
Safety Assessment of 6-Hydroxyindole

as Used in Cosmetics

Status: Tentative Report for Public Comment
Release Date: December 18, 2012
Panel Meeting Date: March 18-19, 2013

All interested persons are provided 60 days from the above release date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

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DRAFT ABSTRACT

The Cosmetic Ingredient Review Expert Panel (the Panel) reviewed the safety of 6-hydroxyindole, which functions as an oxidative hair dye. The Panel reviewed relevant animal and human data provided in this safety assessment, and concluded that 6-hydroxyindole is safe for use in oxidative hair dye formulations.

INTRODUCTION

This report addresses the safety of 6-hydroxyindole. This cosmetic ingredient is used in oxidative hair dyes.

CHEMISTRY

Physical and chemical properties of 6-hydroxyindole are found in Table 1. 6-Hydroxyindole is a bicyclic arylamine, hydroxylated at the six-position of the benzo [b] pyrrole.

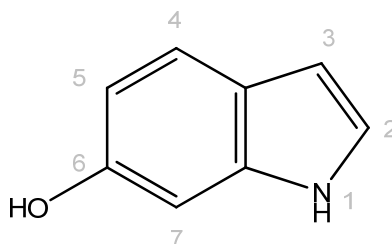
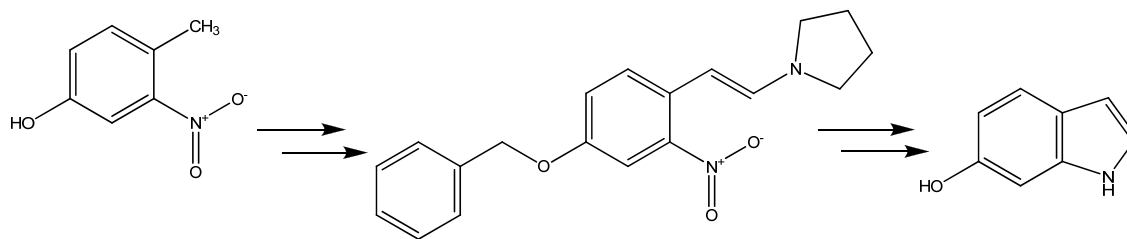


Figure 1. 6-Hydroxyindole

The synthetic process used to manufacture 6-hydroxyindole in hair dyes is the Leimgruber-Batcho indole protocol.^{1,2} The commercially available 4-methyl-3-nitrophenol can be benzyl-protected before condensation with pyrrolidine and *N,N*-dimethylformamide dimethylacetal. Reduction of this intermediate results in cyclization of the pyrrole ring, and benzyl-deprotection then affords 6-hydroxyindole.



Scheme 1. A synthetic pathway to 6-hydroxyindole

Although nitrosamine content has not been reported, 6-hydroxyindole has a secondary amine moiety in a pyrrole ring that potentially can be nitrosated. Indoles are readily nitrosated to form *N*-nitrosamines.⁴ Of concern in cosmetics is the conversion (nitrosation) of secondary amines (R1-NH-R2), such as 6-hydroxyindole (wherein R1 and R2 form the pyrrole ring), into *N*-nitrosamines that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals.⁵ Nitrosation can occur under physiologic conditions.⁶ Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO₂ may also participate in the nitrosation of amines in aqueous solution.⁷ Accordingly, hair dyes with 6-hydroxyindole should be formulated to avoid the formation of nitrosamines.

According to a 2002 study published by COLIPA, the amount of 6-hydroxyindole used in oxidative hair-colouring products throughout the hair dye industry was 1 ton.⁸ For use as a hair dye ingredient, 6-hydroxyindole is oxidized with hydrogen peroxide.³ Oxidation purportedly leads to dimerization, trimerization, and polymerization to eumelanin-like polymers.^{9,10} Linkages predominantly occur at 2, 5, and/or 7-positions, due to electronic effects.

Impurities

Potential impurities in 6-hydroxyindole are reagents and intermediate reaction products. These include 4-methyl-3-nitrophenylamine, 4-methyl-3-nitrophenol, 4-benzyloxy-1-methyl-2-nitrobenzene, 1-[2-(4-benzyloxy-2-nitro-phenyl)-vinyl]-pyrrolidine, and 6-benzyloxy-1H-indole, all at levels below 500 ppm.¹¹

USE

Cosmetic

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), 6-hydroxyindole is used in a total of 105 hair coloring formulations.¹² A survey of use concentrations has reported that 6-hydroxyindole is used at a maximum concentration of 0.5% in hair dyes and colors.¹³

Europe's Scientific Committee on Consumer Products (SCCP) concluded that 6-hydroxyindole would not pose a health risk to the consumer when used as an ingredient in oxidative hair dye formulations, as long as the maximum concentration applied to hair does not exceed 0.5%.¹¹ The European Commission has added 6-hydroxyindole to Annex III List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down using the limitations suggested by the SCCP and has placed the following labeling requirements on this ingredient:

The mixing ratio must be printed on the label. Hair colorants can cause severe allergic reactions. Read and follow instructions. This product is not intended for use on persons under the age of 16. Temporary "black henna" tattoos may increase your risk of allergy. Do not color your hair if you have a rash on your face or sensitive, irritated and damaged scalp, you have ever experienced any reaction after coloring your hair, or you have experienced a reaction to a temporary "black henna" tattoo in the past.¹⁴

Hair Dye Caution Statement - FDA labeling

This ingredient is considered a coal tar hair dye 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 United States' Federal Food, Drug, and Cosmetic 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 a caution statement and patch test instructions for determining whether the product causes skin irritation. The CIR Expert Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 hours after application of the test material and prior to the use of a hair dye formulation.

Earlier this year, Thyssen et al. published a report regarding such self-testing for contact sensitization to hair dyes.¹⁵ These authors concluded that, in its present form, the hair dye self-test has severe limitations. The authors issued the warning that, if the use of a hair dye self-test to predict contact sensitization becomes widespread, there is severe risk that a tool has been marketed that may cause morbidity in European consumers. In accompanying editorial, An Goossens, on behalf of the European Society of Contact Dermatitis, asserted that industry is focussing on predicting the risks from exposure to hair dyes by having millions of European consumers perform a self-test prior to each hair dying and stated that it is the opinion of the ESCD that attention must be given to reducing the risks of serious allergic reactions by improving the safety of the products themselves.¹⁶

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Oral

The plasma pharmacokinetics and excretion balance of 6-hydroxyindole were studied in Wistar Han Ico: WI (IOPS AF/Han) rats.¹⁷ Twelve rats of each sex received a single 300 mg/kg body weight dose of 6-hydroxyindole [benzene ring-U-¹⁴C]-10203 in a suspension of 0.5% aq. carboxymethylcellulose via gavage. For the plasma pharmacokinetics portion of the study, blood samples were collected from 9 rats of each sex before dosing and at 1, 2, 4, 6, 8, 24, 48, and 72 h after dosing. All rats were killed after the last blood sample. No necropsy was performed. In the excretion balance portion of the study in the remaining 3 rats of each sex, urine, feces, and cage washes were collected during a 24-h pre-dosing period and during the periods of 0-6 h, 6-24 h (urine) or 0-24 h (feces), 24-48 h, 48-72 h, 72-96 h, 96-120 h, 120-144 h and 144-168 h (urine and feces) after dosing. Cages were carefully rinsed after each collection of feces. All samples were frozen until analysis. All animals were killed at the end of the study and a wide range of tissues and organs were sampled. For each sample, total radioactivity was evaluated by liquid scintillation counting of plasma, urine, feces, and rinse water.

No mortalities were observed. In the first 24 h, brown greenish urine and piloerection were observed in most animals. Other clinical signs that were observed in some animals about 45 min after dosing and lasting 3-5 h

included tremors, hypersalivation, lacrimation, lateral decubitus, jerking movements, staggering gait, half-closed eyes, and coldness to touch. These observations were more frequent in female animals. The urine remained colored for up to 3 days. Total radioactivity levels in plasma reached a maximum 1 h after dosing with a mean C_{\max} value of $132 \mu\text{g}_{\text{eq}}/\text{ml}$ ($107 \mu\text{g}_{\text{eq}}/\text{ml}$ in males and $157 \mu\text{g}_{\text{eq}}/\text{ml}$ in females). The plasma radioactivity levels decreased in a biphasic manner at a moderate rate until 24 h post-dosing, followed by a slower decline until the last sampling point (mean $0.88 \mu\text{g}_{\text{eq}}/\text{ml}$; $0.77 \mu\text{g}_{\text{eq}}/\text{ml}$ in males and $0.98 \mu\text{g}_{\text{eq}}/\text{ml}$ in females). The mean systemic exposure [AUC (0- ∞)] was $799 \mu\text{g}_{\text{eq}}\text{-h}/\text{ml}$ ($638 \mu\text{g}_{\text{eq}}\text{-h}/\text{ml}$ in males and $954 \mu\text{g}_{\text{eq}}\text{-h}/\text{ml}$ in females). The mean total cumulative excretion (CE) of the radioactive dose over the 168-h period was 95.4%, with most of the dose excreted rapidly at an average of 8.9% within 24 h of dosing. The radioactivity was mainly excreted in the urine (mean 73.8%) and the mean CE in feces was 17.3%. No anomalies were noted in post-mortem examinations. The findings of the study confirm high systemic exposure to 6-hydroxyindole after oral exposure to rats.¹⁷

Dermal/Percutaneous

In an in vitro percutaneous absorption study, [^{14}C] 6-hydroxyindole was applied to human abdominal dermatomed skin in flow-through diffusion cells.^{18,19} The integrity of the skin was tested by measuring trans-epidermal water loss (TEWL) prior to test material application. The test substance was prepared at a concentration of 2% with hydrogen peroxide developer or with water to yield a final concentration of about 1%. The test substance ($20 \text{ mg}/\text{cm}^2$) was applied to the skin samples for 30 minutes and then washed off with water and 2% sodium lauryl sulfate solution. Measurements for radioactivity in the receptor fluid were made 24 h after application. When formulated with hydrogen peroxide, the absorbed amount (epidermis + dermis+ receptor fluid) was $4.70 \pm 2.08 \mu\text{g}/\text{cm}^2$ of the applied dose. When formulated with water, the absorbed amount was $5.86 \pm 2.68 \mu\text{g}/\text{cm}^2$.

The plasma pharmacokinetics, excretion balance, and tissue distribution of 6-hydroxyindole were studied in Wistar Han Ico: WI (IOPS AF/Han) rats.²⁰ Twelve rats of each sex received a single dose of 25 mg/kg body weight 6-hydroxyindole[benzene ring- ^{14}C]-10203 in a suspension of 0.5% aq. carboxymethylcellulose on clipped dorsum skin. The material was applied under a non-occlusive dressing for 30 min and then rinsed with water. Blood samples from designated animals were collected at 1, 2, 4, 6, 8, 24, 48, and 72 h after dressing removal. Urine, feces, and cage washes were collected from designated animals during a 24-h pre-dosing period and during the periods of 0-6 h, 6-24 h (urine) or 0-24 h (feces), 24-48 h, 48-72 h, 72-96 h, 96-120 h, 120-144 h and 144-168 h (urine and feces) after dosing. Cages were carefully rinsed after each collection of feces. All samples were frozen until analysis. All animals were killed at the end of the study and a wide range of tissues and organs were sampled. For each sample, total radioactivity was evaluated by liquid scintillation counting of plasma, urine, feces, ad rinse water. The minimal fraction of oral dose absorbed was derived from excretion data.

Total radioactivity levels in plasma reached a maximum 1 or 2 h after dosing with a mean C_{\max} value of $282 \pm 137 \text{ ng}_{\text{eq}}/\text{ml}$ ($233 \text{ ng}_{\text{eq}}/\text{ml}$ in males and $396 \text{ ng}_{\text{eq}}/\text{ml}$ in females). The plasma radioactivity levels decreased in a biphasic manner. By 4 h post-dosing, plasma levels were 33% of C_{\max} , with a slower decline until the last quantifiable time point at 24 h post-dosing (mean $35.7 \text{ ng}_{\text{eq}}/\text{ml}$; $30.1 \text{ ng}_{\text{eq}}/\text{ml}$ in males and $39.4 \text{ ng}_{\text{eq}}/\text{ml}$ in females). The male rats did have a second minor peak at 6 h. The mean systemic exposure [AUC (0- ∞)] was $2809 \text{ ng}_{\text{eq}}\text{-h}/\text{ml}$ for the sexes combined. The mean total CE of urine, feces, and cage wash over the 168-h period was 5.6% of the radioactive dose (sexes combined). Radioactivity was excreted equally in urine and feces with slow elimination over 96 h (77% mean for both sexes). Most of the administered dose was recovered in the dressing (68%) and in the skin at the application site (11%). The maximal fraction of the absorbed dose was 18% in both sexes (16% in males and 19% in females). Tissue levels of radioactivity were virtually non-quantifiable.²⁰

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral – Non-Human

The acute oral toxicity of 6-hydroxyindole was tested in CrI:CD(SD)BR rats.^{11,21} Groups of 5 male and 5 female rats received a single dose of the test substance at 600 mg/kg body weight in a volume of 10 ml/kg. The vehicle was a 30% aq. solution of PEG-6. The rats were observed twice daily for mortalities and daily for clinical signs of toxicity for 14 days. One male rat died immediately after dosing and a second male died the day after dosing. Clinical signs included hunched posture, hypoactivity and prostration on the day of dosing. No clinical signs of toxicity were observed in the surviving animals after the dosing day. No abnormal weight gain was observed. At necropsy, the animals that survived to day 14 had abnormalities in the spleen of 1 male and in the uterus of 1 female. The remaining animals appeared normal. The study concluded that the maximum tolerated dose

was less than 600 mg/kg body weight and the oral LD₅₀ was greater than 600 mg/kg body weight. In a preliminary range finding study, 4/4 animals died at 1200 mg/kg body weight and 1/4 animals died at 800 mg/kg body weight.

Dermal – Non-Human

The acute dermal toxicity of 6-hydroxyindole was tested in Crl:CD(SD)BR rats.^{11,22} Groups of 5 male and 5 female rats received a single dose of the test substance at 2000 mg/kg body weight on shaved dorsal skin and occluded with a moistened porous gauze dressing. At the end of the 24 h exposure period, remaining test substance was washed off with water. Animals were examined immediately, 30 min, 1 h, 2 h, and 4 h post-treatment and then daily for 14 days. There were no mortalities. Brown staining of the fur at the application site was the only clinical sign. Female weight gain was lower than normal and the uteri of 3/5 females had red discoloration and were distended. These effects were discounted by the study authors. The study concluded that the maximal non-lethal dose of 6-hydroxyindole was greater than 2000 mg/kg.

A study similar to the one above was conducted using Sprague Dawley rats with the same dose of 6-hydroxyindole.²³ No mortalities, clinical signs, or cutaneous reactions were observed. At necropsy, no macroscopic abnormalities were apparent. The LD₅₀ for 6-hydroxyindole in this acute dermal rat study was greater than 2000 mg/kg body weight.

Inhalation – Non-Human

Groups of 5 male and 5 female Wistar rats were utilized in an acute inhalation study of 100% 6-hydroxyindole powder.²⁴ The animals were exposed in a nose-only chamber for 4 h to an aerosol of the test material at 2000 mg/m³. Rats were observed for clinical signs of toxicity for 14 days and body weights were recorded daily. No mortalities or clinical signs were observed. No macroscopic or microscopic abnormalities of the lung or other tissues were observed at necropsy. The LC₅₀ for 6-hydroxyindole was greater than 2000 mg/m³.

Repeated Dose Toxicity

Oral – Non-Human

The potential for oral toxicity to 6-hydroxyindole was investigated in Crl:CD(SD)BR rats.²⁵ Dose groups were comprised of 10 animals of each sex and received 0, 30, 100, or 300 mg/kg body weight of the test material in a 30% aq. PEG-6 solution. The test material was administered by oral gavage once daily for 13 weeks. Clinical signs of toxicity and mortality were observed daily, and feed consumption, and body weights were recorded weekly. Ophthalmoscopic exams were performed before and after the treatment period in the control and high dose group animals. At week 13, complete hematology and blood chemistry investigations and urinalysis were performed. All animals were killed at the end of the treatment period. Major organs were weighed and a detailed necropsy was performed in all animals.

All animals survived until necropsy. Hair loss and scabbing were noted in some animals in all dose groups. No clinical signs of toxicity were observed. No irregularities were observed during the ophthalmoscopic exams. There were no treatment-related changes in body weight gains. Feed consumption was consistently higher in female rats in the high dose group throughout the study, but this was not considered an adverse effect. The female rats in the high dose group had minor hematological changes compared to the controls, with statistically significant dose-related increase in mean cell hemoglobin. There was a 2-fold increase in both alanine aminotransferase and aspartate aminotransferase levels in the plasma of the high dose females. No indication of this effect was noted in the mid-dose group. A significant dose-related elevation of blood cholesterol and inorganic phosphate levels were noted in female rats, but the values were still within historical control ranges. No treatment-related effects were noted in the urinalysis.

Increased absolute and relative liver weights were observed in both sexes in the high dose group when compared to controls (15-18% above controls). Increased absolute and relative spleen and kidney weights were also observed in both high dose groups (13-24% and 11-15% above controls, respectively). However, the changes in the spleen and kidney were within historical control ranges and there were no histopathological changes. No treatment-related macroscopic changes were noted at necropsy. Four out of 10 females in the high dose group had hepatocyte hypertrophy. No other significant abnormalities were observed in the histopathological examination. The authors concluded that the NOAEL was 100 mg/kg body weight/day.²⁵

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a teratogenicity study, mated female Crl:CD(SD)BR rats received 6-hydroxyindole by gavage on days 6-15 of gestation.²⁶ The doses used were based on the results of a range finding study. In the main study, groups of 24 rats received 0, 50, 150, or 450 mg/kg body weight/day of the test material in a suspension of 30% aq. PEG-6 solution. Body weights were recorded on gestation days 0, 6-15, and 20, and feed consumption was measured on gestation days 0-6, 6-9, 9-15, and 15-20. Dams were killed on gestation day 20. Complete necropsy and

macroscopic examination of the organs was performed. Fetuses were removed, sexed, weighed, and examined externally. Number of corpora lutea, implantation sites, resorption sites, and fetuses were recorded. The fetuses were examined for anomalies.

Severe clinical reactions (including hypoactivity, hunched posture, and piloerection) were observed at the onset of dosing in the 450 mg/kg dose group and 14/24 animals were killed. The remaining 10 animals in this group and an additional 14 were given 300 mg/kg/day of the test material from gestation day 6. At 300 mg/kg/day, the maternal effects observed were slightly lower body weight gain and food intake from gestation days 6-9. Increased salivation at 300 mg/kg/day and greenish urine at 150 mg/kg/day were also observed in the dams. Body weight and food consumption were decreased at higher doses. On gestation day 20, the dams did not have any observable treatment-related toxic effects. Pre-implantation losses were similar or lower than the controls. Mean fetal sex ratio was similar in all groups and mean fetal weight was statistically significantly reduced at 300 mg/kg/day. Reduced ossification in most of the fetal bones was noted in the 150 and 300 mg/kg/day dose groups. In the 50 mg/kg/day fetuses, there was a slight, but non-specific indication of reduced ossification. The maternal NOEL was 150 mg/kg body weight and the fetal NOEL was 50 mg/kg body weight in this rat teratology study.²⁶

In another teratogenicity study, mated female Sprague-Dawley rats received 6-hydroxyindole by gavage on days 6-19 of gestation.²⁷ Groups of 24 rats received 0, 15, 60, or 240 mg/kg body weight of the test material in a suspension of 30% aq. PEG-6. Maternal clinical signs were monitored daily. Body weights were recorded every 3 days and feed consumption was measured over 3-day periods. Dams were killed on gestation day 20. Organ abnormalities, pregnancy status, number of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. The fetuses were weighed, sexed, and examined for soft-tissue and skeletal abnormalities.

No mortalities were observed during the study. Ptyalism in 4 rats of the 240 mg/kg/day dose group was observed. No treatment-related changes in body weight, body weight gains or feed consumption were observed. Live fetuses were observed in 23, 24, 24, and 22 females in the 0, 15, 60, and 240 mg/kg dose groups, respectively. No treatment-related findings were observed at necropsy. In the 60 and 240 mg/kg dose groups, preimplantation loss was statistically significant different from the controls, but not considered toxicologically relevant because it was lower than in the control group. The mean numbers of resorptions and dead fetuses and fetal sex ratio were comparable to controls. The mean fetal body weight was slightly lower, but not statistically significant, in the high dose group. No treatment-related visceral anomalies were noted. Retardation of general ossification of fetuses was observed in the high dose group, but there were no bone malformations. The maternal NOEL was 240 mg/kg body weight/day and the fetal NOEL was 60 mg/kg body weight/day in this rat teratology study.²⁷

GENOTOXICITY

In Vitro

The potential of 6-hydroxyindole to induce gene mutation was studied in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 and in *Escherichia coli* strain WP2 uvrA using the reverse mutation assay.²⁸ The assay was performed with and without S9 metabolic activation at concentrations of 8, 40, 200, 1000, or 5000 µg/plate. A dose-dependent increase in revertant colony numbers was observed with and without S9 in TA 1535. It was concluded that 6-hydroxyindole was mutagenic with and without metabolic activation in *S. typhimurium* strain TA 1535.

6-Hydroxyindole was studied for cell mutation in mouse lymphoma L5178Y TK^{+/+} cells in 2 independent experiments.²⁹ Concentrations tested were 10, 40, 60, or 100 µg/ml, and the experiments were performed with and without S9 metabolic activation. In the first experiment, statistically significant positive effects were observed at 10 and 40 µg/ml with S9 activation, but there was no dose-dependent relationship. No statistically significant positive effects with or without S9 activation were observed in the second experiment. Since the results of the first experiment were not reproducible and had no trend, it was concluded that 6-hydroxyindole was not mutagenic in this assay.

The potential of 6-hydroxyindole to induce chromosomal aberrations was studied in Chinese hamster ovary (CHO) cells.³⁰ The study was comprised of 2 experiments, with the concentrations tested with S9 metabolic activation being 10, 50, or 100 µg/ml and the concentrations tested without S9 being 1, 5, and 10 µg/ml for both experiments. Harvest times were 24 and 48 h. In the first experiment, the test material induced a statistically significant increase in the number of cells with structural chromosomal aberrations at 100 µg/ml. The frequency was within historical control ranges, but the number of cells with aberrations was elevated and the types of rearrangements observed were acceptable indicators of clastogenicity. An increased frequency of aberrations was observed at 10 µg/ml without metabolic activation at 48 h. Only 21 cells were scored, but the percentage of aberrations was very high (14%). In the second experiment, an increased number of cells with structural chromosomal aberrations was observed at the highest concentration (not described) with metabolic activation at 48 h

and without metabolic activation at 24 h. Because the study yielded positive results at different concentrations and harvest times but clastogenic properties were observed in the cells, the authors concluded the study was equivocal.

The genotoxic potential of 6-hydroxyindole was studied in a chromosome aberration study using human peripheral blood lymphocytes.³¹ In this 2 part study, the test material was tested up to 67.31 and 892.5 µg/ml, without and with S9 metabolic activation, respectively, in experiment 1, and in experiment 2, the test material was tested up to 25.03 and 900 µg/ml, without and with S9, respectively. Incubation for cells with metabolic activation was 3 h and 20 h for cells without metabolic activation. In both experiments, statistically and biologically significant increased number of cells with structural chromosomal aberrations was observed both with and without metabolic activation. Aberrations included chromatid and chromosome deletions and exchanges. Under the conditions of this study, 6-hydroxyindole was considered clastogenic.

In Vivo

In a bone marrow chromosome aberration test, groups of 5 male and 5 female Sprague-Dawley rats received a single dose of 150, 300, or 600 mg/kg body weight 6-hydroxyindole in 0.5% aq. methylcellulose solution via intragastric gavage.³² The rats were killed 18 or 42 h after dosing. Clinical signs of toxicity were recorded in the 600 mg/kg dose group. No statistically significant or biologically relevant increase in the incidence of cells with chromosome aberrations was observed at any dose and time point. The study concluded 6-hydroxyindole was not clastogenic.

The genotoxic potential of 6-hydroxyindole was studied in a micronucleus test using CD-1 mice.³³ Groups of 5 mice of each sex received single doses of 0, 50, 250, or 500 mg/kg body weight 6-hydroxyindole in 30% aq. PEG via intragastric gavage. Negative and positive controls were within OECD guidelines. Bone marrow was collected at 24, 48, or 72 h. No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells was observed. However, the groups of mice did not exhibit variation of the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE), which would indicate relevant exposure of the bone marrow to the test material. The Scientific Committee on Consumer Products (SCCP) determined this study was inadequate.¹¹

The genotoxic potential of 6-hydroxyindole was studied in a micronucleus test using Sprague Dawley rats.³⁴ Groups of 5 mice of each sex received 0, 75, 150, or 300 mg/kg body weight 6-hydroxyindole via gavage on 2 consecutive days. Control groups were within OECD guidelines. Bone marrow cells were collected at 24 h. There was no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells when compared to the vehicle control values. The treated rats also were not observed to have variation of the PCE/NCE ratio; however, there was no evidence that the test material reached the rats' bone marrow. The SCCP determined this study was inadequate.¹¹

The potential for 6-hydroxyindole to induce unscheduled DNA synthesis (UDS) was assessed using male Wistar HanIbm: WST(SPF) rats.³⁵ Groups of 3 rats received single gavage doses of 150 or 1500 mg/kg body weight 6-hydroxyindole in PEG-6. The animals were killed either 2 h or 16 h post-treatment. The UDS was analyzed by autoradiography. Positive controls were within OECD guidelines. No evidence of UDS induction by 6-hydroxyindole was observed.

CARCINOGENICITY

6-Hydroxyindole was assessed for carcinogenicity in a 105 week study in Wistar Han Ico: WI (IOPS AF/Han) rats.³⁶ The study was preceded by an 8-week toxicity study to determine the dose range. In the main experiment, groups of 50 male and 50 female rats received 0, 6, 25, or 100 mg/kg body weight/day 6-hydroxyindole in a suspension with 0.5% aq. carboxymethylcellulose daily via gavage. The rats were observed daily for clinical signs of toxicity and mortality, while body weight and feed consumption were measured once a week for 13 weeks and then once every 4 weeks. Palpation for possible masses was carried out every 2 weeks from 6 weeks of treatment. Hematological investigations were performed at weeks 52 and 78 (differential white cell count in control and high dose groups) and at study end (red and white cell quantitative and qualitative assessment in all animals). In all groups, 8 additional animals were used for toxicokinetic investigations, with blood sample taken on day 1 and at weeks 13 and 52 for measurement of test material in plasma. The rats were killed and necropsied at the end of the treatment period.

There was a slight increase in mortality rate in the males of the 100 mg/kg dose group; however, these were due to normal causes and suggested a slight shortening of time of onset of death. This did not occur in females. Mortalities in the remaining dose groups were comparable with the control group. Body weight gains in the 6 and 25 mg/kg dose groups and the 100 mg/kg dose group females were comparable to controls. Body weight gain was slightly lower for males in the 100 mg/kg dose group. There were no significant differences in feed consumption between the treated groups and the controls. The only clinical sign of toxicity was increased salivation and/or

regurgitation in all treated animals. Palpable mass incidence, time of onset, and localization in the treated groups were comparable to controls. The only hematological changes were slight decreases in main red blood cell count, packed cell volume and plasma hemoglobin in the 100 mg/kg dose groups, indicating anemia. No quantifiable levels of the test material were observed in the plasma of the treated animals. No gross findings or neoplastic or non-neoplastic lesions attributable to treatment with 6-hydroxyindole were observed. This study concluded that the NOAEL for general toxicity was 25 mg/kg body weight/day and the NOEL for carcinogenicity was 100 mg/kg body weight/day.³⁶

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

The irritation potential of 6-hydroxyindole was assessed in 3 male New Zealand White rabbits.³⁷ Approximately 0.5 g of the test substance (tested as received from the test sponsor) moistened with water was applied to an intact area (6.25 cm²) of skin and then semi-occluded. The patches were removed after 4 h and remaining test substance was rinsed off. No skin reactions were observed in any of the rabbits. A slight yellow staining was observed in 2/3 rabbits during all observation periods. The primary irritation index was calculated to be 0.0. It was concluded that 6-hydroxyindole was non-irritating to rabbit skin.

In a 14-day irritation study, 3 male and 3 female New Zealand White rabbits received 2ml of 6-hydroxyindole in paraffin oil daily on the left flank.³⁸ The test substance was initially applied at a concentration of 5%, but this was reduced to 1.5% on day 10. The right flank received paraffin oil as a control. The sites were not covered. Cutaneous reactions were evaluated in each animal immediately before each application and about 24 h after the last application. From day 3 to the end of the study, very slight or well-defined erythema was observed on almost all of the animals on the treated flank. From days 8-15, moderate erythema was observed in 2/6 rabbits. Slight edema was noted in 2/6 rabbits on day 3 with slight to severe edema persisted in 1 rabbit until day 15. The maximum weekly mean irritation index was calculated to be 2.6. Very slight or well-defined erythema was noted in 3/6 rabbits on the control flank. These reactions were thought to be from contamination of the control with the test substance as the rabbits' ears could touch both flanks as severe erythema and crusts were observed on both ears of all animals from day 9 to study end. Body weight gains in the male rabbits were normal; however, body weight gains in females were decreased. The study concluded that 6-hydroxyindole was not well tolerated in rabbits.

The irritation potential of 6-hydroxyindole was assessed in 5 male and 5 female Hartley CrI: (HA)BR guinea pigs.³⁹ Approximately 0.05 ml of the test substance at 5% w/w in PEG-6 at 30% in purified water was applied to the left clipped flanks of the animals once a day for 14 days. The test site was approximately 2 cm². The test sites were not covered nor were they rinsed. The right flanks received the vehicle as a control. Cutaneous reactions were evaluated in each animal immediately before each application and about 24 h after the last application. No clinical signs of toxicity or mortalities were observed during the study. Body weight gains were within normal parameters. No cutaneous reactions were observed on the control flanks. Very slight erythema was observed on the treated flanks in 5/10 guinea pigs on days 9 and 10, very slight or well-defined erythema was observed in 7/10 guinea pigs on day 11, and then all animals exhibited very slight to moderate erythema from day 12 until study end. No edema was observed. Skin dryness was observed in 1 animal on day 11 and in 3/10 animals on day 12. The maximum weekly irritation index was calculated to be 1.1. It was concluded that 6-hydroxyindole was a slight to moderate skin irritant in guinea pigs.

Ocular

The ocular irritation potential of 6-hydroxyindole was tested in a single male New Zealand White rabbit.⁴⁰ An aliquot of 0.1 g of 6-hydroxyindole (concentration not reported, used as supplied) was administered to the rabbit's right eye. Within 15 min, signs of irritation were observed in the treated eye. One hour after dosing, discharge, moderate hyperemia, and chemosis were observed. Opacity covered most of the cornea, making observation of the iris difficult. The test article was considered to be moderately to severely irritating to the rabbit eye in this study.

6-Hydroxyindole at 5% in 30% aqueous PEG-6 solution was tested for ocular irritation potential in 3 female New Zealand White rabbits.⁴¹ The right eye of each rabbit was instilled with 0.1 ml of the test substance and not rinsed. The untreated eye served as a control. Both eyes were examined at 1, 24, 48, and 72 h post-treatment. No adverse reactions were reported in the eye of any of the rabbits. The study determined that 5% 6-hydroxyindole in 30% PEG-6 solution was not an ocular irritant in rabbits.

Sensitization

Dermal – Non-Human

The local tolerance of 6-hydroxyindole was tested in a 2-week guinea pig study.⁴² Three male and 3 female Dunkin-Hartley guinea pigs received 0.05 ml of 20% (w/w) 6-hydroxyindole in paraffin oil on the left posterior flank daily. The test area was 2 cm² and was not occluded. The right posterior flank received only the vehicle as a control. The concentration was lowered to 10% (w/w) on day 3 because the initial solution was poorly tolerated. The test sites were observed in each animal immediately before each treatment and about 24 h after the last treatment. Because of skin lesions on both flanks, the animals were challenged with the test material and the vehicle after a 1-week rest period. An additional 3 untreated female animals were used as controls. The animals received 0.5 ml of the test material in paraffin oil at 10% on the anterior left flank and paraffin oil alone on the anterior right flank. The test sites were occluded for 6 h and the skin was evaluated for reactions at 24, 48, and 72 h after the dressing was removed.

No clinical signs of toxicity or mortalities were observed during the study. Body weight gains were within normal parameters. Moderate skin reactions were observed on the treated sites during the 2-week period with well-defined to severe erythema in all animals and slight edema in 3/3 males and 1/3 females. The reactions were first observed between days 3 and 6. Dryness of the skin and/or crusts were also noted in all animals. The weekly mean irritation indices were 3.6 for the first week and 2.3 for the second week. Cutaneous reactions, including very slight to moderate erythema in 2/3 males and all females, slight edema in 2/3 males and 1/3 females, and dryness and crusts, were also observed on the flank that received the vehicle only starting on day 6. No cutaneous reactions were observed in the control animals during the challenge. In the treated animals, very slight, well-defined, or moderate erythema was noted in 1/6, 1/6, and 3/6 animals, respectively at 24 h. Dryness of the skin was recorded in 1 animal. These reactions were also observed at 48 h, except 2/6 animals had moderate erythema. The study did not state if the reactions were in the same animals. Dryness of the skin was recorded in 3 animals. Moderate erythema persisted in 1 animal at 72 h. Dryness of the skin was observed in 3 animals which was severe enough to preclude evaluation of erythema in 2 animals. Crusts were also noted in 1 animal. Microscopic examinations indicated that the lesions were due to sensitization. The study concluded that repeated topical applications of 10% and 20% 6-hydroxyindole in paraffin oil were badly tolerated in guinea pigs, possibly due to sensitization.⁴²

The Buehler method was utilized to study the sensitization potential of 20% 6-hydroxyindole in paraffin oil in Dunkin-Hartley guinea pigs.⁴³ There were 10 guinea pigs in the control group (5 of each sex) and 20 guinea pigs in the test group (10 of each sex). During the induction period, animals in the treatment group received a 0.5 ml application of the test material on days 1, 8, and 15 on a 4 cm² area of the anterior left flank that was occluded for 6 h. The anterior right flank of these animals received the vehicle control. After a 2-week rest period, the guinea pigs were challenged with 0.5 ml of 20% test material on the posterior right flank and 0.5 ml of the vehicle control on the posterior left flank. The skin was assessed for cutaneous reactions 24, 48, and 72 h after the challenge patches were removed. No clinical signs of toxicity or mortalities were observed. Very slight erythema was noted in 16/20 and 12/20 animals on days 9 and 16, respectively, of the induction period. No skin reactions were observed in the control animals in the challenge period, but the treated group had slight erythema in 3/20, 5/20, and 3/20 animals at 24, 48, and 72 h, respectively. The study concluded that 20% 6-hydroxyindole in paraffin oil induced sensitization reactions in 25% of the guinea pigs.

A similar Buehler sensitization study was performed using 50% (w/w) 6-hydroxyindole in 30% PEG-6 in purified water for both the induction and challenge phases.⁴⁴ No clinical signs or mortalities were observed during the study. A brown coloration of the skin was observed during the induction period in all animals of the treated group that may have masked erythema reactions. This was also observed at the 24-h challenge reading. Discrete or moderate erythema was noted in 6/20 and 6/20 animals, respectively, at the 48-h reading. Discrete, moderate, or intense erythema was observed in 4/20, 7/20, and 1/20 animals, respectively, at the 72 h reading. Edema was observed in 1 animal. In both the treatment and control groups, skin dryness was observed. The study concluded that 50% 6-hydroxyindole in 30% PEG-6 induced delayed contact hypersensitivity in 55% of the guinea pigs.

In another Buehler sensitization study, 5% (w/w) 6-hydroxyindole in a solution of 30% PEG-6 in purified water was studied in guinea pigs for both the induction and challenge phases.⁴⁵ No clinical signs or mortalities were noted during the study. Discrete erythema reactions were observed in the treated animals during the induction phase. Brown coloration of the skin was observed after the challenge application, which could have masked discrete erythema at the 24-h reading. Discrete or moderate erythema was noted at the 48 h reading in 6/20 and 6/20 animals, respectively. Discrete, moderate, or intense erythema was observed in 4/10, 7/20, and 1/20 animals, respectively, during the 72 h reading. One animal was observed with edema. Dryness of the skin was recorded in a few animals of both groups. The study concluded that 5% 6-hydroxyindole in 30% PEG-6 induced delayed contact hypersensitivity in 30% of the guinea pigs.

The dermal sensitization potential of 6-hydroxyindole in PEG 6/water (85:10) was evaluated with a Magnusson-Kligman maximization study.⁴⁶ The test group consisted of 10 male and 10 female Dunkin Hartley guinea pigs, while the negative control group had 5 animals per sex. A dose of 0.5% was used for the intradermal induction, 5% for topical induction, and 5% for the topical challenge patch. Dose volumes were 0.1 ml for the intradermal induction and 0.5 ml for the topical induction and challenge. Skin was examined for reactions 24 and 48 h after removal of the challenge patch. At the 24-h examination, 2 male and 2 female test animals had slight erythema and 3 females had well-defined erythema. Slight erythema remained in the same 5 females at 48 h, while the reactions had resolved in the males. No reactions were observed in control animals. The author concluded that the responses were individual irritation reactions and the substance was non-sensitizing; however, the SCCP concluded from this study that the substance caused sensitization in 40% of the test animals because there was no irritation during topical induction.

A local lymph node assay (LLNA) was performed using 6-hydroxyindole.⁴⁷ CBA/J female mice were divided into groups of 4 and received 0.2%, 0.5%, 1%, 2%, or 5% of the test material in acetone/olive oil (4:1, v/v) on the ear surface (25 µl) once daily for 3 consecutive days. A positive control group received 25% α -hexylcinnamaldehyde and a negative control group received just the vehicle. Ear thickness was measured on days 1, 2, 3, and 6. On day 6, all animals were injected intravenously with [³H] methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured. The stimulation indices (SI) were calculated.

No clinical signs of toxicity or deaths occurred during the treatment period in any dose group. No cutaneous reaction or increases in ear thickness were observed in the treated animals. Significant dose-related lymphoproliferation was observed in all test groups and the positive control. The SI were 3.77, 4.63, 8.82, 8.07, and 19.17 for the 0.2%, 0.5%, 1%, 2%, and 5% dose groups, respectively. The estimated concentrations for a SI of 3 (EC₃) was not calculated. The study concluded that 6-hydroxyindole induced delayed contact hypersensitivity in this LLNA.⁴⁷

Another LLNA was performed with 6-hydroxyindole using the procedures described above.⁴⁸ CBA/J female mice were divided into groups of 8 and received 0.02%, 0.05%, 0.1%, 0.2%, 0.5% or 1% of the test material in acetone/olive oil (4:1, v/v). No clinical signs of toxicity or deaths occurred during the treatment period in any dose group. No cutaneous reaction or increases in ear thickness were observed in the treated animals. Significant dose-related lymphoproliferation was observed at concentrations \geq 0.2% and the positive control. The SI were 0.77, 0.98, 1.26, 6.78, 10.51, and 8.74 in 0.02%, 0.05%, 0.1%, 0.2%, 0.5% and 1% dose groups, respectively. The EC₃ was not calculated. The study concluded that 6-hydroxyindole induced delayed contact hypersensitivity at concentrations \geq 0.2% in this LLNA.

QSAR

ATOPS-MODE quantitative structure-activity relationship (QSAR) model was utilized to predict the sensitization potential of all hair dye ingredients registered in Europe (229 substances as of 2004).⁸ The model predicted 6-hydroxyindole to be a moderate to strong sensitizer.

Phototoxicity

The phototoxicity and photoallergy potential of 6-hydroxyindole in paraffin oil was studied in Dunkin Hartley guinea-pigs.⁴⁹ The study was comprised of 3 phases. In the first phase, groups of 5 (vehicle control irradiated animals and non-irradiated treated animals) or 10 (irradiated treated animals) received 0% or 5% of the 6-hydroxyindole in paraffin oil at a dose volume of 0.2 ml on the anterior scapula. The test sites were irradiated with 0.1 joules/cm² UVB followed by 9 joules/cm² UVA from a Toxicotronic UV lamp (312/365 nm) 30 minutes after application of the test substance. Cutaneous reactions were evaluated 1, 6, and 24 h. Very slight cutaneous reactions were observed in most of the animals. In the second phase, the animals continued to receive dose applications of 5% for days 2-4; however, the concentration was lowered to 0.1% on days 7 and 8. Weak to severe erythema with edema was noted in all treated animals that were irradiated on days 5, 8, and 9 and in treated animals that were not irradiated on day 5. These reactions were considered to be due to poor local tolerance to the test material following repeated exposure. In the final phase, the animals had a 20 day rest period, after which 0% or 5% challenge applications were made of the distal part of the back. The designated groups were then irradiated 30 minutes later at sub-erythral doses of UVA on the right flank or UVB on the left flank. Cutaneous reactions were evaluated at 1, 6, 24, and 48 h. Well-defined to severe erythema was observed at 48 h in half of the treated animals that were irradiated. No reactions were observed in the other groups. It was determined the 5% 6-hydroxyindole in paraffin oil was a photosensitizer, but not phototoxic.

Another dermal phototoxicity and photoallergenicity study following the same procedures was performed using 2% 6-hydroxyindole in paraffin oil in the challenge phase.⁵⁰ Following the single dermal application on day 1, very slight cutaneous reactions were observed in most animals. Weak to severe erythema with edema was noted in all animals that were treated with the test substance (irradiated and non-) on days 8 and 9. Again, these reactions

were considered to be due to poor local tolerance of the test material following repeated exposure. After the challenge phase, no distinct cutaneous reactions were observed in the irradiated controls and in the vehicle-treated and irradiated controls. In the treated, non-irradiated group, weak to well-defined erythema was observed in most of the animals, and in the treated, irradiated group, weak to severe erythema was observed in over half of the animals. This study concluded that 2% 6-hydroxyindole in paraffin oil was not phototoxic or photosensitizing; however, cutaneous reactions due to the poor tolerance to repeated dermal applications may have masked photosensitization reactions.

A third dermal phototoxicity and photoallergenicity study following the same procedures was performed using 2% 6-hydroxyindole in 30% aq. PEG-6.⁵¹ Following the single dermal application on day 1, very slight cutaneous reactions were observed in most animals. Weak to defined erythema with dryness was noted in all animals that were treated with the test substance (irradiated and non-) on days 8 and 9. After the challenge phase, no distinct cutaneous reactions were observed in the irradiated controls and in the vehicle-treated and irradiated controls. In the treated, non-irradiated group, weak erythema was noted in 1 animal at 1 h and 4 h and another animal had erythema at 24h and 48h. In the treated, irradiated group, questionable or weak erythema was observed in 70% of the animals and weak or well-defined erythema was observed in 1 animal at 1h and 4 h. A weak erythema persisted in 2 animals at 24 h and 48 h. This study concluded that 2% 6-hydroxyindole in PEG-6 was not phototoxic or photosensitizing.

CLINICAL USE

Epidemiology

6-Hydroxyindole is an oxidative hair dye ingredient. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A detailed summary of the available hair dye epidemiology data is available at <http://www.cir-safety.org/cir-findings>.

RISK ASSESSMENT

The CIR has calculated the margin of safety for 6-hydroxyindole to be 530. To obtain this value, the systemic exposure dose (SED) of 0.094 mg/kg was determined based on the maximum absorption through the skin ($8.09 \mu\text{g}/\text{cm}^2$ from an in vitro percutaneous absorption study), typical body weight (60 kg) and skin surface area (700 cm^2 for scalp surface area), which was then divided into the NOAEL from an oral rat developmental study (50 mg/kg).

SUMMARY

6-Hydroxyindole is an oxidative hair dye ingredient with 105 uses reported by the FDA. The European Commission has concluded that 6-hydroxyindole would not pose a health risk to the consumer when used as an ingredient in oxidative hair dye formulations as long as the maximum concentration applied to hair does not exceed 0.5%.

Recent concern has been expressed in Europe regarding the potential induction of sensitization that may result from the currently recommended self-test procedure for hair dyes.

A single oral dose of 300 mg/kg radiolabeled 6-hydroxyindole in a rat pharmacokinetics and excretion study determined a mean plasma C_{max} value of $132 \mu\text{g}_{\text{eq}}/\text{ml}$ at 1 h after dosing and the mean systemic exposure of $799 \mu\text{g}_{\text{eq}}\text{-h}/\text{ml}$. The mean total CE of the radioactive dose over the 168-h period was 95.4%, with most of the dose excreted rapidly in the urine. A single dermal dose of 25 mg/kg radiolabeled 6-hydroxyindole in a similar study determined a mean C_{max} value of $282 \pm 137 \text{ ng}_{\text{eq}}/\text{ml}$ at 1-2 h after dosing and the mean systemic exposure of $2809 \text{ ng}_{\text{eq}}\text{-h}/\text{ml}$. The mean total CE of urine, feces, and cage wash over the 168-h period was 5.6% of the radioactive dose. Radioactivity was excreted equally in urine and feces with slow elimination over 96 h. Tissue levels of radioactivity were virtually non-quantifiable.

An in vitro percutaneous absorption study in human skin samples determined the absorbed amount of radiolabeled 6-hydroxyindole was $4.70 \pm 2.08 \mu\text{g}/\text{cm}^2$ of the applied dose when formulated with hydrogen peroxide and $5.86 \pm 2.68 \mu\text{g}/\text{cm}^2$ when formulated with water.

The maximum tolerated dose of 6-hydroxyindole in an acute oral toxicity study in rats was less than 600 mg/kg body weight and the oral LD_{50} was greater than 600 mg/kg body weight. In dermal acute toxicity studies in

rats, the maximal non-lethal dose of 6-hydroxyindole was greater than 2000 mg/kg. The LC₅₀ for 6-hydroxyindole in an acute inhalation study was greater than 2000 mg/m³.

In an oral repeated dose study of 6-hydroxyindole up to 300 mg/kg/day in rats, the NOAEL was 100 mg/kg body weight/day. Female high dose rats had a statistically significant dose-related increase in mean cell hemoglobin and increases in both alanine aminotransferase and aspartate aminotransferase levels in the plasma. Increased absolute and relative liver weights were observed in both sexes in the high dose group and some females in the high dose group had hepatocyte hypertrophy.

The maternal NOAEL was 150 mg/kg body weight and the fetal NOAEL was 50 mg/kg body weight in an oral rat teratology study of 6-hydroxyindole up to 450 mg/kg/day. High dose dams had slightly lower body weight gains and feed intake and increased salivation. Fetuses had reduced ossification in most fetal bones at 150 and 300 mg/kg/day, as well as statistically significantly reduced mean fetal weight at 300 mg/kg/day. In a second oral teratology study in rats, the maternal NOEL was 240 mg/kg body weight/day 6-hydroxyindole, which was the highest dose tested. The fetal NOEL was 60 mg/kg body weight/day 6-hydroxyindole, with delayed general ossification of fetuses occurring at 240 mg/kg/day.

6-Hydroxyindole yielded positive results in a bacterial reverse mutation assay and a chromosome aberration study of human peripheral blood lymphocytes. Results were equivocal in a CHO chromosomal aberration study. 6-Hydroxyindole was not genotoxic in an in vitro mouse lymphoma cell mutation study and in an in vivo bone marrow chromosome aberration test and an in vivo UDS assay.

In a 105 week carcinogenicity study of 6-hydroxyindole in rats, the NOAEL for general toxicity was 25 mg/kg body weight/day and the NOEL for carcinogenicity was 100 mg/kg body weight/day, the highest dose tested. Decreased body weight gains, main red blood cell count, packed cell volume and plasma were observed in the high dose group.

6-Hydroxyindole was not irritating to rabbit skin in a single application study that tested 0.5 g of the substance neat, but it was not well tolerated in another rabbit study where it was applied daily for 2 weeks at a concentration of 1.55%-5%. A 2 week guinea pig study concluded that 5% 6-hydroxyindole was a slight to moderate skin irritant. 6-Hydroxyindole was moderately to severely irritating to the rabbit eye when tested neat, but was not an ocular irritant when tested at 5% in an aqueous 30% PEG-6 solution.

Positive sensitization reactions were observed in guinea pig studies of 6-hydroxyindole at concentrations of 10-20% in paraffin oil, 5-50% in 30% PEG-6, and 5% in PEG-6/water. Two LLNA studies concluded that 6-hydroxyindole induced delayed contact hypersensitivity. A QSAR model predicted 6-hydroxyindole to be a moderate to strong sensitizer.

6-Hydroxyindole was a photosensitizer in paraffin oil at a concentration of 5% when tested in guinea pigs; however it was not a photosensitizer in paraffin oil at a concentration of 2% or in PEG-6 solution at a concentration of 2%.

Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

A margin of safety for 6-hydroxyindole was calculated by CIR to be 530.

DISCUSSION

The CIR Expert Panel was concerned with data indicating that 6-hydroxyindole caused dermal irritation and sensitization, ocular irritation, and photosensitization. Additionally, the available genotoxicity data indicated the possibility of some genotoxic potential of 6-hydroxyindole. However, no ocular irritation was observed in studies of 6-hydroxyindole at 5%, no photosensitization was observed in studies up to 2%, and no carcinogenicity was observed at 100 mg/kg/day. The Panel determined that none of these adverse effects would be likely at the maximum use concentration of 0.5%.

While nitrosamine content of these hair dyes has not been reported, the Expert Panel noted this ingredient is a secondary amine and potentially can be nitrosated. Accordingly, its use should be restricted to hair dye formulations to avoid formation of N-nitroso compounds.

The Expert Panel recognized that 6-hydroxyindole functions as hair dye ingredients and that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant 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 CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer or other toxicologic endpoints, based on lack of strength of the associations and inconsistency of findings.

The CIR Expert Panel noted that the use of oxidative hair dye formulations involves exposure to precursors and coupling agents as well as to their reaction products. While reaction intermediates may be formed, human exposure is to the precursors and coupling agents and to reaction products, not to reaction intermediates. The exposures to the precursors and couplers are low (they are consumed in the color forming reaction), and the exposures to reaction products are even lower (they are adsorbed into the hair shaft itself and physically retained there). Therefore, safety assessments of oxidative hair dyes are driven by the toxicological evaluation of the ingredients (i.e. precursors and coupling agents), more than by the reaction products formed during use, and not at all by reaction intermediates.

CONCLUSION

The CIR Expert Panel concluded 6-hydroxyindole is safe for use in oxidative hair dye formulations.

TABLES AND FIGURES

Table 1. Physical and chemical properties of 6-hydroxyindole.

Property	Value	Reference
Physical Form	Light-grey powder	¹¹
Molecular Weight g/mol	133.15	¹¹
log P @ 23 °C	1.46	¹¹
Solubility in water at 30 °C (% w/v)	1.5	¹¹
Solubility in ethanol and DMSO (%)	≥ 20	¹¹
Purity titer as determined by HPLC (%)	97.4-99.5	¹¹
Water content (% w/w)	0.05	¹¹
Ash content (%)	< 0.1	¹¹
Heavy metals (ppm)	< 10	¹¹
Chloride ions (% w/w)	< 0.05	¹¹

References

1. Finefield JM and Williams RM. Synthesis of notoamide J: A potentially pivotal intermediate in the biosynthesis of several prenylated indole alkaloids. *J Org Chem.* 2000;75:2785-2789.
2. Personal Care Products Council. 9-20-2012. Confirmation on the method of manufacture of 6-Hydroxyindole. Unpublished data submitted by Personal Care Products Council. 1 pages.
3. Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP). Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning 6-Hydroxyindole. Colipa No. A128. 12-9-2003.
http://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out244_en.pdf. Date Accessed 7-10-2012. Report No. SCCNFP/0667/03.
4. Tiedink HGM, Davies JAR, Visser NA, Jongen WMF, and van Broekhoven LW. The stability of the nitrosated products of indole, indole-3-acetonitrile, indole-3-carbinol and 4-chloroindole. *Fd Chem Toxic.* 1989;27(11):723-730.
5. Shank RC and Magee PN. Toxicity and carcinogenicity of N-nitroso compounds. Chapter: 1. Shank, R. C. In: *Mycotoxins and N-Nitroso Compounds: Environmental Risks*. Boca Raton, FL: CRC Press, Inc.; 1981:185-217.
6. Rostkowska K, Zwierz K, Rozanski A, Moniuszko-Jakoniuk J, and Roszczenko A. Formation and metabolism of N-nitrosamines. *Polish Journal of Environmental Studies.* 1998;7(6):321-325.
7. Challis BC, Shuker DE, Fine DH, Goff EU, and Hoffman GA. Amine nitration and nitrosation by gaseous nitrogen dioxide. *IARC Sci Publ.* 1982;41:11-20.
8. Sosted H, Basketter A, Estrada E, Johansen JD, and Patlewicz GY. Ranking of hair dye substances according to predicted sensitization potency: Quantitative structure-activity relationships. *Contact Dermatitis.* 2004;51:241-254.
9. Napolitano A, d'Ischia M, and Prota G. Oxidation of 4-, 6- and 7-hydroxyindoles. *Tetrahedron.* 1989;45(21):6749-6760.
10. Brown KC, Marlowe E, Prota G, and Wenke G. A novel natural-based hair coloring process. *J Soc Cosmet Chem.* 1997;48:133-140.
11. Scientific Committee on Consumer Products (SCCP). Opinion on 6-Hydroxyindole. Colipa No. A128. European Commission Health & Consumer Protection Directorate-General . 2006.
http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_046.pdf. Date Accessed 6-27-2012. Report No. SCCP/0947/05.
12. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2012. Washington, DC: FDA.
13. Personal Care Products Council. 2012. Concentration of Use by FDA Product Category: 6-Hydroxyindole. Unpublished data submitted by the Personal Care Products Council.
14. European Union. 1976, Council Directive 1976/768/EEC of 27 July 1976 on the Approximation of the Laws of the Member States Relating to Cosmetic Products, as amended through Commission Directive 2012/21/EU. 2012. <http://eur->

lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:208:0008:0016:en:PDF. Internet site accessed August 8, 2012.

15. Thyssen JP, Sosted H, Uter W, Schnuch A, Gimenez-Arnau AM, Vigan M, Rustemeyer T, Granum B, McFadden J, White JM, White IR, Goossens A, Menne T, Liden C, and Johansen JD. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact Dermatitis*. 2012;66:300-311.
16. Goossens A. Self-testing for contact sensitization to hair dyes. *Contact Dermatitis*. 2012;66:299-299.
17. Appleqvist T. 1999. Pharmacokinetics Study in Plasma, Excretion Balance and Tissue Distribution after Single Administration by Oral Route (Gavage) to Rats. CIT Study No. 16435 PAR. Unpublished data submitted by the Personal Care Products Council.
18. Anonymous. 2001. In Vitro Percutaneous Absorption of 6-Hydroxyindole. Study No. 16100. Unpublished data submitted by the Personal Care Products Council.
19. Anonymous. 2004. Amendment to In Vitro Percutaneous Absorption of 6-Hydroxyindole. Study No. 16100. Unpublished data submitted by the Personal Care Products Council.
20. Appleqvist T. 2000. Pharmacokinetics Study in Plasma, Excretion Balance and Tissue Distribution after Single Administration by Cutaneous Route to Rats. CIT Study No. 15585 PAR. Unpublished data submitted by Personal Care Products Council. 28 pages.
21. Hill RE. 1994. Acute Oral Toxicity Study in the Rat (MTD Determination and Limit Test). Toxicol Laboratories Limited Study No. LRL/69/94. Unpublished data submitted by Personal Care Products Council. 10 pages.
22. Brownlie SA. 1994. Acute Dermal Toxicity Study in the Rat (Limit Test). Toxicol Laboratories Limited Study No. LRL/83/94. Unpublished data submitted by Personal Care Products Council. 15 pages.
23. Manciaux X. 2001. Acute Dermal Toxicity Study in Rats. CIT Study No. 21176 TAR. Unpublished data submitted by Personal Care Products Council. 19 pages.
24. Schorsch F. 1998. Study of the Acute Toxicity of 6-Hydroxyindole by Inhalation in the Rat. INERIS-LETE Study No. 97146. Unpublished data submitted by Personal Care Products Council. 18 pages.
25. Brownlie SA. 1994. 13 Week Oral (Gavage) Toxicity Study in the Rat. Toxicol Laboratories Limited Study No. LRL/45/94. Unpublished data submitted by the Personal Care Products Council.
26. Chandler L. 1994. 6-Hydroxyindole Oral (Gavage) Rat Developmental Toxicity Study. Toxicol Laboratories Limited Study No. LRL/46/93. Unpublished data submitted by the Personal Care Products Council.
27. Foulon O. 2004. Prenatal Developmental Toxicity Study by Oral Route (Gavage) in Rats. CIT Study No. 26904 RSR. Unpublished data submitted by the Personal Care Products Council.
28. Kelly MD. 1994. Bacterial Reverse Mutation Test Using 5 Salmonella Strains and 1 E. Coli. Toxicol Laboratories Limited Study No. M/Ames/38798. Unpublished data submitted by the Personal Care Products Council.
29. Kelly MD. 1994. 6-Hydroxyindole. In Vitro Mammalian Cell Gene Mutation Tests: Point Mutation in Mouse Lymphoma (L5178Y) Cells. Toxicol Laboratories Limited Study No. M/PML/39194. Unpublished data submitted by the Personal Care Products Council.

30. Kelly MD. 1994. 6-Hydroxyindole Metaphase Analysis of Chinese Hamster Ovary (CHO) Cells. Toxicol Laboratories Limited Study No. M/CCA/38799. Unpublished data submitted by the Personal Care Products Council.
31. Riley SE. 1994. 6-Hydroxyindole (Batch No. Pil 10): Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes. Molecular Toxicology (Microtest) Hazelton Europe Study No. 413/12. Unpublished data submitted by the Personal Care Products Council.
32. Jouffrey S de. 1996. In Vivo Mammalian Bone Marrow Cytogenetic Test in Rats Chromosomal Analysis. CIT Study No. 13718 MOR. Unpublished data submitted by the Personal Care Products Council.
33. Kelly MD. 1994. 6-Hydroxyindole Mouse Micronucleus Test. Toxicol Laboratories Limited Study No. M/MMN/38797. Unpublished data submitted by the Personal Care Products Council.
34. Jouffrey S de. 1996. Micronucleus Test by Oral Route in Rats. CIT Study No. 13860 MAR. Unpublished data submitted by the Personal Care Products Council.
35. Fautz R. 1994. In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Hepatocytes with 6-Hydroxyindole. Cytotest Cell Research (RCC group) Project No. 473600. Unpublished data submitted by the Personal Care Products Council.
36. Chevalier G. 2003. Carcinogenicity Study by Oral Administration (Gavage) to Rats. CIT Study No. 16461 TCR. Unpublished data submitted by the Personal Care Products Council.
37. Chubb DR. 1993. Acute Dermal Irritation Study in the Rabbit. Toxicol Laboratories Limited Study No. A/S/38794. Unpublished data submitted by Personal Care Products Council. 9 pages.
38. Jouffrey S de. 1997. Local Tolerance Study after Repeated Application for 2 Weeks in Rabbits. CIT Study No. 15543 TSL. Unpublished data submitted by Personal Care Products Council. 15 pages.
39. Manciaux X. 2001. Local Tolerance Study after Repeated Application for 2 Weeks in Guinea-Pigs. CIT Study No. 21092 TSG. Unpublished data submitted by Personal Care Products Council. 3 pages.
40. Chubb DR. 1993. Acute Eye Irritation Study in the Rabbit (6-Hydroxyindole). Toxicol Laboratories Limited Study No. A/E/38792. Unpublished data submitted by the Personal Care Products Council.
41. Chubb DR. 1994. Acute Eye Initiation Study in the Rabbit (6-Hydroxyindole 5% in solution). Toxicol Laboratories Limited Study No. A/FJ40290. Unpublished data submitted by Personal Care Products Council. 16 pages.
42. Jouffrey S de. 1997. Local Tolerance Study after Repeated Application for 2 Weeks in Guinea-Pigs, followed by one Topical Challenge Application for Skin Sensitization. CIT Study No. 14936 TSG. Unpublished data submitted by Personal Care Products Council. 20 pages.
43. Jouffrey S de. 1995. Skin Sensitization Test in Guinea-Pigs (Buehler Test: 3 Applications). CIT Study No. 12386 TSG. Unpublished data submitted by the Personal Care Products Council.
44. Manciaux X. 2001. Skin Sensitization Test in Guinea-Pigs (Buehler Test: 3 Applications). CIT Study No. 21175 TSG. Unpublished data submitted by the Personal Care Products Council.
45. Manciaux X. 2001. Skin Sensitization Test in Guinea-Pigs (Buehler Test: 3 Applications). CIT Study No. 21545 TSG. Unpublished data submitted by the Personal Care Products Council.
46. Clouzeau J. 1991. Skin Sensitization Test in Guinea Pigs. CIT Study No. 7004 TSG. Unpublished data submitted by the Personal Care Products Council.

47. Manciaux X. 2001. Evaluation of Skin Sensitization Potential in Mice Using the Local Lymph Node Assay (LLNA). CIT Study No. 22031 TSS. Unpublished data submitted by the Personal Care Products Council.
48. Manciaux X. 2002. Evaluation of Skin Sensitization Potential in Mice Using the Local Lymph Node Assay (LLNA). CIT Study No. 22836 TSS. Unpublished data submitted by the Personal Care Products Council.
49. Jouffrey S de. 1997. Phototoxic and Photoallergenic Potential by Dermal Route in Guinea-Pigs. CIT Study No. 14935 TSG. Unpublished data submitted by the Personal Care Products Council.
50. Jouffrey S de. 1997. Phototoxic and Photoallergenic Potential by Dermal Route in Guinea-Pigs on the Test Substance Diluted at 2%. CIT Study No. 15545 TSG. Unpublished data submitted by the Personal Care Products Council.
51. Manciaux X. 2002. Phototoxic and Photoallergenic Potential by Cutaneous Route in Guinea-Pigs. CIT Study No. 21174 TSG. Unpublished data submitted by the Personal Care Products Council.