: 60 kg

SED: 2.146 mg ÷ 60 kg = 0.03577 mg/kg bw/d
LOAEL: 50 mg/kg bw/d (90-d, gavage, oral, rat)[2,3]
NOAEL (extrapolated from LOAEL using an assessment factor of 3): 50 mg/kg bw/d ÷ 3 = 16.67 mg/kg bw/d

$$\text{MOE} = \frac{\text{NOAEL}}{\text{SED}} = \frac{16.67 \text{ mg/kg bw/d}}{0.03577 \text{ mg/kg bw/d}} = 466$$

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. 1,2,4-Trihydroxybenzene is reported to be used in oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

1,2,4-Trihydroxybenzene is reported to function as a hair colorant in cosmetics, according to the *Dictionary*. It is an oxidative dye used in permanent hair dye formulations and gradual hair coloring shampoos; this ingredient does not require hydrogen peroxide to activate oxidation and subsequent coupling reactions.

1,2,4-Trihydroxybenzene is a metabolite in biodegradation of aromatic compounds, such as benzene. It also occurs as a biodegradation product of fungi, yeast, and bacteria of aromatic compounds, and is found in roasted coffee beans.

According to 2023 VCRP survey data, 1,2,4-Trihydroxybenzene is reported to be used in 18 hair dye formulations and 1 hair shampoo (coloring). The results of the concentration of use survey conducted by the Council indicate 1,2,4-Trihydroxybenzene is used at up to 2.5% in hair dyes and colors. Under European regulations for cosmetic ingredients, 1,2,4-Trihydroxybenzene, when used as a substance in hair and eyelash dye products, is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe. The SCCS expressed concern for potential genotoxicity of 1,2,4-Trihydroxybenzene when used as an auto-oxidative hair dye component in permanent hair dye formulations. Recent available in vivo genotoxicity data cannot be considered by the SCCS due to the ban on animal testing of cosmetics and cosmetic ingredients in Europe.

In a dermal penetration study, the maximum absorption of a formulation containing 2.78% 1,2,4-Trihydroxybenzene through dermatomed human skin (~ 400 µm) was 0.17 µg/cm^2 or 0.03% after being corrected by + 2 standard deviation from 0.07 $\pm$ 0.05 µg/cm^2 (0.01 $\pm$ 0.01%) due to correction of the concentration tested (originally reported at 3% instead of 2.78%). The total systemically available dose of a hair dye formulation containing 2.5% 1,2,4-Trihydroxybenzene was 1.94 $\mu g_{eq}/cm^2$ (0.393%) with 2.25% *p*-toluenediamine and 1.13 $\mu g_{eq}/cm^2$ (0.226%) without *p*-toluenediamine.

In an acute dermal toxicity study in rats, no mortality was observed following a dermal dose of 2000 mg/kg bw 1,2,4-Trihydroxybenzene. The LD_{50} in an acute oral toxicity rat study of 1,2,4-Trihydroxybenzene was between 350 and 500 mg/kg bw for a preparation containing 1% 1,2,4-Trihydroxybenzene.

No observable toxic effects were noted in mice that received 1.3% (w/w) Trihydroxybenzene in feed mixed with water for 1 wk. In a 4-wk study in which mice received 100 or 500 mg/l 1,2,4-Trihydroxybenzene, feed intake and water consumption decreased, and serum and urinary hydrogen peroxide levels increased with the test material. Reduced blood nitric oxide metabolites and liver *S*-nitrosylated protein levels and decreased whole-body fat utilization were observed following treatment with 1,2,4-Trihydroxybenzene, with the latter occurring in a dose-dependent manner. In another 4-wk oral dietary study, 1.5% 1,2,4-Trihydroxybenzene increased DNA synthesis in a BrDU assay, but was not associated with any hyperplasia changes in glandular stomach mucosa.

In a 90-d gavage study of 1,2,4-Trihydroxybenzene in rats, the NOAEL was determined to be 50 mg/kg bw/d, according to the researchers; this value was determined to be an LOAEL by the SCCP. Statistically significant increases in the absolute weight and/or organ-to-body weight ratios were observed in treated males for the spleen (all dose levels), liver and kidney (100 and 200 mg/kg bw/d), and testes and heart (200 mg/kg bw/d). In the females, statically significant increases in the absolute weight and/or organ-to-body weight ratios were observed for the liver, spleen, and kidneys at 200 mg/kg bw/d.

In a teratogenicity study in which gravid female Sprague-Dawley rats were dosed by gavage with up to 300 mg/kg bw/d of the test article on gestation days 6 through 15, 1,2,4-Trihydroxybenzene was maternotoxic at 300 mg/kg bw/d. Embryotoxicity and teratogenicity were not observed in the fetuses.

1,2,4-Trihydroxybenzene is a compound known to induce DNA damage and is considered genotoxic in vitro. This compound can result in oxidative DNA damage, which is linked to the generation of ROS. However, catalase, peroxidases, and other detoxification (antioxidant) enzymes in the epidermis, along with smaller antioxidant molecules, work to minimize ROS damage within skin cells. Additional detoxification enzyme systems in skin may contribute to the detoxification of 1,2,4-Trihydroxybenzene, 1,2,4-Trihydroxybenzene-derived active oxygen species, and 1,2,4-Trihydroxybenzene-derived quinones, including superoxide dismutase, glutathione peroxidases, glutathione-S-transferases, and quinone reductases.

Genotoxicity was observed in a majority of the in vitro studies where auto-oxidation was not minimized or efforts to reduce auto-oxidization were not reported. Specifically, 1,2,4-Trihydroxybenzene was mutagenic in Ames tests when tested at up to 4000 µg/plate. Genotoxicity of 1,2,4-Trihydroxybenzene was observed in a gene mutation assay of mutants to 6-thioguanine (tested at up to a maximal concentration of 10 µM), DNA strand break tests (at up to 1000 µM), DNA synthetic activity inhibition assays (at up to 24 µM), and SCE assays (at up to 500 µM). 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml). Increases in micronucleus induction were observed in Chinese hamster V79 cells (at up to 25 µM) and human TK6 lymphoblastoid cells (at up to 30 µg/ml). 1,2,4-Trihydroxybenzene was clastogenic and aneugenic in a cytokinesis-block micronucleus test with human lymphocytes at up to 100 µM. Chromosomal damaged was observed in FISH procedures when 1,2,4-Trihydroxybenzene was tested at up to 100 µM.

In studies where degassed solutions were used, 1,2,4-Trihydroxybenzene was mutagenic in an Ames test when tested at up to 5000 µg/plate; however, the mutagenic effect of 1,2,4-Trihydroxybenzene was eliminated in another Ames test when evaluated in the presence of radical scavengers catalase (up to 20,000 IU) and L-glutathione (up to 10 µM). Catalase also reduced the toxicity of 1,2,4-Trihydroxybenzene. In other studies with degassed solutions, 1,2,4-Trihydroxybenzene was not genotoxic in a 3D comet assay (at up to 1250 µg/ml) or in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml). In in vivo micronucleus tests in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at up to 50 mg/kg bw.

The Panel considered the outside expert opinions and analyses of the available genotoxicity data on 1,2,4-Trihydroxybenzene. The positive genotoxic effects observed in vitro are all considered to be the result of the formation of ROS. DNA damage induced by 1,2,4-Trihydroxybenzene is inhibited by enzymes and other hydroxyl radical scavengers. The addition of metabolic activation greatly reduced the observed genotoxic effects, suggesting that the compound is negative with in vivo testing due to rapid inactivation. This is supported by the negative in vivo micronucleus test in mice. The outside experts concluded that 1,2,4, Trihydroxybenzene does not pose a genotoxic risk.

Oxidant-mediated genotoxicity of 1,2,4-Trihydroxybenzene is a multifactorial process. Redox reactions of 1,2,4-Trihydroxybenzene yield oxygen species, semiquinones, and quinones. Copper and iron salts stimulate oxidation of 1,2,4-Trihydroxybenzene, leading to 1,2,4-Trihydroxybenzene-induced genotoxicity.

No conclusion as to the carcinogenic potential of a hair dye formulation containing 0.5% 1,2,4-Trihydroxybenzene could be made in a 2-yr dermal study of mice. 1,2,4-Trihydroxybenzene was cytotoxic in a dose-dependent manner in K562 cells, but it was determined to be less cytotoxic than other benzene metabolites in HL-60 cells. 1,2,4-Trihydroxybenzene has been studied for its role in benzene hematotoxicity, oxidative stress, neuroprotective effects, and potential melanogenesis inhibition. Based on a study in mice, 1,2,4-Trihydroxybenzene may have an adverse effect on both allergen-sensitized type IV and type I allergy responses.

In a dermal irritation study, 3% 1,2,4-Trihydroxybenzene was slightly irritating to rabbit skin. 1,2,4-Trihydroxybenzene was predicted to be a sensitizer in an ARE-Nrf2 luciferase KeratinoSens™ test, and was categorized as an extreme sensitizer in an LLNA when tested at up to 5% in dimethylformamide and at up to 0.5% (solvent not stated). In ocular studies, 3% 1,2,4-Trihydroxybenzene was slightly irritating to rabbit eyes.

An MOE for 2.5% 1,2,4-Trihydroxybenzene in a hair dye was determined to be 466 for a dermal exposure. This calculation uses the LOAEL of 50 mg/kg bw/d from a 90-d oral rat study as a PoD, an assessment factor of 3 for extrapolation from LOAEL to NOAEL, and an SED of 0.03577 mg/kg bw/d. The MOE value is greater than 100, a figure generally accepted as the threshold for considering an ingredient safe to use.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

DISCUSSION

1,2,4-Trihydroxybenzene is reported to function as an oxidative hair dye in hair coloring products. The Panel noted that 1,2,4-Trihydroxybenzene is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe, when used as a substance in hair and eyelash dye products. The SCCS does not consider 1,2,4-Trihydroxybenzene safe due to potential genotoxicity when used as an auto-oxidative hair dye component in permanent hair dye formulations. In vitro genotoxicity studies yielded mixed results, and although in vivo micronucleus studies have yielded negative results, the European Union cannot accept the result of the in vivo studies because of its ban on animal testing. Conversely, the Panel did consider the results of these in vivo studies, along with negative results for other toxicity endpoints, slow absorption through the skin, short exposure time as a rinse-off product, the protective MOE value of 466, and the fact that enzymes present in the skin deactivate harmful ROS following dermal exposure and determined that these mitigated any concerns with the mixed in vitro genotoxicity findings. The Panel concluded the data are sufficient to conclude that 1,2,4-Trihydroxybenzene is safe for use as an oxidative hair dye ingredient in the present practices of use and concentration.

The Panel also noted that 1,2,4-Trihydroxybenzene is a potent suicide substrate of tyrosinase, i.e., the action of tyrosinase on 1,2,4-Trihydroxybenzene may cause the enzyme to self-inactivate. As tyrosinase is a key enzyme involved in the synthesis of the pigment melanin, the inactivation of tyrosinase by 1,2,4-Trihydroxybenzene may play a role in skin depigmentation. The Panel noted that depigmentation is considered to be a drug effect in the US and should not occur during the use of cosmetic products. Accordingly, cosmetic formulators should only use 1,2,4-Trihydroxybenzene in products in a manner that does not cause depigmentation.

The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the FD&C Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to scientifically support a causal relationship between hair dye use and cancer or other toxicological endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

The Panel's respiratory exposure resource document (available at https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery

systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	light-medium beige powder	[4]
Molecular Weight (g/mol)	126.11	[2]
Melting Point (ºC)	139 - 150	[2,3]
Water Solubility (g/l @ 20 ºC)	486	[3]
Other Solubility (g/100 ml @ 22 ºC)	ethanol: > 1, < 10 DMSO: > 10, < 20	[3]
log P_{ow}	0.2 (estimated)	[2]
UV/Visible Spectrum (λ_{max}; nm)	291	[3]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO						
1,2,4-Trihydroxybenzene (purity not stated)	2.5 - 40 µg/plate (without metabolic activation); 1 – 2000 µg/plate (with metabolic activation)	not reported	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535	Bacterial reverse mutation test performed with and without metabolic activation	Mutagenic; test substance induced gene mutation in strain TA104 with metabolic activation	[45]
1,2,4-Trihydroxybenzene; 98.1% pure	6.25 - 4000 µg/plate	purified water	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Mutagenic; test material induced gene mutations in strains TA98 and TA100 without metabolic activation; toxic effects (i.e., reduction in the number of revertant colonies and/or thinning of the bacterial lawn) was observed at higher concentration with and without metabolic activation in nearly all strains used	[2,3]
1,2,4-Trihydroxybenzene; 97.8 - 99.5% pure	6.7 - 5000 µg/plate	degassed water	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Mutagenic; test material induced positive mutagenic response in strain TA1537 without metabolic activation; results were negative for strains TA98, TA100, TA102, and TA1535	[4,5]
1,2,4-Trihydroxybenzene; 98.1% pure	100 - 500 µg/plate	degassed water	*S. typhimurium* strain TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; without S9 metabolic activation; study evaluated the effect of radical scavengers, catalase (1000 - 20,000 IU) and L-glutathione (5 – 15 µM), with the test material	Mutagenic; effect observed in the study described above was repeated in test strain without metabolic activation; mutagenic effect was eliminated in presence of 5 and 10 µM L-glutathione and in the presence of 1000 - 20,000 IU of catalase	[4,5]
1,2,4-Trihydroxybenzene; 99.4% pure	Test 1: up to 20 µg/ml without metabolic activation and up to 160 µg/ml with metabolic activation Test 2: up to 22.5 µg/ml without metabolic activation and up to 240 µg/ml with metabolic activation	not reported	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *hprt* locus in accordance with OECD TG 476; with and without metabolic activation; appropriate negative and positive controls used	Not genotoxic; no statistically significant increase in mutant frequency was observed at any dose level tested, with or without metabolic activation; positive and negative controls yielded expected results	[2,3]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene (purity not stated)	At least 5 concentrations used; optimal/maximal concentration of 10 µM; no further details provided	not reported	Chinese hamster V79 cells	Gene mutation assay; cells were incubated with test material for 24 h; frequency of mutants to 6-thioguanine (7 µg/ml) determined after an expression period of 6 d	Genotoxic; gene mutations induced at optimal/maximal concentration of 10 µM	[45]
1,2,4-Trihydoxybenzene; purity not reported	6, 12, or 24 µM	not reported	ICR mouse bone marrow cells	DNA strand break test; cells treated with test material for 1 h; alkaline DNA elution method (pH > 9.5) used; fractions collected every 24 min over a total of 120 min; cells exposed to test material also evaluated for protective effects of glutathione (350 µg/ml) and catalase (130 IU/ml) tested in parallel	Genotoxic; concentration-dependent increase in alkali-labile DNA single strand breaks observed, with a 42% increase at the highest concentration tested; double-strand breaks were not observed, but a significant pH-dependent increase in DNA elution rate was observed in treated cells when the elution pH increased from 9.6 to 12.6; DNA damage by test material was 53% blocked by glutathione and completely blocked by catalase	[3,46]
1,2,4-Trihydroxybenzene; 99.4% pure	Test 1: 1.25 - 5 µg/ml without metabolic activation and 3.75 - 15 µg/ml with metabolic activation Test 2: 2.5 - 7.5 µg/ml without metabolic activation and 10 - 20 µg/ml with metabolic activation	not reported	human lymphocytes	Mammalian chromosome aberration test in accordance with OECD TG 473, with and without S9 metabolic activation; appropriate negative and positive controls used	Not clastogenic; test material did not induce any significant increase in aberrant cell frequency, with or without metabolic activation; however, test concentrations did not induce required degree of cytotoxicity and an insufficient number of cells was evaluated in some cases	[2]
1,2,4-Trihydroxybenzene; purity not reported	24 µM	not reported	Crl:COBS CD-1 ICR BR mice bone marrow cells	DNA synthetic activity inhibition assay; DNA synthesis evaluated via the addition of [^{3}H]thymidine into DNA	Genotoxic; the test substance inhibited 64% of nuclear DNA synthetic activity; IC_{50} determined to be 19.4 µM	[47]
1,2,4-Trihydroxybenzene; purity not reported	0 - 24 µM	not reported	cell-free DNA assay system	DNA synthetic activity inhibition assay; test substance incubated with reaction mixture containing DNA polymerase (either DNA polymerase α or DNA polymerase I) for 30 min, followed by the addition of ^{3}H-TTP; filtered precipitated DNA evaluated for radioactivity	Genotoxic; when DNA polymerase α was used as the source of DNA polymerase, a dose-related inhibition of DNA synthesis was observed (IC_{50} = 15 µM); no inhibitory effect observed when DNA polymerase I was used	[47]
1,2,4-Trihydroxybenzene; purity not reported	1 - 1000 µM	not reported	L5178YS mouse lymphoma cells	DNA strand break test; cells exposed to test substance, followed by alkaline denaturation method, including hydroxylapatite chromatography, to separate single- and double-stranded DNA in order to examine DNA strand breaks	Genotoxic; the test substance produced DNA breaks in a dose-related fashion; ED_{50} values for induction of single-stranded DNA was 55µM	[33]
1,2,4-Trihydroxybenzene (purity not stated)	At least 5 concentrations used up to 25 µM, no further details provided	not reported	Chinese hamster V79 cells	Micronucleus assay; cells were incubated with test material for 24 h	Genotoxic; elevated frequencies of micronucleated cells observed at 25 µM	[45]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene; 98.1% pure	Range finding: 0.50 - 200 µg/cm^2 Test 1: 1.5 - 200 µg/cm^2 Test 2: 12 – 224 µg/cm^2 Test 3: 3 - 224 µg/cm^2	degassed acetone	MatTek EpiDerm™ human reconstructed skin tissue	Micronucleus assay; 3 tissue models per concentration in Tests 1, 2, and 3; tissue was exposed to 10 µl of test material in solution and incubated for 24 h twice (48 h total) before harvesting in Tests 1 and 2; Test 3 was confirmatory and 72 h in duration	Not genotoxic; induction of micronuclei did not occur in reconstructed skin	[4,5]
1,2,4-Trihydroxybenzene; 97.8% pure	Range finding: 0.126 - 1260 µg/ml, with and without metabolic activation 4 h exposure + 20 h: 1.26 - 150 µg/ml, with and without metabolic activation 24 h exposure: 0.1 - 100 µg/ml without metabolic activation	degassed water	human lymphocytes	Micronucleus test in accordance with OECD TG 487, with and without S9 metabolic activation; appropriate negative and positive controls used	Not genotoxic; percentage of cells with micronuclei in treated group not significantly increased relative to vehicle control at any dose level, with or without metabolic activation; positive and negative controls yielded expected results; substantial cytotoxicity (55 $\underline{+}$ 5% or greater reduction in cytokinesis-blocked proliferation index relative to vehicle control at dose levels $\underline{\geq}$ 50 µg/ml in non-activated 4 h treatment group, at dose levels $\underline{\geq}$ 100 µg/ml metabolic activated 4 h treatment group, and at dose levels $\underline{\geq}$ 30 µg/ml in the non-metabolic activated 24 h treatment group	[4,5]
1,2,4-Trihydroxybenzene; purity not reported	15, 20, 30 µg/ml	DMSO and water	human TK6 lymphoblastoid cells	Flow cytometry assay used to measure relative survival, apoptotic/necrotic cells, and micronucleus induction in parallel with application of TGx-28.65 genomic biomarker; lymphoblastoid cell exposures to test substance occurred in the presence of 2% hepatic S9 fraction; vehicle used as negative control; benzo[*a*]pyrene used as positive control; TK6 cells exposed for 4 h, rinsed, re-suspended, and re-incubated for additional 3 - 4 h for gene expression analysis and 20 h for flow cytometry analysis note: TGx-28.65 genomic biomarkers were developed by the authors were previously based on a database of gene expression profiles derived from human TK6 cells exposed to 28 well-known compounds; the biomarker comprises 65 genes that can classify chemicals as DNA damaging or non-DNA damaging	Genotoxic; dose-dependent declines in relative survival and increase in apoptosis; strong significant increase in micronucleus induction at all concentrations; the test substance was considered to be genotoxic at all three test concentrations. Controls gave expected results. TGx-28.65 analysis classified the test substance as genotoxic.	[48]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydoxybenezene; purity not reported	10, 25, 50 or 100 µM	not reported	human lymphocytes	Cytokinesis-block micronucleus test in combination with a fluorescence in situ hybridization technique with specific centromeric probes for chromosomes 7 and 8; lymphocytes were treated for 48 h with test material; 1000 binuclear cells scored for presence of micronuclei	Clastogenic and aneugenic; concentration-dependent and statistically significant increase in number of lymphocytes with micronuclei observed; concentration-dependent and statistically significant induction of aneuploidy of chromosomes 7 and 8 observed, with aneuploidy 8 being more frequent; non-disjunction of chromosomes 7 and 8 also observed	3,50
1,2,4-Trihydroxybenzene (purity not stated)	Optimal/maximal concentration was 10 µM, no further details provided	not reported	Chinese hamster V79 cells	SCE assay; cells were incubated with test material for 27 h; a total of 30 metaphases were scored for SCE per data point	Genotoxic; statistically significant ($p > 0.01$) number of SCE per cell above background at an optimal/maximal concentration of 10 µM	45
1,2,4-Trihydroxybenzene; 99% pure	5, 50, 70, 100, 300, or 500 µM	RPMI 1640 medium	human lymphocytes	SCE assay; lymphocytes treated with test material for 48 h in presence of BrdU	Genotoxic; concentration-dependent decrease of mitotic activity observed; concentration-dependent increase in SCE observed	3,49
1,2,4-Trihydroxybenzene; 97.8% pure	Range finding: 0.1 - 100 mg/ml or 1.6 - 1600 µg/cm^2 Test 1: 0.125 - 1 mg/ml or 2 - 16 µg/cm^2 Test 2: 0.25 - 1.25 mg/ml or 4 - 20 µg/cm^2	degassed acetone	Phenion® full thickness human skin model consisting of human primary keratinocytes and fibroblasts from single donor origin	3D skin comet assay; application volume was 16 µg/cm^2; total exposure time was 48 h; negative and positive controls were run in parallel	Not genotoxic; test material did not induce DNA damage to human skin cells after topical application; controls yielded expected results; cytotoxicity observed at and above 50 µg/cm^2 in adenylate kinase release and at 16 µg/cm^2 based on lactate dehydrogenase release during range finding study, in test 2, cytotoxicity observed at 20 µg/cm^2 based on measurement of intracellular adenosine triphosphate	4,5
1,2,4-Trihydroxybenzene; 99% pure	0, 5, 10, 20, 50. 80, or 100 µM	PBS	human HL60 cells	FISH procedure to detect aneuploidy; cells treated with test material at 24 h after culture initiation in duplicate and harvested after 48 h of exposure; centromeric probes specific for chromosomes 7 and 9 utilized; microtubule staining was also performed after 1 h of exposure	Genotoxic; treatment with 5 µM test material increased hyperdiploidy for chromosome 9 approximately 3-fold and 50 µM increased hyperdiploidy approximately 4-fold; similar results observed with chromosome 7 probe; majority of hyperdiploidy induced was trisomy; staining with anti-tubulin antibodies showed that the test material disrupted microtubule organization	37
1,2,4-Trihydroxybenzene; 99% pure	10, 25, and 50 µM	PBS	human peripheral lymphocytes	FISH procedure; cells treated with test material at 24 h after culture initiation in duplicate and harvested after 48 h of exposure; metaphase spreads prepared and hybridized with centromeric probes for chromosomes 1, 5, and 7, and sequence specific probes for 5q31 and 7q36-qter	Genotoxic; test material significantly increased monosomy 5 and 7 by 3-5 fold ($p < 0.0001$); test material also significantly increased the rate of del(5q) and del(7q) by 8-12 fold ($p < 0.0001$); chromosome 7 was especially susceptible to aneusomy induction at lower dose: treatment at 10 µM increased monosomy of chromosome 7, but not of chromosome 1 or 5	36

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene; 99% pure	10, 25, and 50 µM	PBS	human peripheral blood	Blood exposed to test substance, and ploidy status of 9 different chromosomes evaluated using FISH of metaphase spreads; 48-h chemical exposure	Genotoxic; test substance produced a dose-dependent increase in monosomy of chromosomes 5, 7, 8, and 9, but not of chromosomes 1 and 21; more profound effect observed on monosomy 5 and 7 compared to other chromosomes, and at low levels of exposure; the test substance also induced trisomy in all evaluated chromosomes in a dose-dependent manner	[38]
IN VIVO						
1,2,4-Trihydroxybenzene; 99.4% pure	50 mg/kg bw	water	5 Swiss OF1 mice per sex	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; single intraperitoneal dose; appropriate negative and positive controls used	Not genotoxic; number of micronucleated polychromatic erythrocytes did not differ statistically from the vehicle control values; ratio of polychromatic to normochromatic erythrocytes decreased significantly ($p < 0.05$) 24 h after treatment and ($p < 0.001$) 48 h after treatment, indicating a toxic effect of the test material to bone marrow cells; SCCP noted test not in accordance with current OECD TG as only 1 dose was tested	[2]
1,2,4-Trihydroxybenzene; 99% pure	6.3, 12.5, or 25 mg/kg (low and mid-concentrations did not meet acceptance criterion for % of targe, actual values achieved were 5.32 and 8.15 mg/kg); range finding doses were 25, 50, and 100 mg/kg	degassed deionized water	groups of 6 Hsd:ICR (CD-1) male mice; range finding study used groups of 3 males and 3 females mice of same strain	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; mice received single intraperitoneal injection (5 ml/kg); blood samples taken 1-h post dosing in 3 mice/dose group; micronucleated cells scored at 24 and 48 h post-dosing; appropriate negative and positive controls used	Not genotoxic; no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes observed at either time point relative to the vehicle control; positive control yielded expected results; no detectable presence of test article in blood samples; in range finding study, all but 1 female died in the high dose group and mice in mid-dose group had severe clinical signs of toxicity	[5,51]

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