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# Revised Test Plan for
## 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt

## CAS No. 25956-17-6

## Consortium Registration Number

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**Submitted to the EPA under the HPV Challenge Program by:**

**The International Association of Color Manufacturers/HPV Committee**

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# List of Member Companies

**Colorcon**

**Noveon, Inc.**

**Sensient Colors, Inc.**

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# Test Plan for
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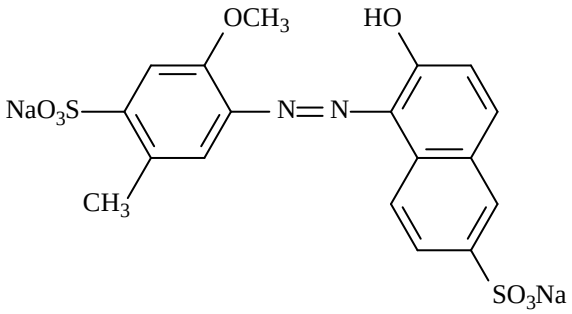
## 1 IDENTITY OF SUBSTANCES




**2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt**

**CAS No. 25956-17-6**

**Synonyms:**
FD&C Red No. 40
Allura Red
CI Food Red 17

# 2 CATEGORY ANALYSIS

## 2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries presented represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

## 2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for FD&C Red No. 40. FD&C Red No. 40 is a red powder that is soluble in water and is used to color gelatins, puddings, custards, alcoholic and nonalcoholic beverages, sauces, topping, candy, sugars, frostings, fruits, juices, dairy products, bakery products, jams, jellies, condiments, meat and poultry. FD&C Red No. 40 is also used to color drugs and cosmetics.

FD&C Red No. 40 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility.

## 2.3 REGULATORY STATUS

FD&C Red No. 40 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

**Table 1. US FDA Specifications**

FD&C Red No. 40 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.340):

- Sum of volatile matter (at 135° C) and chlorides and sulfates (calculated as sodium salts), not more than 14.0 percent.
    - Water-insoluble matter, not more than 0.2 percent.
    - Higher sulfonated subsidiary colors (as sodium salts), not more than 1.0 percent.
    - Lower sulfonated subsidiary colors (as sodium salts), not more than 1.0 percent.
- Disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenoxy)-2-naphthalenesulfonic acid, not more than 1.0 percent.
- Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid (Schaeffer's salt), not more than 0.3 percent.
    - 4-Amino-5-methoxy-o-toluenesulfonic acid, not more than 0.2 percent.
- Disodium salt of 6,6'-oxybis (2-naphthalene-sulfonic acid), not more than 10 parts per million.
    - Lead (as Pb), not more than 10 parts per million.
    - Arsenic (as As), not more than 3 parts per million.
    - Total color, not less than 85.0 percent.

FD&C Red No. 40 was first listed for food use in the United States in 1971. In 1994, 1,477,651 kg of FD&C Red No. 40 dye and 257,752 kg of FD&C Red No. 40 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) has also evaluated the safety of FD&C Red No. 40 used as a coloring agent in food. An average daily intake (ADI) of 0-7 mg/kg bw/d was assigned by JECFA in 1981 based on the extensive human toxicological information available that indicated FD&C Red No. 40 did not possess carcinogenic potential (see Table 2 below).

**Table 2. Regulatory Approvals/Consumption Limits[1]**

| | |
|---|---|
| USA | GMP (21 CFR 74.340) |
| EEC | GMP (EC Journal No. L237; 1994) |
| JECFA | ADI of 0-7 mg/kg body weight (25th Report, 1981) |

Based on the long history of use of FD&C Red No. 40 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Red No. 40, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical "Right to Know" Program.

## 2.4 STRUCTURAL CLASSIFICATION

FD&C Red No. 40 is principally the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4sulfophenyl) azo]-2-naphthalenesulfonic acid (US FDA-21 CFR 74.340). FD& C Red No. 40 is a monoazo dye. The diazo nucleus (-N=N-) contains a benzene ring

[1] IACM, 2003

substituted with *o*-methoxy, *m*-methyl, and *p*-sulfonic acid groups and a naphthalene ring substituted with *o*-hydroxy and *p*'-sulfonic acid groups.

## 2.5 INDUSTRIAL PRODUCTION

FD&C Red No. 40 is manufactured by coupling diazotized 5-amino-4-methoxy-2-toluenesulfonic acid with 6-hydroxy-2-naphthalene sulfonic acid.

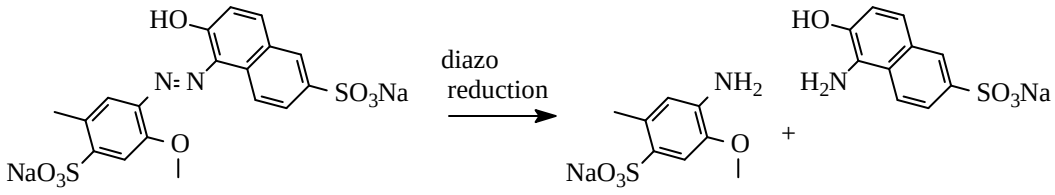
## *2.6* PHARMACOKINETICS AND METABOLISM

Orally administered FD&C Red No. 40 is poorly absorbed by dogs and rats. Dogs (2) were pretreated with unlabeled 100 mg/kg bw/d FD&C Red No. 40 in capsules for five days and with 100 mg/kg bw/d $^{35}$S radiolabelled FD&C Red No. 40 on the sixth day. The animals were studied for up to 72 hours following the last dose. After 72 hours, no significant radioactivity could be detected in any of the organs and tissues assayed. The major excretory route was through the feces. Approximately 85% of the administered dose (75 and 95% in each) was excreted in the feces within 24 hours. Minor amounts (1.1 and 0.3%) were excreted during the following 24-48 hour collection period. The urinary excretion of FD&C Red No. 40 was minimal with an average recovery of 3% in dogs. The total recovery of radioactivity from all tissues assayed was insignificant, with the exception of the intestine and its contents [Hazelton Laboratories, 1975a].

Five male rats were pretreated with unlabeled 200 mg/kg bw/d FD&C Red No. 40 *via* gavage for five days and with 200 mg/kg bw/d $^{35}$S radiolabelled FD&C Red No. 40 on the sixth day. Two controls received the vehicle only. The animals were studied for up to 72 hours following the last dose. Similar to dogs, the majority of the radioactivity was excreted in the feces (76-92% in 72 hours) with minimal amounts excreted in the urine (approximately 8%[2]) as the parent substance or its metabolites [Hazelton Laboratories, 1975a].

[2] 19.8% of the radioactivity administered was excreted in the urine in one test animal, but given the value was nearly double that of any other animal, and the fact that lung damage was observed at necropsy, the authors reported the animals was improperly dosed and that the value was excessive.

In a separate follow-up study in both rats and dogs using the protocol described above, the principal urinary metabolite in both species was determined to be 4-amino-5-methoxy-o-toluene sulfonic acid (cresidine sulphonic acid), indicating diazo reduction and cleavage to yield two amine fragments. Cresidine sulphonic acid was also the principal urinary metabolite (greater than 90%). However, total urinary metabolites only accounted for approximately 7% of the total administered dose. Analysis of the rat-fecal extracts revealed an unknown metabolite (35.4%); unchanged FD&C Red No. 40 at (14.9%); and cresidine sulphonic acid (25.6%). The formation of cresidine sulfonic acid in the feces may arise *via* microfloral reduction of the parent dye. A similar metabolic pattern was reported following the analysis of the dog-fecal extract. The authors commented that the polar unknown metabolite is apparently a glucuronide or sulfate conjugate, possibly formed from one of the two diazo reduction products, 1-amino-2-naphthol-6-sulfonic acid. The polar unknown is not absorbed, given that only 0.6% of the dose was found at the origin following thin layer chromatography analysis of the rat urine [Hazleton Laboratories, 1975b].




Red No.40

cresidine sulphonic acid

1-amino-2-naphthol-6-sulfonic acid

In summary, FD&C Red No. 40 is predominantly not absorbed by animals. It is excreted mainly in the feces either unchanged or as polar metabolites (*e.g.*, cresidine sulfonic acid) formed by diazo reduction FD&C Red No. 40. Fecal excretion occurs predominantly within the first 24 hours. Small amounts of cresidine sulfonic acid are also detected in the urine.

# 3 TEST PLAN

## 3.1 CHEMICAL AND PHYSICAL PROPERTIES

### 3.1.1 Melting Point

FD&C Red No. 40 is a solid and did not melt when heated to 300 °C [Hazelton Laboratories, 1970]. Accordingly, the melting point of FD&C Red No. 40 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000].

### 3.1.2 Boiling Point

The boiling point of FD&C Red No. 40 was calculated to be 872 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

### 3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Red No. 40 has been reported to be $1.25 \times 10^{-23}$ mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Red No. 40 (496.43) and the estimated Henry's law constant for azo dyes of $10^{-15}$ atm-$m^3$/mol it is highly unlikely that FD&C Red No. 40 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than $1 \times 10^{-20}$ mm Hg.

### 3.1.4 Octanol/Water Partition Coefficients

Log $K_{OW}$ value for FD&C Red No. 40 is –0.55 [KOWWIN EPI Suite, 2000]. The experimental log Kow value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Red No. 40 is very water soluble (220,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (1 mg/L) [Marmion, 1991], it is anticipated that the log Kow value for this substances would exceed 6.0.

### 3.1.5 Water Solubility

FD&C Red No. 40 has a reported water solubility of 180,000 mg/L at 20 °C, 220,000 mg/L at 25 °C, and 260,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Red No. 40 in 100% glycerol is 3,000 mg/L at 25 °C while the solubility in ethanol is reported to be 1 mg/L at 25 °C [Marmion, 1991, robust summary not included]. The solubility of FD&C Red No. 40 in octanol is expected to be less than 1 mg/L.

### 3.1.6 New Testing Required

None.

## 3.2 ENVIRONMENTAL FATE AND PATHWAYS

### 3.2.1 Photodegradation

Direct and indirect photolysis experiments were conducted on FD&C Red No. 40 using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for hydroxyl radical reactions is 18.2 hours [AOPWIN EPI Suite, 2000].

### 3.2.2 Stability In Water

FD &C Red No. 40 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc*.) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 $^{o}$C. These conditions would not typically be encountered in the environment. Therefore, FD &C Red No. 40 and its corresponding salts are anticipated to be stable in water.

### 3.2.3 Biodegradation

The biodegradability of azo dyes substituted with a phenolic OH and two sulfonic acid groups consistently show that these substances are not absorbed onto activated sludge and, therefore, are not biodegradable [Shaul *et al*., 1990]. Incubation of 1.0 or 5.0 mg/L of a structurally related azo dye, (1-naphthalenesulfonic acid, 4-hydroxy-3-[(4-sulfo-1-

naphthalenyl)azo]-, disodium salt)[3] with activated sludge from a sewage treatment plant revealed that the concentration of dye remained essentially constant in the influent flow, primary effluent, and activated sludge effluent. Essentially no azo dye was absorbed by activated sludge. Two other azo dyes ring-substituted with sulfonic acid groups (Acid Orange No. 10 and Acid Red No. 1) exhibited a similar behavior in these experiments.

FD&C Red No. 40 was not predicted to be readily degradable by BIOWIN model calculations [AOPWIN EPI Suite, 2000].

### 3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 [Trent University, 2002]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log $K_{OW}$.

As expected, the model predicts that FD&C Red No. 40 is distributed completely to the water compartment (greater than 100%). Consistent with the extremely high water solubility and low log $K_{OW}$ data, FD &C Red No. 40 showed no significant distribution to the soil compartment ($2 \times 10^{-14}$%). Based on this physiochemical model, the ratio for distribution of FD&C Red No. 40 between water (greater than 100%) and fish ($4.9 \times 10^{-6}$ %) is greater than seven orders of magnitude suggesting essentially no bioaccumulation in fish. These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

### 3.2.5 New Testing Required

None.

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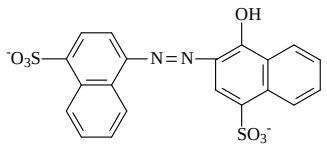


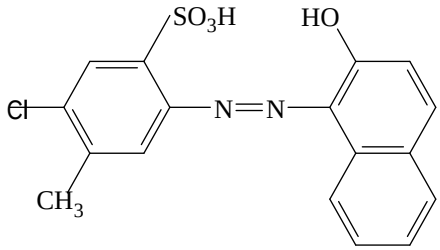
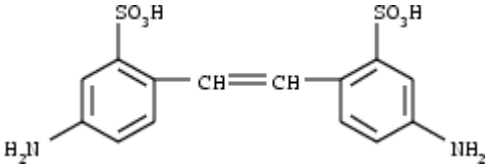
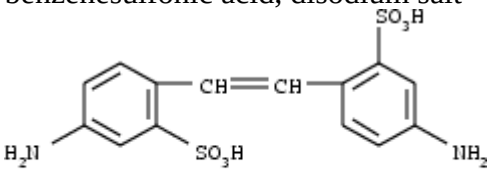
## 3.3 ECOTOXICITY

### 3.3.1 Acute Toxicity to Fish

Based on input parameters for molecular weight (496.43), water solubility (220,000 mg/L at 25 °C), and melting point (350 °C), the calculated 96-hour LC50 for FD&C Red No. 40 is 2,714 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Red No. 40 is to a large extent, a function of the presence of aromatic sulfonic acid and phenolic ring substituents. The presence of more than one aromatic sulfonic acid groups enhances water solubility and decreases absorption by aquatic species. Two structurally related substituted azo colorants containing naphthalene sulfonic acid and benzene sulfonic acid residues have been the subject of ecotoxicity studies in fish. The closest structural relative is the barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid. In two fish species (*Brachydanio rerio* and *Oryzias latipes*), the 96 hr- LC50 exceeded 500 mg/L, one in a semi-static test and the other in a static test (Hoechst AG, 1992). In other acute fish toxicity tests, the structurally related azo dye, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt showed an96-hr LC50=33 mg/L in Orange killifish (MITI, Japan, 1992). These data support the conclusion that Red No. 40 exhibits a low order of toxicity for fish.

The extensive studies on the ecotoxicity of aromatic sulfonic acids also indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of stilbene sulfonic acid derivatives result in an 96-hour LC50 value greater than 10,000 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

**Table 3**

| Name | Acute Toxicity to fish |
|---|---|
| barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid | 96-hour LC50: >500 mg/L<br>96-hour LC50: >500 mg/L |
|  | |
| 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid | 48-hour LC50: 200 mg/L |
|  | |
| 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt | 72-hour LC50: greater than 1000 mg/L |
|  | |

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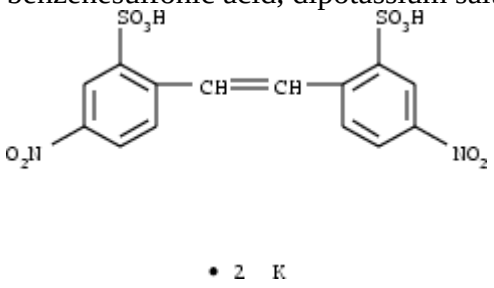
|---|---|
| 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt  | 96-hour LC50: greater than 10,000 mg/L |

Given the high-calculated LC50 values from the ECOSAR model, the experimentally measured toxicity of aromatic sulfonic acid derivatives, and the difficulties inherent in acute aquatic testing with dyes, no additional testing is requested.

### 3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Red No. 40 in daphnids is 295 mg/L based on input parameters for molecular weight (496.43), water solubility (220,000 mg/L at 25 °C), and melting point (350 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. Experimental data for the two azo colorants containing a benzene sulfonic acid or naphthalene sulfonic acid (barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid and 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt ) show low levels of toxicity in *Daphia magna.* In an OECD 202 guideline study, the EC50 is reported to be 280 mg/L (EA, Japan, 1992). The extensive water solubility and limited lipophilicity of FD&C Red No. 40 is to a large extent, a function of the presence of aromatic sulfonic acid phenolic ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids also indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994].

These experimental values (100-280 mg/L) are consistent with calculated values (295 mg/L).

### 3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (496.43), water solubility (220,000 mg/L at 25 °C), and melting point (350 °C), the calculated EC50 for FD&C Red No. 40 with green algae is 44,524 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced measurable toxicity. Given the high-predicted value for acute toxicity to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

### 3.3.4 New Testing Required

None.

## 3.4 HUMAN HEALTH TOXICITY

### 3.4.1 Acute Toxicity

Pre-GLP acute toxicity studies were conducted on FD&C Red No. 40 in rats and dogs. Six groups of five male and five female Sprague-Dawley rats were each administered the test substance in a 10% weight/volume solution. The dosage levels tested were 215, 464, 1,000, 2,150, 4640, and 10,000 mg/kg bw. Observations were made immediately following dosing, at 1, 4, 24, 48-hours and once daily thereafter up to 14 days. Following the observation period, the animals were weighed, sacrificed by cerebral concussion and necropsied. Clinical observations were normal with the exception of red-colored feces in both sexes at all dose levels and red-colored urine at the three highest dose levels in the female animals. There were no deaths at any dose level tested. The acute LD50 was determined to be greater than 10,000 mg/kg bw/day for adult male and female Sprague-Dawley albino rats administered FD&C Red No. 40 *via* gavage [Hazelton Laboratories, Inc., 1965a].

Two male Mongrel dogs were administered the test substance in an aqueous solution at a dose level of 5,000 mg/kg bw. Observations were made immediately following dosing and daily thereafter for 7 days. Following the observation period, the animals were weighed, sacrificed and necropsied. Red diarrhea was observed 30 minutes following dosing in one animal, which was followed by emesis. Red urine was reported for the other animal. Red stools were reported for both dogs one day following dosing. From the third day until the seventh day, both animals appeared normal with respect to appearance, behavior, appetite and elimination. Gross necropsy revealed fibrotic changes and decreased weight in a kidney of one test animal. This finding was not considered treatment related, but was rather considered to be a chronic lesion. The spleen also appeared enlarged in this test animal. In the other test animal, hookworms were observed in the gastrointestinal tract. There were no deaths at the dose level tested (5,000 mg/kg bw). The acute LD50 was determined to be greater than 5,000 mg/kg bw/day for male

Mongrel dogs administered FD&C Red No. 40 *via* gavage [Hazelton Laboratories, Inc. 1965b].

### 3.4.2 *In vitro* and *In vivo* Genotoxicity

#### *3.4.2.1 In vitro*

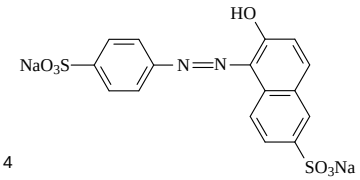
FD&C Red No. 40 tested negative in reverse mutation assay using TA1535, TA1537, TA98, TA100; and *Saccharomyces cerevisiae* strain D4 with and without metabolic activation at concentrations up to 5,000 micrograms/plate [Brusick, 1976; Muzzall and Cook, 1979].

#### *3.4.2.2 In vivo*

*In vivo* genotoxicity data are available for the structurally related azo dye, FD&C Yellow No. 6 (6-hydroxy-5-[(4-sulfophenyl)azo]-2-napthalenesulfonic acid, disodium salt)[4], which is the same structure as FD&C Red No. 40, except without the 2-methoxy and 5-methyl functional groups on the single ring. FD&C Yellow No. 6 tested negative in the rat micronucleus test at a single dose level of up to 1,000 mg/kg bw/day [Westmoreland and Gatehouse, 1991].

### 3.4.3 Repeat Dose Toxicity

In a Lifetime Toxicity/Carcinogenicity Study, FD&C Red No. 40 was provided in the diet as an admixture to Sprague-Dawley rats. In the *in utero* phase, 240 male and female rats were randomly assigned (30/group) to the control, low dose (0.37%), mid-dose (1.39%) or high dose (5.19%) groups, providing daily intake levels of 180, 701 or 2,829

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mg/kg bw/day for males and 228, 901 or 3,604 mg/kg bw/day for females. These parental ($P_1$) rats received the test material one week prior to mating, during the three-week mating period and during the gestation and lactation periods. The offspring of these animals were randomly selected and put into groups of fifty male and female weanling rats each. These groups were administered the test substance in the diet of the male animals for 118 weeks and the diet of female animals for 121 weeks at levels of 0, 0.37, 1.39 to 5.19 % corresponding to the dietary levels used in the *in utero* phase. Parameters included survival, clinical signs, body weight and food consumption, gross and microscopic pathology. Gross necropsies were performed on all animals dying during the study, all animals found in a moribund condition, and all animals killed at study termination. Complete histological examinations were performed on all animals in both the control and high-dose groups. The tissues examined histologically included: brain, pituitary, thoracic spinal cord, eyes, esophagus, thyroid, thymus, heart, lungs, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph node, kidneys, adrenal, urinary bladder, uterus, prostate, ovaries, testes with epididymides, seminal vesicles, skin, rib junction, bone marrow, nerve with muscle, and any tissue masses or lesions. Histological examination was also performed on animals from any group with observable masses or lesions. If a potential effect was seen recurrently in a tissue, than that tissue was examined in all animals.

Food consumption was elevated among high dose males and females, but was not statistically significant. Red-tinted fur was reported among all treated animals, and red-tinted feces were reported for mid- and high-dose male and females. Group mean body weights of treated males and females were decreased compared to control animals at study termination, with the exception of mid-dose treated male rats that experienced an increase in mean body weight. However, the decrease in mean body weight was only statistically significant in female rats at the high dose level (3,604 mg/kg bw/day). Clinical chemistry and urinalysis parameters revealed no treatment related effects.

Histopathological examination revealed lesions in both control and treated animals at similar prevalence, and thus not attributed to test substance administration. No biologically significant adverse effects were reported following administration of FD&C

Red No. 40, with the exception of decrease mean body weights for high-dose female rats at study termination. The authors attributed this effect to the large amount of non-nutritive material in the diet at the intake level [Borzelleca *et al*., 1991a].

A similar lifetime/carcinogenicity study was also performed in Charles River HaM/ICR (CD-1) mice and in CD-1 outbred mice. In the *in utero* phase, 50 male and female CD-1 mice each (study A) or 70 male and female CD-1 outbred mice each (study B) were randomly assigned to the control, low dose (0.37%), mid-dose (1.39%) or high dose (5.19%) groups, providing daily intake levels of 507, 1,877 or 7,422 mg/kg bw/day for males and 577, 2,043 or 8,304 mg/kg bw/day for females (study A) and 492, 1,821, or 7,318 mg/kg bw/day (males) and 526, 2,057 or 8,356 mg/kg bw/day (females) (study B). These $F_0$ groups received the test material one week prior to mating, during the three week mating period and during gestation and lactation periods.

Groups of fifty male and female weanling CD-1 albino mice were randomly selected from the litters at 21 days of age and administered the FD&C Red No. 40 in the diet of study A animals for 104 weeks and the diet of study B animals for 109 weeks at levels of 0, 0.37, 1.39 or 5.19 %. These animals were the $F_1$ offspring of parental rats ($F_0$), which were treated at the corresponding levels. Study A had one control group while study B had two control groups. Parameters included survival, clinical signs, body weight and food consumption, gross and microscopic pathology. Gross necropsies were performed on all animals dying during the study, all animals found in a moribund condition, and all animals killed at study termination. Complete histology was conducted on all mice from all groups in study A and on 10/sex/group for the two control groups and the highest-dose group from study B. The tissues examined histologically included: brain, pituitary, thoracic spinal cord, eyes, esophagus, thyroid, thymus, heart, lungs, liver, spleen, pancreas, stomach, small and large intestine, mammary glands (study B only), mesenteric lymph node, kidneys, adrenal, urinary bladder, uterus, prostate, ovaries, testes with epididymides, seminal vesicles, skin, rib junction, bone marrow, nerve with muscle, and any tissue masses or lesions. Histological examination was also performed on animals from any group with observable masses or lesions. If a potential effect was seen recurrently in a tissue, than that tissue was examined in all animals.

No treatment-related effects on survival were found. The authors reported decreased food consumption among the mid- and high-dose females for week 62-106 in study B. However, no consistent statistically significant effects on food consumption were reported in either study. Localized alopecia, labored respiration, colored hair coat, lacrimation and thinness were reported in similar incidences in both control and treated mice at all dose levels. Distended abdomens were noted in both mid- and high-dose females, while palpable masses were reported in control and treated groups at a similar incidence. Hematological and clinical chemistry parameters revealed few differences among treated and control groups. No significant gross pathological changes were reported among treated groups compared to control groups. An increase in absolute and relative thyroid weights in study B in the high-dose males and females was reported, but the signficance was questioned because there was no accompanying histopathology, nor was it dose-dependent and it appeared to be species-specific.

The authors reported an earlier appearance of lymphatic lymphomas among treated groups in study A compared to control groups. No increases in incidence or appearance of lymphocytic lymphomas were reported in study B. The authors noted that study B was conducted using a different strain of mouse to further investigate if FD&C Red No. 40 had an effect on the appearance of lymphocytic lymphomas, and it revealed no relationship between the incidence of lymphocytic lymphomas and FD&C Red No. 40 [Borzelleca *et al.*, 1991b].

### 3.4.4 Developmental Toxicity

Four groups of female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD&C Red No. 40 in the drinking water at intake levels of 0, 0.2, 0.4 or 0.7% for the first 20 days of gestation. These intake levels correspond to daily doses of 0, 273.58, 545.68 or 939.29 mg/kg bw/day [Collins *et al*., 1989a]. On day 20, the animals were examined for gross abnormalities followed by euthanasia. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of *corpora lutea* and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were

divided and assigned to skeletal or soft tissue examination. No clinical findings were reported and no deaths occurred during treatment. Mean fluid consumption was significantly increased in animals at the 0.2 and 0.4% intake levels, but only on days 14-20. Because fluid consumption was not increased at the 0.7% level, the findings were not considered significant. No other effects were reported.

A significant increase in the incidence of litters containing fetuses with missing sternebrae occurred in the 0.4% group, but not in the group receiving 0.7%. No dose related increases were reported for any sternebral variations. The number of fetuses with at least one type of sternebral variations was greater in all treated groups, but only significantly greater in the 0.4 and 0.7% groups. The percentage of total fetuses with at least one sternebral variation was greater in all of the treated groups compared to the control group, but the differences were not significant. The number of fetuses with more than one skeletal variation were similar among treated and control groups. The incidence of reduced ossification of the hyoid bone was significantly increased at the 0.7% intake level. Significant dose related increases were reported at the highest intake level for the average number of fetuses per litter with at least two skeletal variations and the number of litters containing them.

The authors questioned the biological significance of the reduced ossification of the hyoid bone given the lack of effect seen in a gavage study using higher dose levels. The increased incidence was slightly above that found in the historical controls, and the control group was noted as having a lower incidence compared to the historical controls [Collins *et al*., 1989a].

Four groups of female Osborne-Mendel (FDA strain) rats (42-43 per group) were administered FD & C Red No. 40 *via* gavage at dose levels of 0, 30, 75, 150, 300, 600 or 1000 mg/kg bw/day for the first 19 days of gestation. On day 19, the animals were examined for gross abnormalities followed by euthanization. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of corpora lutea and implantation sites. All live fetuses were promptly

weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination.

No clinical findings were reported and no deaths occurred during treatment. No other dose related findings were reported. The only significant skeletal anomaly found was an increase in 14th rib buds at the 300 mg/kg bw/day dose level but was not seen at the higher dose levels. No other soft-tissue or sternebral variations were reported. The NOAEL's for maternal and fetal toxicity were 1000 mg/kg bw/day [Collins *et al*., 1989b].

### 3.4.5 Reproductive Toxicity

Groups of male (10) and female (20) Charles River rats were administered FD&C Red No. 40 in the diet at 0, 3700, 13,900, or 51,900 ppm for 27 weeks prior to initiation of the first breeding phase. This $P_1$ parental generation was individually housed. Clinical observations included food consumption, appearance, individual body weights and behavior and were made weekly.

During the breeding phase of the $P_1$ generation, two females and one male were placed in a breeding cage. At weekly intervals during the mating period, the males were rotated among the females in each group. Following mating, the females were placed in individual cages to produce the first (FIA) litters. Twenty-four hours following the birth of the pups the first litters (FIA) were arbitrarily reduced to 8 maximum per mother. The number of conceptions, number of litters, live births, stillbirths, size of natural and nursing litters, deaths during the period of lactation, and number of pups weaned were recorded. The body weights of each pup were recorded at 24 hours and at weaning. Gross signs of toxicity were monitored. After 21 days of nursing, random pups were sacrificed and gross necropsies performed. Twenty-four females and twelve males remaining from each test group and control group were selected at random and designated the $P_2$ generation. Following the weaning of the F1A animals, the $P_1$ generation was remated to produce their second litters referred to as F1B, according to the procedures described above.

The $P_2$ generation was housed 4-5 per cage and was maintained on the same dietary levels as their parents. The procedures outlined above for the $P_1$ generation were maintained for the $P_2$ generation. The litters of the $P_2$ animals were referred to as the F2A litters. Body weights of the F2A pups were monitored 24 hours following the birth and at weaning. Gross signs of toxicity were recorded. Following a 21 day nursing period, all pups were weaned and sacrificed. One week following the weaning period of the F2A litter, the $P_2$ generation was remated to produce their second litters (F2B). Two females were placed in a cage with a male from the corresponding dose group. Males were rotated weekly, and females were examined daily for presence of spermatozoa for a maximum of 21 consecutive days. The first day that sperm were observed was designated as day 0 of gestation. The females were then placed in individual cages. Half of the females (12) were sacrificed on day 19 or 20 of gestation and Caesarean sections were performed. Observations included number and placement of implantation sites, resorption sites, and live and dead fetuses, individual fetal weight and length (crown to rump), and external fetal anatomical structure. Gross necropsies were performed on each female including examination of uterus and visceral structures. The remaining 12 females were allowed to litter normally. The fetuses of both females delivering normally and *via* Caesarean section were necropsied.

Fertility indices for the control and test animals of both F1A and F1B were considered low. The authors attributed this to the advanced age of the animals upon mating. The fertility index of the 3,700 ppm test group in the F2A breeding cycle as well as the 3700 and 51,900 ppm test groups in the F2B breeding cycle were reported to be low in comparison to control animals and historical control data. Growth suppression, characterized as slight, was also reported for the low-level F1B pups, and the high-level F1A and F1B pups and the F2A and F2B breeding cycles when compared with controls. All other measured parameters were comparable to controls in each generation and among the two filial generations. The authors concluded that FD&C Red No. 40 caused meaningful growth suppression in the pups whose parents received the high level diets. The authors reported a no observable adverse effect level (NOAEL) for reproductive

toxicity following administration of FD&C Red No. 40 as 13,900 ppm [Hazelton Laboratories, 1969].

### 3.4.6 New Testing Required

None.

## 3.5 TEST PLAN TABLE

| Chemical | Physical-Chemical Properties | | | | |
|---|---|---|---|---|---|
| | Melting Point | Boiling Point | Vapor Pressure | Partition Coefficient | Water Solubility |
| 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt CAS No. 25956-17-6 | A | Calc | Calc | Calc | A |

| Chemical | Environmental Fate and Pathways | | | |
|---|---|---|---|---|
| | Photodegradation | Stability in Water | Biodegradation | Fugacity |
| 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt CAS No. 25956-17-6 | A, Calc | NA | R | Calc |

| Chemical | Ecotoxicity | | |
|---|---|---|---|
| | Acute Toxicity to Fish | Acute Toxicity to Aquatic Invertebrates | Acute Toxicity to Aquatic Plants |
| 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt CAS No. 25956-17-6 | R, Calc | R, Calc | R, Calc |

| Chemical | Human Health Data | | | | | |
|---|---|---|---|---|---|---|
| | Acute Toxicity | Genetic Toxicity *In Vitro* | Genetic Toxicity *In Vivo* | Repeat Dose Toxicity | Repro-ductive Toxicity | Develop-mental Toxicity |
| 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt CAS No. 25956-17-6 | A | A | R | A | A | A |

| Legend | |
|---|---|
| **Symbol** | **Description** |
| R | Endpoint requirement fulfilled using category approach, SAR |
| Test | Endpoint requirements to be fulfilled with testing |
| Calc | Endpoint requirement fulfilled based on calculated data |
| A | Endpoint requirement fulfilled with adequate existing data |
| NR | Not required per the OECD SIDS guidance |
| NA | Not applicable due to physical/chemical properties |

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