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)
DMSO	dimethyl sulfoxide
DNCB	2,4-dinitrochlorobenzene
EC1.5	interpolated concentration resulting in a 1.5-fold luciferase induction
EC_3	estimated concentrations of an SI of 3
ED_{50}	median effective dose
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
Fe^{3+}	iron (III)
GC	gas chromatography
HPLC	high-performance liquid chromatography
^{3}H-TTP	thymidine triphosphate
IC_{50}	50% inhibitory concentration
IU	international units
LLNA	local lymph node assay
LNC	lymph node cell
LOAEL	lowest-observable-adverse-effect-level
LPS	lipopolysaccharide
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOAEL	no-observable-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
ROS	reactive oxygen species
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SI	stimulation index
TBARS	thiobarbituric acid-reactive substances
TG	test guideline
TGx	toxicogenomics
TMA	trimellitic anhydride
UV	ultraviolet light
VCRP	Voluntary Cosmetic Registration Program
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)

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 (*wINCI; *Dictionary)*, this ingredient, also called 1,2,4-benzenetriol or 4-hydroxycatechol, functions 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This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated.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 conducted September 2023. 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 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]

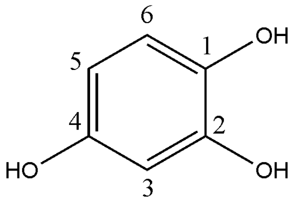

Figure 1. 1,2,4-Trihydroxybenzene

1,2,4-Trihydroxybenzene is an auto-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.

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 5th carbon position on the ring (see Figure 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. 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.

1,2,4-Trihydroxybenzene, at physiological pH, can be oxidized spontaneously (auto-oxidation) or enzymatically.[5] 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. If any such metal salts are present, any apparent "auto-oxidation" may actually be catalyzed oxidation.

The auto-oxidation of 1,2,4-Trihydroxybenzene produces reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals.[5] In the process, 1,2,4-Trihydroxybenzene is oxidized to 2-hydroxy-1,4-benzoquinone. (See Figure 2.)

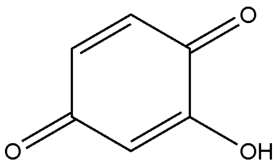

Figure 2. 2-hydroxy-1,4-benzoquinone

Chemical Properties

Chemical properties for 1,2,4-Trihydroxybenzene are summarized in Table 1. 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.[2-4]

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.[6] 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 high-performance liquid chromatography (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: benzene-1,2,4,5-tetraol; 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.[7] 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.[8,9]

1,2,4-Trihydroxybenzene also occurs as a biodegradation product by fungi, yeast, and bacteria of catechin, resorcinol, and other aromatic chemicals.[10-12] 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.[13-16]

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 were submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based 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).[17] 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.[18]

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.

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.[19] The SCCS considers 1,2,4-Trihydroxybenzene unsafe when used as an auto-oxidative hair dye component in permanent hair dye formulations.[4]

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 (draft). 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 (phosphate buffered saline (PBS) containing 0.01% sodium azide) was pumped at a speed of approximately 1.5 ml/h. Application of the test material was terminated by rinsing with water (10x), 2% sodium dodecyl-sulfate 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). The maximum absorption (dermal delivery) was 0.17 µg/cm^2 or 0.03% after being corrected by + 2 standard deviation from 0.07 ± 0.05 µg/cm^2 (0.01 ± 0.01%) due to correction of the concentration tested (original reported at 3% instead of 2.78%; the SCCP commented that degradation of circa 8% within 1 wk of the test substance (content 3%) in the test formulation was indicated, even though the test item was stored under an inert atmosphere.

In another dermal penetration study, [^{14}C]1,2,4-Trihydryoxybenezene (98.3% pure; 2.00 MBq/mg) was incorporated at a final concentrations of 2.5% (w/w) into hair dye formulations, with and without 2.25% *p*-toluenediamine.[4] The study was performed in accordance with OECD TG 428. The formulations were applied to dermatomed human skin (thickness not specified) 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 ± 0.58 μg_{eq}/cm^2 or 0.226%. The total systemically available dose from the test formulation with *p*-toluenediamine was 1.94 ± 1.76 μg_{eq}/cm^2 or 0.393%.

Absorption, Distribution, Metabolism, and Excretion

Benzene is metabolized in the liver via benzene epoxide to phenol, which is then further hydroxylated to catechol, hydroquinone, and 1,2,4-Trihydroxybenzene.[5] Metabolites of benzene, including 1,2,4-Trihydroxybenzene, may mediate the myelotoxicity and carcinogenicity of benzene.

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] 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.

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.[20] 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.[21] 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 increasing concentration of 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 increasing 1,2,4-Trihydroxybenzne concentrations, 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-nitroslyated 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. 1,2,4-Trihydroxybenzene treatment also decreased fatty acid oxidation in mouse primary liver cells.

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] 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-observable-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-observable-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] 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. Exencephaly was already noted in fetuses coming from dams treated with a non-teratogenic substance. This incidence 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]

GENOTOXICITY STUDIES

1,2,4-Trihydroxybenzene is a compound known to induce DNA damage and is considered genotoxic. This compound can result in oxidative DNA damage, which is linked to the generation of ROS.[22-27] 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.[28,29] 1,2,4-Trihydroxybenzene may inhibit mitochondrial DNA replication.[30] Furthermore, several studies have elucidated the role of 1,2,4-Trihydroxybenzene in causing chromosomal damage and subsequent biological consequences.[31-33]

In vitro and in vivo genotoxicity studies on 1,2,4-Trihydroxybenzene summarized here are detailed in Table 2. 1,2,4-Trihydroxybenzene was mutagenic in several Ames tests when tested at up to 5000 µg/plate.[2-4,34] 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), sister chromatid exchange (SCE) assays (at up to 500 µM), and in a human lymphocyte study using fluorescence in situ hybridization of metaphase spreads (at up to 50 µM).[3,28,33-38] 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) or in a 3D skin comet assay (at up to 1250 µg/ml), and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml).[2-4] Genotoxicity was not observed in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml), but 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).[4,34,37] 1,2,4-Trihydroxybenzene was clastogenic and aneugenic in a cytokinesis-block micronucleus test with human lymphocytes at up to 100 µM.[3,39] In an in vivo micronucleus test in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at 50 mg/kg bw.[2]

Mechanism

Oxidant-mediated genotoxicity of 1,2,4-Trihydroxybenzene is a multifactorial process.[5] Redox reactions of 1,2,4-Trihydroxybenzene yield oxygen-derived active species 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 to Cu^{2+}-mediated 2-electron transfer, which correlates with the changes in the pattern of 1,2,4-Trihydroxybenzene-induced micronucleus formation from kinetochore-positive to kinetochore-negative. (Kinetochore is a complex of proteins associated with the centromere of a chromosome during cell division, to which the microtubules of the spindle attach.)

CARCINOGENICITY STUDIES

Dermal

The dermal carcinogenicity potential of a semi-permanent hair dye formulation containing 0.5% 1,2,4-Trihydroxybenzene (purity not reported) was evaluated in groups of 50 male and 50 female Swiss Webster mice.[2,3] The test material (0.05 ml) was applied once weekly for 21 - 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 and the study was terminated after 23 mo. 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. There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 7 and 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.

Oral

In an oral study, 5 male and 5 female F344 rats received 1.5% 1,2,4-Trihydroxybenzene in diet continuously for 4 wk.[40] A control group of the same composition of animals received regular diet. At wk 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.

OTHER RELEVANT STUDIES

Cytotoxicity

The cytotoxicity of 1,2,4-Trihydroxybenzene was investigated in K562 erythroleukemia cells.[41] 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.[42] 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.[43] 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 hematoxicity.[42,44,45] 1,2,4-Trihydroxybenzene (3.1×10^{-6} to 500×10^{-6}) was studied for its effect on the ability of stomal cells to influence granulocyte/monocyte colony growth after incubation with the test material for 3 d.[44] 1,2,4-Trihydroxybenzene inhibited colony growth at doses $\geq 100 \times 10^{-6}$ M. K562 cells were used to determine the effects of 1,2,4-Trihydroxybenzene on erythroid differentiation.[42,45] 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.[20] 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.[46] 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 reported to be a substrate of tyrosinase and a strong suicide substrate.[47] This may contribute to the depigmentation property of hydroquinone.

Immunomodulatory Effects

The effect of 1,2,4-Trihydroxybenzene on type IV and type I allergy responses was studied using male BALB/c mice.[20] Type IV allergy responses were investigated through contact sensitization responses induced by 2,4-dinitrochlorobenzene (DNCB). Groups of 3 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. Control groups received normal feed mixed with untreated water, with or without exposure to nitrogen dioxide. The mice then underwent a local lymph node assay (LLNA) where both ears were treated with 25 µl of 1% (w/v) DNCB in acetone/olive oil (4:1 v/v). Lymph node weight was significantly lowered (p not reported) in 1,2,4-Trihydroxybenzene-treated mice, 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 1,2,4-Trihydroxybenzene in both nitrogen dioxide exposed and non-exposed groups.

Type I allergy responses were measured in serum IgE levels in the mice treated with the test material as described above. The mice were then sensitized with 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 1,2,4-Trihydroxybenzene may have an adverse effect on both allergen-sensitized type IV and type I allergy responses.[20]

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] 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] 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. 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% 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.

SUMMARY

1,2,4-Trihydroxybenzene is reported to function as a hair colorant in cosmetics, according to the *Dictionary*. It is an auto-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.

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.

No observable toxic effects were observed 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 increasing concentrations of the test material. Reduced blood nitric oxide metabolites and liver *S*-nitroslyated 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. 1,2,4-Trihydroxybenzene treatment also decreased fatty acid oxidation in mouse primary liver cells.

In a 90-d gavage study of 1,2,4-Trihydroxybenzene in rats, the NOAEL were 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, 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. This compound can result in oxidative DNA damage, which is linked to the generation of ROS. 1,2,4-Trihydroxybenzene was mutagenic in several Ames tests when tested at up to 5000 µg/plate. Genotoxicity of 1,2,4-Trihydroxybenzene was observed in a gene mutation assay of mutants to 6-thioguanine (tested at 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), SCE assays (at up to 500 µM), and in a human lymphocyte study using fluorescence in situ hybridization of metaphase spreads (at up to 50 µM). 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) or in a 3D skin comet assay (at up to 1250 µg/ml), and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml). Genotoxicity was not observed in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml), but 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. In an in vivo micronucleus test in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at 50 mg/kg bw.

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. In a 4-wk oral dietary study, 1.5% 1,2,4-Trihydroxybenzene was not associated with any hyperplasia changes in glandular stomach mucosa.

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. 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% without dimethylformamide. In ocular studies, 3% 1,2,4-Trihydroxybenzene was slightly irritating to rabbit eyes.

INFORMATION SOUGHT

The following information on 1,2,4-Trihydroxybenzene, as used in cosmetics, is being sought for use in the resulting safety assessment:

- Additional impurities data
- Clarity on the chemistry and risks of use in a coloring shampoo
- Further genotoxicity studies and/or clarification of currently included genotoxicity studies
- Further dermal sensitization data, such as human repeated insult patch tests, quantitative risk assessments (2.0), or equivalent new approach methodologies.
- Any additional toxicological studies that will help inform the Panel to make a conclusion on safety

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 (nm)	λ_{max} 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	34
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	4
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
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
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	34
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	3,35

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
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	36
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	36
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	28
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	34
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
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	4

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
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.	37
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	3,39
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	34
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	3,38
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	4

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 fluorescence in situ hybridization 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	33
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

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