AIHA® GUIDELINE FOUNDATION | Occupational Exposure Limits

Benzenesulfonic Acid, 5-Chloro-2-((2-Hydroxy-1-Naphthalenyl)-Azo)-4-Methyl, Barium Salt (2:1)

Document History
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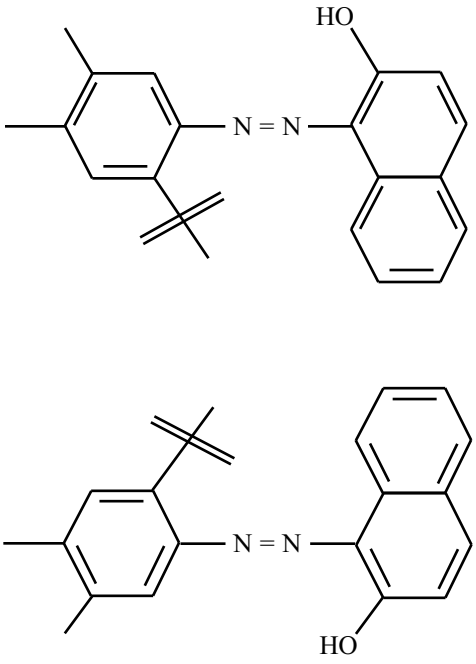
I. IDENTIFICATION

Chemical Name: Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1)
Synonyms: D&C Red No. 9 (when purity ≥87%)
C.I. Pigment Red 53:1 (no minimum purity standards)
Red Lake C
5-Chloro-2-[(2-hydroxy-1-naphthyl)azo]-p-toluenesulfonic acid, barium salt
1-(4-Chloro-o-sulfo-5-tolyazo)-2-naphthol, barium salt
CAS Number: 5160-02-1
UN Number: Not Applicable
Chemical Family: Barium salt of monoazo dye; aromatic amine
Molecular Formula: $(C_{17}H_{13}ClN_2O_4S)_2 \cdot Ba$
Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES[1–4]

Physical State and Appearance: Red to orange-red crystalline powder
Odor Description: Not available
Molecular Weight: 888.9
Conversion Factors: Not applicable
Melting Point:
a) 343–345°C (649–53°F); decomposes
b) 356-392°C (673-738°F), decomposes
c) Decomposes below melting point of 330°C (626°F)
Boiling Point: Not applicable
Autoignition Temperature: >340°C (>644°F)
Specific Gravity: 1.66 g/cm³ (temperature not reported)
Solubility in Water: Practically insoluble (0.002–0.1 mg/mL at 20°C [71°F])
Other Solubility: Insoluble in toluene, ethyl ether, and butyl acetate; practically insoluble in glycerol, petroleum, stearic acid, oleic acid, mineral oil, and mineral wax; insoluble to very sparingly soluble in acetone, methanol, and ethanol.
Stability: No data for compound alone. When mixed with dietary feed, stable for two weeks at up to 45°C (113°F)
Octanol-water partition coefficient (log P_{ow}) = -0.49

III. USES

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as C.I. Pigment Red 53:1, is primarily used as a colorant in printing inks, as well as for rubber, plastics, artists' crayons, baking enamels, stoving finishes, and for coating paper. It has also been used as a tracer to monitor the diffusion of air pollutants from point source emissions. As D&C Red No. 9, it is a banned substance in the United States and the European Union, but is approved for use as a colorant for cosmetics in Japan (when appropriately labeled as a barium salt).[1,5]

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity and Irritancy

1. Oral Toxicity

Rat LD_{50} 400–3200 mg/kg[1]

Rat LD_{50} >10,000 mg/kg[5]

Mouse LD_{50} >10,000 mg/kg[5]

No signs of toxicity were seen in F344 rats and $B_6C_3F_1$ mice fed benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, for 24 hours, at dietary concentrations up to 100,000 ppm (doses expressed in mg/kg were not reported).[4]

2. Eye Irritation

Not irritating to the rabbit eye (method not described).[5]

3. Skin Absorption

No information availble.

4. Skin Irritation

Not irritating to the rabbit skin (method not described).[5]

5. Skin Sensitization:

It was not sensitizing in the Guinea Pig Maximization Test.[5] An earlier report of contact sensitization associated with exposure to benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9[6], is thought to have been caused by impurities in the test substance, and not from exposure to the pure dye.[5]

6. Inhalation Toxicity

No information available.

B. Subacute Toxicity

There were no deaths observed when F344 rats were treated with benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily for two weeks, at dietary concentrations up to 100,000 ppm (equivalent to 5400 mg/kg/day). However, 4/5 and 3/5 deaths occurred in male and female B6C3F1 mice, respectively, fed a diet containing ≥25,000 ppm (approx. 4300 mg/kg/day) for two weeks. Gross examination of all treated animals revealed dark red coloration and enlargement of the spleen, as well as dark red to reddish-tan discoloration of the liver and kidneys. No histopathological examinations were performed.[4]

C. Subchronic Toxicity

Groups of F344 rats (10 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 90 days, at doses of 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm (equivalent to 0, 163, 325, 678, 1357, and 2714 mg/kg/day). One 6,000-ppm male and one 3,000-ppm female died on study. Spleens of all treated animals were darkened and enlarged to 2–5 times their usual size; splenic congestion and lymphoreticular hyperplasia were seen in all treated females and in all males dosed at ≥6,000 ppm (80% at 3,000 ppm). Pigment deposition in the renal tubular epithelium was also seen at all doses. Lymphoreticular hyperplasia of the thymic lymph nodes occurred in 75–100% of all treated females except for those in the low-dose group, in which the incidence was 0%. This finding was also seen in 70-100% of treated males except for those in the high-dose group, in which the incidence was 43%. Hemosiderosis of the liver was seen in all treated females and occurred in a dose-dependent manner in males between 3,000 ppm (30%) and 12,500 ppm (90%). No NOAEL was identified in this study; the LOAEL was 3,000 ppm in diet (163 mg/kg/day).[4]

Groups of B6C3F1 mice (10 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 90 days, at doses of 0, 600, 1,250, 2,500, 5,000, or 10,000 ppm (equivalent to 0, 103, 214, 429, 857, or 1714 mg/kg/day). No deaths were observed at any dose. Splenic congestion was seen in 92% (55 of 60) animals treated at ≥2,500 ppm. Hemosiderosis (tissues not specified, but assumed to be of the liver) was seen in all females at ≥2,500 ppm and in males at ≥1,250 ppm. No other findings were reported. NOAELs in this study were identified as 600 and 1,250 ppm in diet (103 and 214 mg/kg/day) in male and female rats, respectively.[4]

Groups of Osborne-Mendel rats (5 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 20 weeks, at doses of 0, 2,500, 5,000, 10,000, or 20,000 ppm (approx. 0, 136, 272, 543, or 1086 mg/kg/day). No mortality was observed. Treat-

* Unless otherwise indicated, ppm to mg/kg/day equivalences were estimated using standard United States Environmental Protection Agency (USEPA) reference tables.

ment-related findings consisted of decreased hemoglobin and hematocrit (doses not specified), splenomegaly at all doses, and liver enlargement at 5,000 and 10,000 ppm only. No NOAEL was identified in this study; the LOAEL was 2,500 ppm in diet (136 mg/kg/day).[5]

D. Chronic Toxicity/Carcinogenicity

Groups of Osborne-Mendel rats (25 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for two years, at doses of 0, 0.01, 0.05, 0.25, or 1% (reported as approximately 0, 5, 25, 125, and 500 mg/kg/day). There were no deaths at any dose level. A slight decrease in hemoglobin levels along with abnormal (not otherwise described) shape of red blood cells were seen at 0.25%. Slight and moderate splenomegaly was also seen at 0.25 and 1%/day, respectively, and slight bone marrow hyperplasia was seen at both doses. At 1%, splenic hemosiderosis and splenic infarcts (seen in some animals only) were also observed. No increased tumor incidence was seen; however, only a small number of animals were histopathologically examined. The NOAEL for target organ toxicity in this study was identified as 0.05% (5 mg/kg/day).[5,7]

Groups of B6C3F1 mice (50 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 103 weeks (with a 1-week recovery period), at doses of 0, 1,000, or 2,000 ppm (calculated as approximately 0, 222, and 448, or 0, 248, or 473 mg/kg/day, in males and females, respectively, based on actual body weight and food consumption data). No effects on body weight, food consumption, clinical signs of toxicity, or survival rate were observed. While an increased incidence of undifferentiated sarcomas, generally in the subcutaneous tissue of skin of the back, was seen in low-dose males, this type of tumor is not uncommon in male mice, and, in addition, was not seen in high-dose males. Hepatocellular carcinomas were also seen in some male animals from all treatment groups (incidence 4/50, 9/50, and 11/50, respectively by dose group). Though the incidence was increased relative to this particular control group, it was not significantly different from the historical record at this laboratory for male mice. The study authors concluded that there was no increased tumor incidence in male mice that could be unequivocally linked to treatment. No increased incidence of any tumor type was seen in females. The NOAEL for carcinogenicity in this study was identified as 2,000 ppm (473 mg/kg/day) in females, whereas no NOAEL was definitively identified in males (LOAEL = 1,000 ppm, or 66 mg/kg/day).[4]

Groups of F344 rats (50 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 103 weeks (with a 1-week recovery period), at doses of 0, 1,000, or 3,000 ppm (calculated as approximately 0, 51 or 152 mg/kg/day, and 0, 66, or 193 mg/kg/day, in males and females, respectively, based on actual body weight and food consumption data). With the exception of increased survival rate seen in low-dose males only, no effects on body weight, food consumption, clinical signs of toxicity, or survival rate were observed. Non-neoplastic lesions (including congestion of the splenic parenchyma, focal/ multifocal or diffuse fibrosis occasionally with large pigmented areas, and areas of fatty metamorphosis) were seen in high-dose males and females. There was a statistically significant increase in splenic fibrosarcomas in high-dose males (incidence 0/50, 0/50, and 26/48), as well as neoplastic liver nodules in males at both doses (incidence 0/50, 6/50, and 7/49, respectively by dose group), with a statistically significant trend ($p < 0.05$) toward increased neoplastic liver nodules in high-dose females (incidence 1/50, 1/50, and 5/50, respectively by dose group). There were no other observed increases in any other tumor type, in either sex. The NOAEL for carcinogenicity in this study was identified as 1,000 ppm (50 mg/kg/day) in females, while no NOAEL was identified in males (LOAEL = 1,000 ppm, or 66 mg/kg/day).[4]

As a follow-up to the study described above, groups of F344 rats (50 per sex) were administered benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, daily in the diet for 104 weeks at doses of 0, 1,000, or 3,000 ppm (equivalent to 0, 54, or 163 mg/kg/day). No effects on body weight gain, clinical signs, or changes in survival rates were observed. Splenomegaly and splenic lesions (fatty metamorphosis, splenic fibrosis, and capsule hyperplasia) were seen at 3,000 ppm in both males and females, as well as dose-dependent splenic hemorrhage in all treatment groups. Fibrosarcomas and neoplastic nodules of the liver were seen in high-dose male rats only. No NOAELs were identified in this study (LOAEL for both sexes was 1,000 ppm, or 66 mg/kg/day). The authors concluded that the non-neoplastic splenic lesions preceded the induction of the splenic sarcomas, and were precursors to

the neoplastic process. Additionally, they speculated that the splenic effects might have resulted from N-oxidation of the dye to form a mutagenic N-hydroxylamine metabolite.[8,9] More recent studies have also shown that a metabolite or metabolites of benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) is responsible for the genotoxic/carcinogenic properties of the dye.[10,11]

No increased incidence over control animals of skin or other tumors was seen when 1 mg benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, was applied in 0.1 mL water to the clipped back skin (approximately 6 cm^2) of ICR (Swiss Webster-derived) mice, 50 per sex, twice weekly for 18 months.[3,5,12]

E. Reproductive/Developmental Toxicity

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, was administered to 35-day-old Charles River CD rats in the diet for 30 months, at daily doses of 0 or 10,000 ppm (equivalent to 0 or 543 mg/kg/day). After nine weeks of dosing, animals were mated by pairing for 7 days. Although there was general toxicity manifested by decreased red blood cell parameters, increased reticulocytes, and effects on the spleen (including increased weight, hemosiderosis, congestion, and fibrosis), no adverse effects on fertility, gestation length, or fetus/pup viability (from start of gestation through Day 21 of lactation) were observed. The NOAEL for reproductive toxicity was identified as >10,000 ppm (>543 mg/kg/day), while the NOAEL for general toxicity was identified as <10,000 ppm. A NOAEL of >10,000 ppm was also reported for reproductive toxicity in F1 offspring; however, no additional information was provided.[5]

No evidence of teratogenicity in was seen in oral gavage studies of benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, with rats and rabbits. Doses used in these studies (conducted as part of studies with 25 food coloring agents) were reportedly based on "the highest NOEL [unspecified] identified for rats and dogs in previous two-year feeding studies."[13]

No evidence of reproductive toxicity was seen through the F2B litter in a dietary multi-generation rat study of benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9. Doses used in this study (conducted as part of studies with 25 food coloring agents) were based on "multiples of the ADI [unspecified] or of the projected safe dose determined from data of previous long-term feeding studies in rats and dogs…no doses in excess of 1,000 mg/kg/day were used." Based on the study results, the NOAEL for reproductive toxicity was reported as >1,000 mg/kg/day.[14]

F. Genotoxicity/Mutagenicity

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) was not mutagenic in the Ames bacterial mutagenicity assay (*S. typhimurium* strains TA98 and TA100) in the presence of metabolic activation only.[1,5] A negative result in the Ames assay was also observed in studies with lipsticks containing Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9.[15,16]

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) was not mutagenic when tested in both the *in vitro* CHO/HGPRT assay and in cultured mouse V79 lung fibroblasts.[1,5]

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, was not genotoxic in the Mouse Lymphoma assay, and when tested for sister-chromatid exchanges in CHO cells or for unscheduled DNA synthesis (UDS) in rat hepatocytes.[1,3,5]

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, did not cause chromosomal aberrations or SCEs tested up to toxic concentrations, with or without metabolic activation, in cultured CHO-WBL cells.[3,17]

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D & C Red No. 9, was not genotoxic when tested in in vivo rat and mouse micronucleus and rat UDS assays.[3,5,18]

Although the dye itself has not been shown to have genotoxic properties, 1-amino-2-naphthol, a metabolite of benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), was mutagenic in the TA100 strain of *S. typhimurium*, and weakly mutagenic in the TA98 strain, when tested in the Ames bacterial mutagenicity assay.[10,11]

G. Metabolism/Pharmacokinetics

Most water-soluble azo dyes are readily reduced by intestinal microflora to their corresponding amines.[1] Even though it is water-insoluble,

benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) also undergoes reduction of the sulfonic acid group to yield 1-amino-2-naphthol; this cleavage takes place in the gut by caecal bacteria or, in certain species, under other anaerobic condi- tions.[11,19] Evidence has also demonstrated that reductase enzymes in the liver are capable of metabolizing water-insoluble azo dyes.[20,21]

No other information on the metabolism and pharmacokinetics of benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) was available. However, D&C Orange No. 4 (CAS No. 633-96-5), which is structurally similar to benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), undergoes reductive cleavage of the sulfonic acid group to 1-amino-2-naphthol. It is speculated that D&C Orange No. 4 is absorbed unchanged from the gut and returned to the intestine through the bile as water-soluble conjugates, which can undergo reductive cleavage. Following reduction, the metabolites are then absorbed from the gut, further metabolized, and excreted in the urine.[1,20] Additionally, studies with rats and rabbits have demonstrated that the naphthalene moiety of Orange II may be metabolized to 1-amino-2-naphthylsulfate and 1-amino-2-naphthylglucosiduronic acid, and excreted as sulfanilic acid and p-acetamidobenzenesulfonic acid.(22) A similar pharmacokinetics profile might be predicted for benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1).

V. HUMAN USE AND EXPERIENCE

a. Workplace Exposure

No information available.

b. Other Exposure Data

According to NIOSH, occupational exposures to benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) occur primarily in paper coating/glazing and commercial printing. Although exposure levels were not reported, airborne workplace concentrations in 1982 were predicted to be low due to the non-volatility of the dye.[1]

Between 1979 and 1988, benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), as D&C Red No. 9, was provisionally approved in the U.S. for use in drugs and in externally applied cosmetic and hygiene products. The maximum amount of dye permitted in externally applied drugs and skin cosmetics, lipsticks/other lip cosmetics, mouthwashes, and toothpastes was 6.0, 3.0, 0.005, and 0.002% by weight, respectively. Use of this dye in drugs for ingestion was also approved, at a maximum level of 0.1 mg of pure dye reasonably expected to be ingested in one day. Direct consumer use was estimated to result in a maximum ("worst-case") possible ingestion exposure of 0.2 mg/day, if an individual were to have continuously used drugs and cosmetic products containing this dye. No Acceptable Daily Intake (ADI) was determined/reported.[1,4,5,23]

There are no known reports of any adverse effects in humans who were exposed to benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1), either as D&C Red No. 9 or as C.I. Pigment Red 53:1.

VI. RATIONALE

Benzenesulfonic acid, 5-chloro-2-((2-hydroxy-1-naphthalenyl)-azo)-4-methyl, barium salt (2:1) has a relatively low order of toxicity following acute administration, and no evidence for reproductive, developmental, or definitive genotoxic potential has been observed. Although repeat-dose studies with several animal species have demonstrated a potential for splenic toxicity, and lifetime administration of low doses to male rats caused both non-neoplastic and neoplastic lesions of the spleen and liver (along with liver carcinomas in male mice that could not be unequivocally linked to treatment), there are no known reports of any adverse effects in humans resulting from exposure to this dye; consequently, the relevance of this finding to humans is unclear. It has been suggested that the carcinogenic potential of this dye in animals is attributable to a weakly mutagenic metabolite, despite generally negative genotoxicity results in studies with the parent compound itself.

Non-neoplastic and neoplastic lesions of the spleen and liver, seen in two 2-year rat studies, were chosen as the basis for the OEL. From these studies, a LOAEL of 1,000 ppm in diet (approximately 50 mg/kg/day) and a NOAEL of 0.05% in diet (approximately 5 mg/kg/day) was identified. Extrapolation based on body weight gives an equivalent dose of 2700 mg/day for a 55-kg person, which is achieved after exposure to an airborne concentration of approximately 340 mg/m^3 (if one assumes a respiratory volume of 8 m3 per 8-hour workday for a 55-kg individual). Based on these data, an OEL of 1 mg/m^3 is expected to provide a reasonable margin of safety against possible adverse effects in humans. This value is consistent with one derived by extrapolation to a "benchmark dose," according to standard methodology adopted by the USEPA. (Crump, 2002)

VII. RECOMMENDED OEL

8-Hour time-weighted average: 1 mg/m^3 (as the barium salt).

VII. REFERENCES

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