Robust Summaries for

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

CAS No. 25956-17-6

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The International Association of Color Manufacturers/HPV Committee

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Colorcon

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Sensient Colors, Inc.

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The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 MELTING POINT

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	Measured
GLP	No
Year	1970
Decomposition	300 °C
Remarks for Results	Decomposes without melting.
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Hazelton Laboratories (1970) Petition to FDA.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	Calculated
Melting Point	350 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.2 BOILING POINT

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	Calculated
Boiling Point	872 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.3 VAPOR PRESSURE

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	1.25 X 10^{-23} mm Hg
Temperature	25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.4 N-OCTANOL/WATER PARTITION COEFFICIENTS

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	Calculated
Log Pow	-0.55
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) US Environmental Protection Agency.

1.5 WATER SOLUBILITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Purity not listed
Method/Guideline	Experimental
GLP	Ambiguous
Year	1991
Value (mg/L) at Temperature	180,000 mg/L at 20 °C; 220,000 mg/L at 25 °C; 260,000 mg/L at 60 °C
Description of Solubility	Not given
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Marmion D.M. (1991) Handbook of U.S. Colorants: Foods, Drugs, and Cosmetics and Medical Devices. 3rd Ed. New York, John Wiley & Sons, Inc.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 PHOTODEGRADATION

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	FD&C Red No. 40
Method/guideline	Not given
Test Type	Experimental
GLP	Ambiguous
Year	1991
Light Source	15-watt General Electric germicidal lamps
Light Spectrum (nm)	Ultraviolet
Remarks for Test Conditions	The concentration of the dye solution was measured before and after the photolysis using the Hewlett-Packard 8452A diode-array UV/Visible Spectrophotometer. FD & C Red No. 40 was prepared in an initial concentration of 5 mg/l. In the first part of the study, photolysis experiments were conducted using two 15-W (30 Watts total) General Electric germicidal lamps as the ultraviolet light source. The distance between the light source and the reaction vessels was approximately 2.5 cm. Both direct photolysis and indirect photolysis experiments were conducted. The indirect photolysis experiment used acetone as the sensitizer for indirect photodegradation.
Concentration of Substance	5 mg/L
Direct photolysis	7% degradation after 50 minutes
Indirect photolysis	99% degradation after 20 minutes
Sensitizer	Acetone
Concentration of sensitizer	5 mg/L
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Pasin B. and Rickabaugh J. (1991) Destruction of Azo Dyes by Sensitized Photolysis. Hazard. Ind. Wastes, 359-367.

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

CAS No.	25956-17-6
Method/guideline	Calculation
Test Type	AOPWIN
Halflife t1/2	18.2 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 BIODEGRADATION

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Data are for structurally related substance, C.I. Acid Red 14, 1-Naphthalenesulfonic acid, 4-hydroxy-3-[(4-sulfo-1-naphthalenyl)azo]-, disodium salt (CAS No. 3567-69-9)
Method	Not given
GLP	Ambiguous
Year	1993
Contact time (units)	24 hour
Innoculum	Activated sludge
Remarks for Test Conditions	Screened raw wastewater was used as the influent in three pilot scale activated sludge biological treatment systems. Each water soluble dye was tested at doses of 1 mg/L for low spike systems and 5 mg/L for high spike systems of influent flow. Before the data collection, dye analytical recovery studies were conducted by dosing the purified dye compound into organic free water, influent wastewater, and mixed liquor. These studies were run in duplicate and each recovery study was repeated at least once to ensure that the dye compound could be extracted. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization.

	The INF, primary effluent (PE), and ASE were filtered and the filtrate was passed through a column packed with resin. The filter paper and resin were soaked in an ammonia acetonitrile solution and then Soxhlet extracted with ammonia-acetonitrile. The extract was concentrated and brought up to 50 mL volume with a methanol/dimethylformamide solution. The mixed liquor samples were separated into two components, the filtrate or soluble fraction (SOL) and the residue (RES) fraction. The SOL fraction was processed similar to these samples but he resin adsorption step was omitted. All extracted samples were analyzed by HPLC with and ultraviolet-visible detector. Total suspended solids analyses were also performed on the INF, PE, ML, and ASE samples. All systems were operated for at least three times the solids retention time to ensure acclimation prior to initiation of data collection. All samples were 24 hr. composites made up of 6 grab samples collected every 4 hr. and stored at 4 deg Celsius.
Results	Percent recovery as measured: Organic Free Water: 101% at 1 mg/L and 90% at 5 mg/L; Wastewater: 98% at 1mg/L and 97% at 5 mg/L; Mixed Liquor: 88% at 1mg/L and 92% at 5 mg/L Mass Balance Data Summary: Low spike: 116% recovered, 1% adsorbed; High spike: 148% recovered, less than 1% adsorbed.
Remarks fields for results	Since the majority of the test substance was recovered, the authors assumed that this compound was not biodegraded. The authors based this assumption on preliminary data indicating little or no problems in recovering the compounds from the sample matrix. Additionally the results also indicate that the material was not adsorbed. The authors attributed the high sulfonic acid substitution on the test substance as the reason why the material was not removed by the microbial cells or cell byproducts and subject to aerobic biodegradation.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Shaul G.M., Holdsworth T.J., Dempsey C.R., and Dostal K.A. (1990) Fate of water soluble azo dyes in the activated sludge process. Chemosphere 22, p107-119.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method	Calculated
Classification	Not readily biodegradable
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

2.3 FUGACITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Air-Water Partition Coefficient
Absorption coefficient	1.13E-18
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Fish-Water Partition Coefficient
Absorption coefficient	0.0491
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Aerosol-Air Partition Coefficient
Absorption coefficient	2.87E+15
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Air
Estimated Distribution and Media Concentration	3.68E-24%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald

	(1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Soil-Water Partition Coefficient
Absorption coefficient	0.0201
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Sediment-Water Partition Coefficient
Absorption coefficient	0.0403
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Suspended Sediment-Water Partition Coefficient
Absorption coefficient	0.201
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Water
Estimated Distribution and Media Concentration	100%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Soil
Estimated Distribution and Media Concentration	1.64E-14%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Sediment
Absorption coefficient	0.0403%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

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

CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Suspended Sediment
Estimated Distribution and Media Concentration	1.01E-4%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.70 Level III
Input Parameters	MW, log Kow, water solubility, MP & VP
Media	Fish
Estimated Distribution and Media Concentration	4.91E-6%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Trent University (2002) Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70. Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

3 ECOTOXICITY

3.1 ACUTE TOXICITY TO FISH

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Data are for structurally related substance, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid (CAS No. 81-11-8)
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Exposure Period	48 hour
Endpoint value	LC50 = 200 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
Reference	Greim H., Ahlers J., Bias R., Broecker B., Hollander H., Gelbke H.P., Klimisch H., Mangelsdorf I., Paetz A., Schone N., Stropp G., Vogel R., Weber C., Ziegler-Skylakakis K., and Bayer E. (1994) Toxicity and ecotoxicity of sulfonic acids: structure-activity relationship. Chemosphere, 28, 2203-2236.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Data are for structurally related substance, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt (CAS No. 7336-20-1)
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Exposure Period	72 hour

Endpoint value	LC50 greater than 1000 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
Reference	Greim H., Ahlers J., Bias R., Broecker B., Hollander H., Gelbke H.P., Klimisch H., Mangelsdorf I., Paetz A., Schone N., Stropp G., Vogel R., Weber C., Ziegler-Skylakakis K., and Bayer E. (1994) Toxicity and ecotoxicity of sulfonic acids: structure-activity relationship. Chemosphere, 28, 2203-2236.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Data are for structurally related substance, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt (CAS No. 78447-91-3)
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Endpoint value	LC50 greater than 10000 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
Reference	Greim H., Ahlers J., Bias R., Broecker B., Hollander H., Gelbke H.P., Klimisch H., Mangelsdorf I., Paetz A., Schone N., Stropp G., Vogel R., Weber C., Ziegler-Skylakakis K., and Bayer E. (1994) Toxicity and ecotoxicity of sulfonic acids: structure-activity relationship. Chemosphere, 28, 2203-2236.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, 350 °C, Water solubility, 220,000 mg/L at 25 deg C

Endpoint value	LC50 = 2714 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Data are for structurally related substance 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt (CAS No. 7336-20-1)
Test Type	Experimental
Species/Strain/Supplier	*Daphnia magna*
Test Details	24 hour
EC50, EL50, LC0, at 24,48 hours	EC50 = 100 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4.Only secondary literature (review, tables, books, etc.).
Reference	Griem H., Ahlers J., Bias R., Broecker B., Hollander H., Gelbke H.P., Klimisch H., Mangelsdorf I., Paetz A., Schone N., Stropp G., Vogel R., Weber C., Ziegler-Skylakakis K., and Bayer E. (1994) Toxicity and ecotoxicity of sulfonic acids: structure-activity relationship. Chemosphere, 28, 2203-2236.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6

Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Input parameters: Melting point, 350 °C, Water solubility, 220,000 mg/L at 25 deg C
EC50, EL50, LC0, at 24,48 hours	LC50 = 295 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.3 ACUTE TOXICITY TO AQUATIC PLANTS

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Test Substance	The test substance was an unidentified sulphonic acid substituted azo dye.
Test Type	Experimental
GLP	Ambiguous
Year	1996
Species/Strain/Supplier	Green algae, *Selenastrum capricornutum*
Exposure Period	96 hour
Remarks for Test Conditions	Algal chronic toxicity test were performed according the method of EPA, 1988. Three replicates were performed for each dye at a nominal concentration of 1 mg/l for the active colorant. One ml of dye stock solution was added to 50 mg/l of algal assay medium in 125 ml Erlenmeyer flasks. S. capricornutum in continuous culture provided the initial innoculum (10,000 algal cells/ml). The cells were incubated in the solution for 96 hours. The diluent and negative control were algal assay medium. AAM was prepared by adding 1 ml from each of five stock

	solutions to 900 ml of deionized water. After spiking, the total volume was brought to 1 liter with deionized water. Population growth was used to establish potential toxicity. If the dye inhibited algal growth by more than 50% of that of the negative controls, a definitive test using several dilutions of the dye was performed to allow for determination of an EC50 concentration.
Endpoint value	Average yield: 36.6% with 95% C.I. (34.9-38.4).
Biological observations	26.4% stimulation of population growth compared to control.
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Yes, Dunnett's test
Remarks fields for results	Not statistically significant.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Greene J. C. and Baughman G.L. (1996) Effects of 46 dyes on population-growth of fresh-water green-alga *selenastrum-capricornutum*. Textile Chemist And Colorist, 28, 23-30. Green J.D. et al. (1988) Protocols for short term toxicity screening of hazardous waste sites. Report to EPA 600/3-88-029. U.S. Environmental Protection Agency. Corvallis, Oregon.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, 350 °C, Water solubility - 220,000 mg/L at 25 deg C
Endpoint value	EC50 = 44,524 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) US Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

4 HUMAN HEALTH TOXICITY

4.1 ACUTE TOXICITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	FD&C Red No. 40; purity not given; dark red in color
Method/guideline	Not given
Test Type	Acute Oral LD50
GLP	No
Year	1965
Species/strain	Rat/Sprague-Dawley albino
Sex	Male and Female
# of animals per sex per dose	5 male and 5 female
Vehicle	Water
Route of Administration	Oral-Gavage
Remarks for Test Condition	Six groups of five male and five female Sprague-Dawley rats each were administered the test substance in a 10% weight/volume solution. The dosage levels tested were 215, 464, 1000, 2150, 4640, and 10,000 mg/kg bw. The animals were fasted for 3-4 hours prior to dosing. Following dosing, the animals were housed in metal cages suspended above the droppings. Food and water were available *ad libitum*. 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.
Value LD50 or LC50 with confidence limits	Greater than 10,000 mg/kg bw
Number of deaths at each dose level	There were no deaths at any dose level tested.
Remarks for results	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.
Conclusion remarks	The acute LD50 was determined to be greater than 10,000 mg/kg bw/d for adult male and female Sprague-Dawley albino rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Hazelton Laboratories, Inc. (1965a) Acute oral administration-rats. Five experimental non-toxic red colors. Unpublished Report No. 165-114.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	FD&C Red No. 40; purity not given; dark red in color
Method/guideline	Not given
Test Type	Acute Oral LD50
GLP	No
Year	1965
Species/strain	Dog/Mongrel
Sex	Male
# of animals per sex per dose	2 males
Vehicle	Water
Route of Administration	Oral-Gavage
Remarks for Test Conditions	One groups of two male Mongrel dogs was administered the test substance in an aqueous solution at a dose level of 5 g/kg bw. Two concurrent control animals receiving 300 ml of water each were also maintained. Each test animal was individually housed. Food and water were available *ad libitum*. Observations were made immediately following dosing and daily thereafter for 7 days. Following the observation period, the animals were weighed, sacrificed and necropsied. Necropsies were not performed on control animals.
Value LD50 or LC50 with confidence limits	Greater than 5,000 mg/kg bw
Number of deaths at each dose level	There were no deaths at the dose level tested (5000 mg/kg bw).
Remarks for results	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.

Conclusion remarks	The acute LD50 was determined to be greater than 5,000 mg/kg bw/d for male Mongrel dogs.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Hazelton Laboratories, Inc. (1965b) Acute oral administration-dogs. Five experimental non-toxic red colors. Unpublished Report.

4.2 GENETIC TOXICITY

4.2.1 In vitro Genotoxicity

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Purity not given; red powder.
Method/guideline	Ames, McCann and Yamasaki (1975) Plate Test (Overlay method)
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1983
Species/Strain	*Salmonella typhimurium* TA98, TA1535, TA1537; *Saccharomyces cerevisiae* strain D4
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.625, 1.25, 2.5, 5.0% or 10, 100, 1000, or 5000 micrograms per plate
Statistical Methods	Not given
Remarks for Test Conditions	Toxicity tests were conducted to identify the 12.5%, 25% and 50% killing doses. If no toxicity was found, a maximum dose of 5% was used as the highest dose concentration. The same doses were used for both activation and non-activation assays. Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molter agar supplemented with biotin and a trace of histidine.

	For tests with activation, the rat liver 9000 x g tissue supernatant and required cofactors were added to the overlay tubes. The four dose levels of the test substance were added to the overlay tubes, followed by mixing and pouring over minimal agar plates. The plates were then incubated for 48-72 hours at 37 deg Celsius and scored for colonies. Positive and negative (solvent only) controls were run with each assay. Positive controls for the non-activation assays were ethylmethanesulfonate (EMS); methylnitrosoguanidine (MNNG); 2-nitrofluorene (NF); quinacrine mustard (QM). Postive controls with activation included 2-anthramine (ANTH); 2-acetylaminofluorene (AAF); 8-aminoquinoline (AMQ); dimethylnitrosamine (DMNA).
Results	The maximum dose of 5% was used, since 50% survival was not determined. Slight toxic effects noted at 5%.
Cytotoxic concentration	Slight toxic effects at 5%.
Genotoxic Effects	Negative at all concentration levels.
Appropriate statistical evaluations?	None given
Remarks for results	The substance was determined to be soluble.
Conclusion Remarks	The test substance did not exhibit genotoxic activity with or without metabolic activation in the AMES assay using SAL TA98, TA1535 or TA1537 plate overlay method.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Brusick D. (1976) Mutagenicity evaluation of NTR-Z-4576. Unpublished report.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	Purity not given
Method/guideline	Ames test
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	10-250 mg/plate
Statistical Methods	Not given

Remarks for Test Conditions	The test substance was dissolved in DMSO. The test was considered positive if 2 fold increase in revertants was observed. Positive controls included 9-aminoacridine; 2-aminoflourine; N-methyl-N-nitrosoguanidine.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations?	None given
Remarks for Results	Negative
Conclusion Remarks	No evidence of genotoxicity was reported.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muzzall J.M. and Cook W.I. (1979) Mutagenicity test of dyes used in cosmetics with the Salmonella/mammalian microsome test. Mutations Research 67, 1-8.a

4.2.2 In vivo Genotoxicity

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Test Substance	Test substance was the structurally related substance, FD&C Yellow 6, 6-hydroxy-5-[(4-sulfophenyl)azo]-2-napthalenesulfonic acid, disodium salt (CAS No. 2783-94-0).
Method/guideline	Rodent Micronucleus Test
GLP	Ambiguous
Year	1991
Species/Strain	Rat/PVG
Sex	Male
Route of Administration	Oral-Gavage
Doses/Concentration	10 ml/kg bw

Exposure Period	Single dose
Remarks for Test Conditions	Male PVG rats received a single oral dose of 500, or 1000 mg/kg of sunset yellow 6. Bone marrow samples were taken at 24 and 48 hours later.
Genotoxic effects	No significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species and there was no effect on the % PE (polychromatic erthyrocytes).
Appropriate statistical evaluations?	Yes
Remarks for results	No effects.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Westmoreland C. and Gatehouse D.G. (1991) The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 in vivo in the rodent micronucleus test (observations on species and tissue specificity). Carcinogenesis 12 (8), 1403-8.

4.3 REPEATED DOSE TOXICITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	FD&C Red No. 40; 88% purity
Method/guideline	Lifetime Toxicity/Carcinogenicity Study
GLP	Ambiguous
Year	1991
Species/strain	Rat/Sprague-Dawley
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0.37, 1.39 or 5.19%
Exposure Period	118 (males) or 121 weeks (females)

Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	In a Lifetime Toxicity/Carcinogenicity Study, FD & C Red 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 2829 mg/kg bw/d for males and 228, 901 or 3604 mg/kg bw/d for females. These parental (P1) 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.
NOAEL(NOEL)	5.19% or 2829 mg/kg bw/d (males); 1.39% or 901 mg/kg bw/d (females)
LOAEL(LOEL)	Greater than 5.19% or 2829 mg/kg bw/d (males); 5.19% or 3604 mg/kg bw/d (females)
Actual dose received by dose level and sex	180, 701 or 2829 mg/kg bw/d (males); 228, 901 or 3604 mg/kg bw/d (females)
Toxic Response/effects by Dose Level	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 was reported among 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, which 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 (3604 mg/kg bw/d). 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.

Appropriate statistical evaluations?	Yes
Conclusion Remarks	No biologically significant adverse effects were reported following administration of FD&C Red 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.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J.F., Olson J.W. and Reno F.E. (1991a) Lifetime toxicity/ carcinogenicity studies of FD&C Red No. 40 (Allura Red) in Sprague Dawley Rats. Food and Chemical Toxicology, 27, 701-705.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Test Substance	FD&C Red No. 40; 88% purity
Method/guideline	Lifetime Toxicity/Carcinogenicity Study
GLP	Ambiguous
Year	1991
Species/strain	Mice\Charles River CD1 (study A) and outbred CD-1 (study B)
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0.37, 1.39 or 5.19%
Exposure Period	104 weeks (Study A) or 109 weeks (Study B)
Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	In the in utero phase, 50 male and female mice each (study A) or 70 male and female 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, 1877 or 7422 mg/kg bw/d for males and 577, 2043 or 8304 mg/kg bw/d for females (study A) and 492, 1821, or 7318 mg/kg bw/d (males) and 526, 2057 or 8356 mg/kg bw/d (females) (study B). These Fo mice 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 Charles River mice each were administered the test substance 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 Fo

	offspring of parental mice (P1), 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.
NOAEL(NOEL)	Greater than 5.19%
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	507, 1877 or 7422 mg/kg bw/d for males and 577, 2043 or 8304 mg/kg bw/d for females (study A) and 492, 1821, or 7318 mg/kg bw/d (males) and 526, 2057 or 8356 mg/kg bw/d (females) (study B).
Toxic Response/effects by Dose Level	No treatment -related effects were observed for any parameter evaluated at any dose level.
Appropriate statistical evaluations?	Yes.
Remarks for Results	No treatment-related effects were reported on survival. The authors reported decreased food consumption among the mid- and high-dose females for wk 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 significance was questioned because there was no accompanying histopathology, and were not dose-dependent and were species-specific. The authors also 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 was reported in study B. However, statistical analyses of the data revealed no statistical significance in the finding of an apparent acceleration of lymphocytic lymphomas development.

Conclusion Remarks	No treatment-related adverse effects were reported at any dose level following lifetime administration of FD & C Red 40 to male and female mice. The second study, study B, conducted using a different strain of mouse to further investigate if FD&C Red 40 had an effect on the appearance of lymphocytic lymphomas, revealed no relationship between the incidence of lymphocytic lymphomas and FD&C Red 40.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J.F., Olson J.W. and Reno F.E. (1991b) Lifetime toxicity/ carcinogenicity studies of FD&C Red No. 40 (Allura Red) in mice. Food and Chemical Toxicology, 29, 313-319.

4.4 DEVELOPMENTAL TOXICITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	FDA Teratology Study
GLP	Yes
Year	1989
Species/strain	Rat/Osborne-Mendel (FDA strain)
Sex	Female
Route of Administration	Oral-drinking water
Duration of Test	20 days
Doses/concentration Levels	0, 0.2, 0.4 or 0.7%
Exposure Period	20 days
Frequency of Treatment	*ad libitum*
Control Group and Treatment	Yes

Remarks for Test Conditions	Four groups of female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD & C Red 40 in the drinking water at intake levels of 0, 0.2, 0.4 or 0.7% for the first 20 days of gestation. On day 20, 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.
NOAEL(NOEL) maternal toxicity	.7% or 939.29 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	Not determined
NOAEL (NOEL) developmental toxicity	273.58 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	545.68 mg/kg bw/d
Actual dose received by dose level and sex	0, 273.58, 545.68 or 939.29 mg/kg bw/d
Maternal data with dose level	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 biologically significant. No other effects were reported.
Fetal Data with Dose Level	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.
Appropriate statistical evaluations?	Yes, ANOVA, Fisher's Exact Test, t-test.
Remarks for results	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 also just outside that found in the historical controls, and the control group was noted as having a lower incidence compared to the historical controls.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References	Collins T., Black T.N., Welsch J.J., and Brown L.H. (1989a) Study of the teratogenic potential of FD & C Red No. 40 when given in drinking water. Toxicology and Industrial Health 5, 937-948.

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Method/guideline	FDA Teratology Study
GLP	Yes
Year	1989
Species/strain	Rat/Osborne-Mendel (FDA strain)
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	19 days
Doses/concentration Levels	0, 30, 75, 150, 300, 600 or 1000 mg/kg bw/d
Exposure Period	19 days
Frequency of Treatment	Daily
Control Group and Treatment	Yes
Remarks for Test Conditions	Four groups of female Osborne-Mendel (FDA strain) rats (42-43 per group) were administered FD & C Red 40 via gavage at dose levels of 0, 30, 75, 150, 300, 600 or 1000 mg/kg bw/d 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.
NOAEL(NOEL) maternal toxicity	1000 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	Not determined
NOAEL (NOEL) developmental toxicity	1000 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	Not determined
Appropriate statistical evaluations?	Yes, ANOVA, Fisher's Exact Test, t-test.
Actual dose received by dose level and sex	0, 30, 75, 150, 300, 600 or 1000 mg/kg bw/d
Maternal data with dose level	No clinical findings were reported and no deaths occurred during treatment. No other dose related findings were reported.

Fetal Data with Dose Level	The only significant skeletal anomaly found was an increase in 14th rib buds at the 300 mg/kg bw/d dose level but was not seen at the higher dose levels. No other soft-tissue or sternebral variations were reported.
Conclusion remarks	The NOAEL's for maternal and fetal toxicity were 1000 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Collins T., Black T.N., Welsch J.J., and Brown L.H. (1989b) Study of the teratogenic potential of FD & C Red No. 40 when given by gavage to rats. Fd. Chem. Toxic. Vol 27, pp 707-713.

4.5 REPRODUCTIVE TOXICITY

Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
CAS No.	25956-17-6
Remarks for Substance	FD&C Red No. 40; fine dark red powders without noticeable odor
Method/guideline	Not given
Test Type	Two generation reproductive study
GLP	Ambiguous
Year	1969
Species/strain	Rat/Charles River Caesarean albino
Sex	Male and Female
Route of Administration	Oral-Diet
Duration of Test	Two parental generations and two two-litter filial generations
Doses/concentration Levels	3700, 13,900 and 51,900 ppm
Premating Exposure period for males	27 weeks
Premating Exposure period for females	27 weeks
Frequency of Treatment	Daily

Control Group and Treatment	Yes, basal diet
Remarks for Test Conditions	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. These P1 parental generations were individually housed. Clinical observations included food consumption, appearance, individual body weights and behavior and were made weekly. The F1A weanling rats designated P2 generation were kept 4-5 to a cage according to sex and maintained on the same concentration level as their parents until reaching maturity. During the breeding phase of the P1 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 P2 generation. Following the weaning of the F1A animals, the P1 generation was remated to produce their second litters referred to as F1B, according to the procedures described above. The P2 generation was housed 4-5 per cage and was maintained on the same dietary levels as their parents. The procedures outlined above for the P1 generation were maintained for the P2 generation. The litters of the P2 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 P2 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.
NOAEL(NOEL)	13,900 ppm
LOAEL(LOEL)	51,900 ppm
Actual dose received by dose level and sex	Not given
Parental data and F1 as appropriate	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 3700 ppm test group in the F2A breeding cycle as well as the 3700 and 51900 ppm test groups in the F2B breeding cycle were reported to be low in comparison to control animals and historical control data.
Offspring toxicity F1 and F2	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 40 caused meaningful growth suppression in the pups whose parents received the high level diets.
Appropriate statistical evaluations?	Not given
Conclusion remarks	The authors reported a NOAEL for reproductive toxicity following administration of FD&C Red 40 as 13,900 ppm.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Hazelton Laboratories Inc. (1969) Two-generation reproductive study in rats. Red Z4576 (FD&C Red 40). Unpublished report 165-125.