201-15443

July 9, 2004

Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Administrator:

On behalf of the member companies of the HPV Committee, the International Association of Color Manufacturers is pleased to submit the test plan and robust summaries for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3). The IACM HPV Committee has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. A hard copy of this submission is available upon request. The EPA registration number for the IACM HPV Committee is

Please feel free to contact me with any questions or comments you might have concerning the submission (tadams@therobertsgroup.net or 202-331-2325).

Sincerely,

Timothy Adams, Ph.D.
Technical Contact Person for IACM HPV

201-15443A

Test Plan for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3)

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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List of Member Companies

Colorcon

Noveon, Inc.

Sensient Colors, Inc.

.

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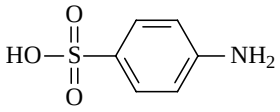
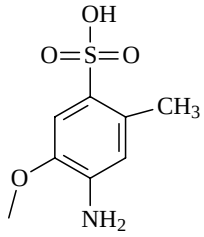
Test Plan for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3)

1 IDENTITY OF SUBSTANCES



Sulfanilic acid
CAS No. 121-57-3



o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)

CAS No. 6471-78-9

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 sulfanilic acid (CAS No. 121-57-3) and for the related substance, o-toluene sulfonic acid, 4-amino-5-methoxy- (p-cresidine sulfonic acid) (CAS No. 6471-78-3). Both are used as intermediates in the production of azo dyes. In anaerobic conditions, azo dyes undergo bacterial azo reduction in the gastrointestinal tract of rats, rabbits and humans to yield the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid (Allan & Roxon, 1974; Chung et al., 1978; Dubin & Wright, 1975; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980). These data support the conclusion that human health data on FD&C Yellow 5 and 6, and FD&C Red 40 is relevant to sulfanilic acid and p-cresidine sulfonic acid in that FD&C Yellow 5 and 6 and FD&C Red 40 form sulfanilic acid and p-cresidine sulfonic acid, respectively, in animals, which is then absorbed, metabolized and excreted.

2.3 STRUCTURAL CLASSIFICATION

Sulfanilic acid and p-cresidine sulfonic acid are both aromatic aminosulphonic acids. p-Cresidine sulfonic acid is sulfanilic acid substituted with *o*-methoxy, and a *m*-methyl groups.

2.4 PHARMACOKINETICS AND METABOLISM

Sulfanilic acid is principally excreted unchanged in the urine and in the feces with lesser amounts excreted as the N-acetyl derivatives of the amine functional group. N-acetylation occurs both in the gastrointestinal tract and following absorption as shown by N-acetyl conjugates appearing in the urine and in the feces.

Following oral administration of 25 mg of sulfanilic acid to rats, the 24-hr urine showed approximately 40% excreted unchanged in the urine with approximately 15% excreted in the conjugated form which was presumed to be the N-acetyl derivative. Approximately 40% (30 % excreted unchanged and 10% excreted in the conjugated form) was excreted in the feces by 48 hours (Scheline and Longberg, 1965).

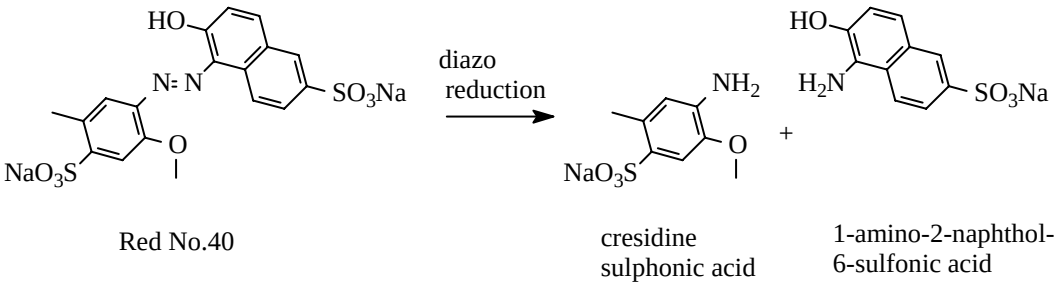
In a study comparing sulfanilic acid in the rat, rabbit and guinea-pig, 10 mg/animal was administered via gavage to the rats (10 animals) and guinea-pigs (10 animals), while 120 mg/animal was administered to the rabbit (8 animals). The remaining two rabbits received 630 mg/kg bw via gavage. All three animals excreted sulfanilic acid in the urine as either the free material or as an N-acetyl derivative. The rat excreted 36 and 13%, the guinea-pig 12 and 36% and the rabbit 76 and 16% as the unchanged material and metabolite, respectively. The authors reported 26, 74 and 17 % as the percentage of acetylated compound in the recovered material (McMahon and Reilly, 1969).

Azo dyes undergo bacterial azo reduction in the anaerobic environment of the gastrointestinal tract of rats, rabbits and humans to the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid. Studies across species have shown that FD&C Yellow 5 is reduced to sulfanilic acid and a pyrazalone metabolite, which is further reduced by intestinal bacteria first to p-phenylhydrazinesulfonic acid and then to sulfanilic acid; FD&C Yellow 6 is reduced to sulfanilic acid and amino-2-naphthol-6-sulfonic acid; and FD&C Red 40 is reduced to p-cresidine sulfanilic acid and amino-2-naphthol-6-sulfonic acid in the intestines (see Figure 1) (Allan & Roxon, 1974;

Chung et al., 1978; Dubin & Wright, 1975; Hazleton Laboratories, 1975b; Honohan *et al*., 1977; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980).

Figure 1. Reduction of azo dyes

Metabolism of FD&C Red 40

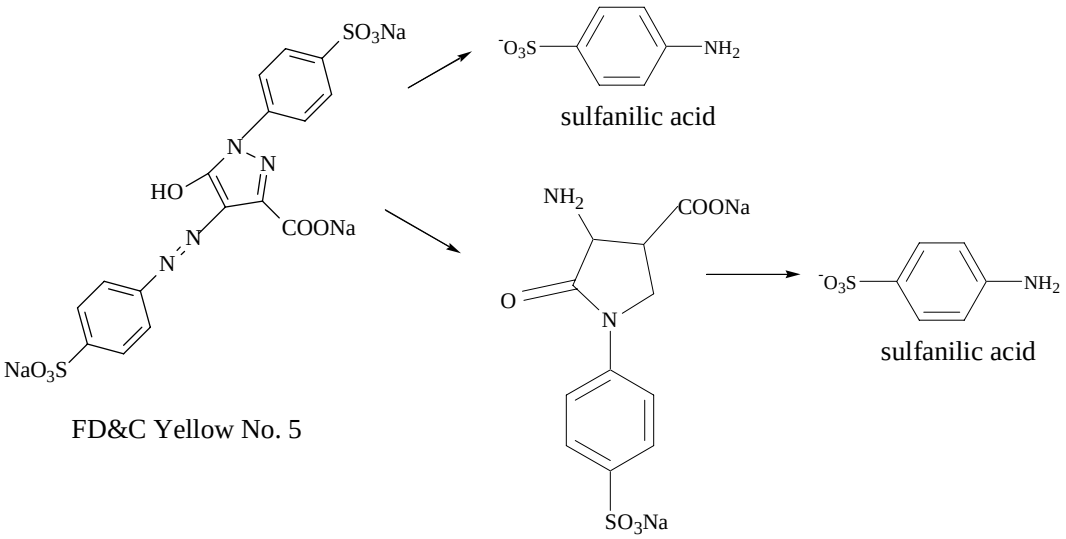


Red No.40

cresidine sulphonic acid

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

Metabolism of FD&C Yellow 5



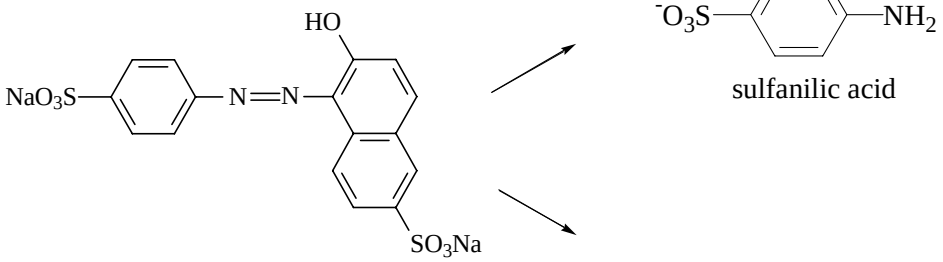
FD&C Yellow No. 5

sulfanilic acid

sulfanilic acid

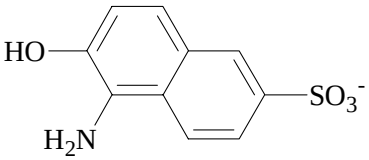
4-amino-5-oxo-1(p-sulphophenyl)-2-pyrazoline-3-carboxylic acid

Metabolism of FD&C Yellow 6



FD&C Yellow No. 6

sulfanilic acid



amino-2-naphthol-6-sulfonic acid

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

Sulfanilic acid decomposed without melting when heated to 288 °C (Merck, 1997). p-Cresidine sulfonic acid has a calculated melting point of 152.63 °C (MPBPVPWIN EPI Suite, 2000).

3.1.2 Boiling Point

The boiling point of sulfanilic acid was calculated to be 363.26 °C while the boiling point of p-cresidine sulfonic acid was calculated to be 398.51 °C (MPBPVPWIN EPI Suite, 2000).

3.1.3 Vapor Pressure

The calculated vapor pressure for sulfanilic acid has been reported to be 2.62 X 10^{-9} mm Hg at 25°C while the vapor pressure of p-cresidine sulfonic acid is calculated to be 1.02 X 10^{-8} mm Hg at 25°C (MPBPVPWIN EPI Suite, 2000). Based on the presence of polar sulfonic acid and amine functional groups, a very low vapor pressure is anticipated for these substances.

3.1.4 Octanol/Water Partition Coefficients

The experimental log K_{OW} value for sulfanilic acid is –2.16 (Okamoto et al., 1991). The calculated log K_{OW} value closely matches the experimental value for sulfanilic acid and is -2.08 (KOWWIN EPI Suite, 2000). An experimental value is not available for p-cresidine sulfonic acid, however the calculated value is -1.45 (KOWWIN

EPI Suite, 2000). Given the presence of sulfonic acid and amine functional groups on both sulfanilic acid and p-cresidine sulfonic acid, similar log K_{OW} values are anticipated.

3.1.5 Water Solubility

Sulfanilic acid has a reported water solubility of 10,800 mg/L at 20 °C (Yalkowsky and Dannenfelser, 1992). The calculated value for sulfanilic acid is 41,530 mg/L at 25 °C (WSKOW EPI Suite, 2000). An experimental value is not available for p-cresidine sulfonic acid, however the calculated value is 6208 mg/L at 25 °C based on the calculated log K_{OW} (WSKOW EPI Suite, 2000). The calculated values for both sulfonic acid derivatives are consistent with the experimental value available for sulfanilic acid.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life for hydroxyl radical reactions is 5.5 and 2.4 hours for sulfanilic acid and p-cresidine sulfonic acid, respectively (AOPWIN EPI Suite, 2000). These calculated short half-lives are consistent with ready abstraction of the sulfonic acid hydrogen by hydroxyl radicals.

3.2.2 Stability in Water

Potential reactivity in water would involve desulfonation of the aromatic sulfonic acid. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at high temperatures (i.e. 100 to 175 ^{o}C). These conditions would not typically be encountered in the environment. Therefore, sulfanilic acid and p-cresidine sulfonic acid are anticipated to be stable in water.

3.2.3 Biodegradation

In a study assessing biodegradability by manometric respirometry conducted by the Commission of European Communities, five of the eight laboratories employed in the study determined sulfanilic acid was <20% biodegradable. A sixth laboratory where the inoculum was adapted to sulphonic acids found sulfanilic acid to be 70% biodegradable at 10 days and 90% biodegradable at 28 days. A seventh laboratory found duplicates on one occasion to give no removal while a single determination yielded 12% degradation. The eighth laboratory reported 6, 14 and 62% biodegradation after 28 days in three triplicates (Commission of European Communities, 1983). Based on results of the majority of eight separate laboratory determinations, it can be concluded that both sulfonic acid derivatives are not readily biodegradable.

Sulfanilic acid and p-cresidine sulphonic acid were predicted not 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 (EPIWIN EPI Suite, 2000). 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 sulfanilic acid and p-cresidine sulfonic acid are distributed completely to the water and soil compartments. These data are consistent with experimental ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L (Alstoffe, 1992).

3.2.5 New Testing Required

None.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Extensive studies on the ecotoxicity of benzene sulfonic acids have been conducted and indicate a low order of toxicity to fish (Alstoffe, 1992). An experimental 96-hour LC50 value is available for sulfanilic acid and was reported to be 100.4 mg/L (Alstoffe, 1992). Based on input parameters for molecular weight, water solubility, and melting point, the calculated 96-hour LC50s for sulfanilic acid and p-cresidine sulfonic acid are 5.39 X 10^5 and 2.31 X 10^5 mg/L, respectively (ECOSAR EPI Suite, 2000) indicating a very low order of acute toxicity to fish. Based on the slight increase in K_{OW} (-1.45) for p-cresidine sulfonic acid compared to that of sulfonic acid (-2.08) and the small difference in calculated 96-hour LC50 values, a 96-hour LC50 value slightly less than 100 mg/L is expected for p-cresidine sulfonic acid.

3.3.2 Acute Toxicity to Aquatic Invertebrates

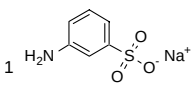
An experimental 24-hour EC50 value with *Daphnia* is available for sulfanilic acid and was reported to be 109.13 mg/L (Alstoffe, 1992). The calculated 48-hour LC50 values for sulfanilic acid and p-cresidine sulfonic acid in daphnids (151 and 128 mg/L, respectively) based on input parameters for molecular weight, water solubility, and melting point (ECOSAR EPI Suite, 2000) also indicating a low order of acute toxicity. These values are consistent with the experimental value available for sulfanilic acid. Based on the consistency in the measured and calculated values, the EC50 of both substances is concluded to be >100 mg/L.

3.3.3 Acute Toxicity to Aquatic Plants

Experimental values are available for the closely related substance, 3-amino-benzenesulfonic acid, monosodium salt[1]. The 72-hr EC50 for this substance was reported to be >500 mg/L in algae (Alstoffe, 1992). Calculated EC50's are not available. The calculated chronic toxicity value (ChV) for 3-amino-benzenesulfonic acid, monosodium salt, to green algae is 14,287 mg/L (log Kow = -3.87). Similarly, the calculated chronic toxicity value for sulfanilic acid is 11,234 mg/L to green algae while the chronic toxicity value for p-cresidine sulfonic acid is 6005 mg/L based on input parameters for molecular weight, water solubility, and melting point (ECOSAR EPI Suite, 2000)

3.3.4 New Testing Required

None.

1 

3-amino-benzenesulfonic acid, monosodium salt

3.4 HUMAN HEALTH TOXICITY

In anaerobic conditions, azo dyes undergo bacterial azo reduction in the gastrointestinal tract of rats, rabbits and humans to the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid. Studies across species have shown that FD&C Yellow 5 is reduced to sulfanilic acid and a pyrazalone metabolite, which is further reduced by intestinal bacteria first to p-phenylhydrazinesulfonic acid and then to sulfanilic acid; FD&C Yellow 6 is reduced to sulfanilic acid and amino-2-naphthol-6-sulfonic acid; and FD&C Red 40 is reduced to p-cresidine sulfanilic acid and amino-2-naphthol-6-sulfonic acid in the intestines (see Figures) (Allan & Roxon, 1974; Chung et al., 1978; Dubin & Wright, 1975; Hazleton Laboratories, 1975b; Honohan *et al*., 1977; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980).

Few human health toxicity studies have been carried out on aromatic amino sulfonic acids (AASA) such as the two considered here most likely due to the expected low toxicity of these materials. Jung et al. has proposed that it is reasonable to consider the carcinogenicity data available on the dyes when reviewing the safety of aromatic amino sulfonic acids (Jung et al., 1992) given that hypothetically the dyes can only form AASAs on reductive cleavage of the azo group. In the case of FD&C Red 40, and FD&C Yellow 5 and 6, numerous human health studies are available and have been submitted and deemed adequate for fulfilling the HPV endpoints by the EPA. Given the very high doses employed in these tests, substantial amounts of either sulfanilic acid or p-cresidine sulfonic acid are presumably formed in the gastrointestinal tract during these assays.

3.4.1 Acute Toxicity

Acute toxicity data are available for the parent compounds.

FD&C Yellow No. 5

In reports submitted to the World Health Organization, the acute oral LD50 in mice was reported to be 12,750 mg/kg bw for FD&C Yellow No. 5 (National Institute of Hygienic Sciences of Japan, 1964). In rats, the LD50 for FD&C Yellow No. 5 by intraperitoneal injection was reported to be 2,000 mg/kg bw and the LD50 by intravenous injection was reported to be 1,000 mg/kg bw (Deutsche Forschungsgemeinschaft, 1957).

FD&C Yellow No. 6

The low acute oral toxicity of FD&C Yellow No. 6 is reflected by LD50 values greater than 2,000 mg/kg (Lu and Lavalle, 1964) and 10,000 mg/kg (Gaunt *et al*., 1967) in rats, and greater than 6,000 mg/kg in mice (Gaunt *et al*., 1967).

In a pre-GLP acute toxicity study, adult male Wistar rats were administered 2000 mg/kg bw of FD&C Yellow No. 6 *via* stomach tube. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw (Lu and Lavalle, 1964).

In another pre-GLP acute toxicity study, groups of five male and female rats each were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 10,000 mg/kg bw. Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours

prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 6000 mg/kg bw Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 in mice was determined to be greater than 6000 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 3800 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 5500 mg/kg bw (Gaunt *et al.*, 1967).

FD&C Red No. 40

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 FD&C Red No. 40 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

Sulfanilic acid tested negative in several reverse mutation assays using *Salmonella typhimurium* TA1538, TA1535, TA 97, TA98, TA100 up to 10,000 micrograms/plate with and without metabolic activation (Chung et al., 1978; Zeiger, 1988; Litton Bionetics, 1985). Negative assay results were also reported in a GLP sister chromatid exchange assay (SCE) when sulfanilic acid was incubated with Chinese

Hamster Ovary cells with and without metabolic activation up to 5.0 mg/ml (Litton Bionetics, 1985). Sulfanilic acid was considered inactive in a GLP mouse lymphoma forward mutation assay with and without metabolic activation at concentrations up to 5000 micrograms/ml (Litton Bionetics, 1985).

3.4.2.2 In vivo

In vivo genotoxicity data are available for the parent compound, FD&C Yellow 5 and Yellow No. 6. In an *in vivo* UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw. FD&C Yellow No. 5 *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested (Kornbrust and Barfknecht, 1985). 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

FD&C Yellow No. 5

Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD&C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, while detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses.

Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus, thyroid gland including parathyroid, trachea, and urinary bladder. Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. The no observable adverse effect level (NOAEL) of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/day was established for male and female mice under the conditions of this study (Borzelleca and Hallagan, 1988b).

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. Animals were housed individually and fed the test diet *ad libitum*.

Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of FD&C Yellow 5 was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.

During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no treatment related effects on pup survival.

In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the

females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. A NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/day and 3348 mg/kg/day for male and female rats, respectively, was reported under the conditions of this study (Borzelleca and Hallagan, 1988a).

FD&C Yellow No. 6

Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD&C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level. The NOAEL's were reported to be 50,000 ppm and less than 6,000 ppm for male and female mice, respectively (NTP, 1981).

Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined animals at the 50,000 or 100,000 ppm intake levels. The NOAEL's were reported to be 6000 ppm for female rats and 12,500 ppm for male rats (NTP, 1981).

Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD&C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not

considered clearly related to administration of the test material given the variability in tumor occurrence in control male B6C3F1 mice and because the incidence of these tumors was not significantly increased in the high dose male mice. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in B6C3F1 mice (NTP, 1981).

Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included the adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in F344/N rats (NTP, 1981).

FD&C Red No. 40

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 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 significance 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

FD&C Red No. 40

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

FD&C Yellow No. 5

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups (Borzelleca and Hallagan, 1988a).

FD&C Yellow No. 6

In a three-generation reproduction study, 150 Charles River CD rats (10 males and 20 females/group/generation) received FD&C Yellow No. 6 at dietary levels of 0, 5, 50, 150, or 500 mg/kg/day. No treatment-related effects were observed in the parental rats or the pups receiving oral doses of up to 500 mg/kg bw/day (International Research and Development Corporation, 1974).

FD&C Red No. 40

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**
Sulfanilic acid CAS No. 121-57-3	A, Calc	Calc	Calc	A, Calc	A, Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	Calc	Calc	Calc	Calc	Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Sulfanilic acid CAS No. 121-57-3	Calc	NA	A, Calc	Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Sulfanilic acid CAS No. 121-57-3	A, Calc	A, Calc	R, Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	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**
Sulfanilic acid CAS No. 121-57-3	R	A	R	R	R	R
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	R	R	R	R	R	R

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

4 REFERENCES FOR TEST PLAN AND ROBUST SUMMARIES

Allan R. & Roxon J. (1974) Metabolism by intestinal bacteria: The effect of bile salts on tartrazine azo reduction. *Xenobiotica* 4, 637-643.

Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt as *cited in* 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.

Ames B.N., McCann J. and Yamasaki E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research*, **31**, 347.

AOPWIN EPI Suite (2000) U S Environmental Protection Program.

AOPWIN EPI Suite (2000) US Environmental Protection Agency.

BIOWIN EPI Suite (2000) US Environmental Protection Agency.

Borzelleca J. and Hallagan J. (1988a) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in mice. *Food and Chemical Toxicology* 26, 189-194.

Borzelleca J. and Hallagan J. (1988b) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in rats. *Food and Chemical Toxicology* 26, 179-187.

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.

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.

Chung K.T., Fulk G.E., & Andrews A.W. (1978) The mutagenicity of methyl orange and metabolites produced by intestinal anaerobes. *Mutation Research,* 58, 375-379.

Chung K.T., Fulk G.E., & Andrews A.W. (1981) Mutagenicity testing of some commonly used dyes. *Applied and Environmental Microbiology* 42, 641-648.

Collins T., Black T.N., Brown L.H., and Bulhack P. (1990) Study of the teratogenic potential of FD&C Yellow No. 5 when given by gavage to rats. *Fd. Chem. Toxic*. **28**, 821-827.

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.

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. *Food Chemical* Toxicology, 27, 707-713.

Commission of European Communities (1983) Ring-test programme 1981-82. Assessment of biodegradability of chemicals in water by manometric respirometry. National Technical Information Service. OTS0516839.

Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany, Farbstoff Kommission (1957) Mitteilung 6.

Dubin P. & Wright S. (1975) Reduction of azo food dyes in cultures of Proteus vulgaris. Xenobiotica. 5, 563-571.

ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet

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.

Hazelton Laboratories Inc. (1969) Two-generation reproductive study in rats. Red Z4576 (FD&C Red 40). Unpublished report No.165-125.

Hazelton Laboratories, Inc. (1965a) Acute oral administration-rats. Five experimental non-toxic red colors. Unpublished Report No. 165-114.

Hazelton Laboratories, Inc. (1965b) Acute oral administration-dogs. Five experimental non-toxic red colors. Unpublished Report.

International Research and Development Corporation (1972) Teratology study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-004.

International Research and Development Corporation (1974) Multi-generation reproduction study in rats. Compound FD&C Yellow No. 6. Unpublished report no.

Klimisch H. J., Andreae, M. and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Journal of Regulatory Toxicology and Pharmacology, 25, 1-5.

Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Enviromental Mutagenesis 7, 101-120.

KOWWIN EPI Suite (2000) U S Environmental Protection Agency.

Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the SCE assay in Chinese Hamster Ovary Cells. Unpublished report to IACM.

Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the AMES Salmonella Plate test. Unpublished report to IACM.

Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the Mouse Lymphoma Forward Mutation Assay. Unpublished report to IACM.

Lu F. and Lavalle C. (1964) The acute toxicity of some synthetic colours used in drugs and foods. Canadian Pharmaceutical Journal 9

McMahon K.A. and O'Reilly W.J. (1972) The metabolism of sulfanilic acid in the rat, rabbit and guinea pig. *Food and Cosmetic Toxicology* **7**, 493-496.

Merck (1997) Merck Index. Whitehouse Station, NJ.

MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

National Institute of Hygienic Sciences of Japan. Unpublished data submitted to WHO, 1964 cited in ILSI report on FD&C Yellow 5 6/2/83.

NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

Okamoto H., Hahida M. and Sezaki H. (1991) Effect of 1-alkyl- or 1-alkenylazacycloalkanone derivatives on the penetration of drugs with different lipophilicities through guinea pig skin. *Journal of Pharmaceutical Sciences*, 80, 39-45.

Roxon J., Ryan A. and Wright S. (1967a) Enzymatic reduction of tartrazine by Proteus vulgaris from rats. Food and Cosmetic Toxicology 5, 645-656.

Roxon J., Ryan A. and Wright S. (1967b) Reduction of water-soluble azo dyes by intestinal bacteria. Food and Cosmetic Toxicology 5, 367-369.

Scheline R. and Longberg B. (1965) The absorption, metabolism and excretion of the sulphonated azo dye, acid yellow, by rats. *Acta pharmacol. et toxicol.* **23**, 1-14.

Watabe T., Ozawa N., Kobayashi F. & Kurata H. (1980) Reduction of sulphonated water-soluble azo dyes by micro-organisms from human feces. *Food and Cosmetic Toxicolog* **18**, 349-352.

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),** 1403-1408.

WSKOW EPI Suite (2000) U S Environmental Protection Agency.

Yalkowsky and Dannenfelser (1992) Aquasol database of aqueous solubility. Version 5.; College of Pharmacy, University of Arizona. Tucson, AZ. Pc Version.; 1992 Cited in SRC PhysProp Database. Syracuse Research Corporation 2004.

Zeiger E., Anderson B., Haworth S., Lawlor T., and Mortelmans K. (1988) (Salmonella Mutagenicity Tests: IV. Results from the testing of 300 chemicals. Environmental and Molecular Mutagenesis 2, 1-158.

201-15443A

Robust Summaries for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3)

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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.

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Robust Summaries for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3)

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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for substance	Not given
Method/guideline	Experimental
GLP	Ambiguous
Year	1997
Remarks for Test Conditions	
Melting Point	
Decomposition	288 C (decomposes without melting)
Sublimation	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Merck (1997) Merck Index. Whitehouse Station, NJ.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Melting Point	
Decomposition	152.63 deg
Sublimation	
Remarks for Results	
Conclusion Remarks	
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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Boiling Point	363.26 deg C
Pressure	
Pressure Unit	
Decomposition	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Boiling Point	398.51 deg C
Pressure	
Pressure Unit	
Decomposition	
Remarks for Results	
Conclusion Remarks	
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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for substance	
Method/guideline	Calculated/Mean of Antoine & Grain
GLP	No
Year	
Remarks for Test Conditions	
Vapor Pressure	2.62×10^{-9} mm Hg
Temperature	25 C
Decomposition	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for substance	
Method/guideline	Calculated/Mean of Antoine & Grain
GLP	No
Year	
Remarks for Test Conditions	
Vapor Pressure	1.02 X 10^{-8} mm Hg
Temperature	25 C
Decomposition	
Remarks for Results	
Conclusion Remarks	
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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Log Pow	-2.08
Temperature	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for substance	
Method/guideline	Experiemental
GLP	Ambiguous

Year	1991
Remarks for Test Conditions	Partition coefficients between n-octanol and water were determined by dissolving 0.1 mM in water saturated with n-octanol. Each solution contained 0.5 uCi of radio-labeled compound per 10 mL of solution. Two milliliter aliquots of each solution were withdrawn and mixed with the same volume of the counterphase solvent. The mixture was shaken for 10 min, centrifuged and left at 37 deg C for 48 hr. After equilibration, a 1 ml aliquot of each pahse was withdrawn into a vial. The partition coefficients were calculated as the ratio of radioactivity in the organic phase to that in the aqueous phase.
Log Pow	-2.16
Temperature	37 deg C
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Okamoto H., Hashida M. and Sezaki H. (1991) Effect of 1-Alkyl- or 1-alkenylazacycloalkanone derivatives on the penetration of drugs with different lipophilicities through guinea pig skin. Journal of Pharmaceutical Sciences 80, 39.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Log Pow	-1.45
Temperature	
Remarks for Results	
Conclusion Remarks	
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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Value (mg/L) at temperature	41,530 mg/L at 25 deg C
Description of Solubility	
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOW EPI Suite (2000) U S Environmental Protection Agency.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	Experimental
GLP	Ambiguous
Year	1992
Remarks for Test Conditions	Not given
Value (mg/L) at temperature	10,800 mg/L at 20 deg C
Description of Solubility	
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Yalkowsky and Dannenfelser (1992) Cited in SRC PhysProp Database. Syracuse Research Corporation 2004.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Value (mg/L) at temperature	6,208 mg/L at 25 deg C
Description of Solubility	
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOW EPI Suite (2000) U S Environmental Protection Agency.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 PHOTODEGRADATION

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	
Test Type	AOPWIN
GLP	
Year	
Light Source	
Light Spectrum (nm)	
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	
Concentration of Substance	
Temperature	
Direct photolysis	
Halflife t1/2	5.5 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data	Code 4. Calculated.

Reliability	
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	
Test Type	AOPWIN
GLP	
Year	
Light Source	
Light Spectrum (nm)	
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	
Concentration of Substance	
Temperature	
Direct photolysis	
Halflife t1/2	2.4 hours
Degradation % after	
Quantum yield	
Indirect photolysis	
Sensitizer	
Concentration of sensitizer	
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
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

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method	
Test Type	Calculated

GLP	
Year	
Contact time (units)	
Innoculum	
Remarks for Test Conditions	
Degradation % after time	
Results	
Kinetic	
Time required for 10% degradation	
10 day window criteria	
Total degradation	
Classification	Not readily biodegradable
Breakdown products	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method	
Test Type	Calculated
GLP	
Year	
Contact time (units)	
Innoculum	
Remarks for Test Conditions	
Degradation % after time	
Results	
Kinetic	

Time required for 10% degradation	
10 day window criteria	
Total degradation	
Classification	Not readily biodegradable
Breakdown products	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method	Manometric respirometry
Test Type	Experimental
GLP	Ambiguous
Year	1983
Contact time (units)	28 days
Innoculum	Sludge grown on sewage
Remarks for Test Conditions	Eight laboratories were used in this ring-test programme to determine reliability of a method based on a UK adaptation of the Japanese MITI test to assess biodegradability.
Degradation % after time	
Results	Five of the eight laboratories employed in the study determined sulfanilic acid was <20% biodegradable. A sixth laboratory where the inoculum was adapted to sulphonic acids found sulfanilic acid to be 70% biodegradable at 10 days and 90% biodegradable at 28 days. A seventh laboratory found duplicates on one occasion to give no removal while a single determination yielded 12% degradation. The eighth laboratory reported 6, 14 and 62% biodegradation after 28 days in three triplicates.
Kinetic	
Time required for 10% degradation	
10 day window criteria	
Total degradation	
Classification	<20% biodegradable
Breakdown products	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References	Commission of European Communities (1983) Ring-test programme 1981-82. Assessment of biodegradability of chemicals in water by manometric respirometry. National Technical Information Service. OTS0516839.

2.3 FUGACITY

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Air
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.000974%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III

Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Sediment
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.0755%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Soil
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	54.6%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Water
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	45.3%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Air
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.00000585%

Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Sediment
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.0918%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Soil
absorption coefficient	
Desorption	
Volatility	
Model data and results	

Estimated Distribution and Media Concentration	50.1%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Water
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	49.8%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

3 ECOTOXICITY

3.1 ACUTE TOXICITY TO FISH

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	
Test Type	Experimental
GLP	Ambiguous

Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 greater than 100.4 mg/L
Reference substances	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt as cited in 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.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	
Analytical monitoring	
Exposure period (unit)	96 hr.
Remarks for Test Conditions	Input parameters: molecular weight, water solubility, and melting point
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 5.39 X 10^{5} mg/L
Reference substances	

Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	
Analytical monitoring	
Exposure period (unit)	96 hr.
Remarks for Test Conditions	Input parameters: molecular weight, water solubility, and melting point
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 2.31 X 10^5 mg/L
Reference substances	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

3.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	
Test Type	Experimental

GLP	
Year	
Analytical procedures	
Species/Strain	Daphnia magna
Test details	24 hour
Remarks for Test Conditions	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
EC50, EL50, LC0, at 24,48 hours	EC50 = 109.13 mg/L
Biological observations	
Control response satisfactory	
Appropriate statistical evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt as cited in 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.

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Analytical procedures	
Species/Strain	Daphnia magna
Test details	48 hours

Remarks for Test Conditions	Input parameters: molecular weight, water solubility, and melting point
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
EC50, EL50, LC0, at 24,48 hours	EC50= 151 mg/L
Biological observations	
Control response satisfactory	
Appropriate statistical evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Analytical procedures	
Species/Strain	Daphnia magna
Test details	48 hours
Remarks for Test Conditions	Input parameters: molecular weight, water solubility, and melting point
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	

EC50, EL50, LC0, at 24,48 hours	EC50= 128 mg/L
Biological observations	
Control response satisfactory	
Appropriate statistical evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

3.3 ACUTE TOXICITY TO AQUATIC PLANTS

CAS	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Green algae
Endpoint basis	
Exposure period (duration)	
Analytical monitoring	
Remarks for Test Conditions	Input parameters: Water solubility; Molecular weight, Melting Point
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	Chronic toxicity value: 11,234 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory	
Appropriate statistical	

evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency.

CAS	6471-78-9
Substance Name	o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)
Remarks for Substance	
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Green algae, Selenatrum capricornutum
Endpoint basis	
Exposure period (duration)	
Analytical monitoring	
Remarks for Test Conditions	Input parameters: Water solubility; Molecular weight, Melting Point
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	Chronic toxicity value: 6005 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory	
Appropriate statistical evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency.

CAS	1126-34-7
Substance Name	3-amino-benzenesulfonic acid, monosodium salt
Remarks for Substance	
Method/guideline	Experimental
Test Type	
GLP	
Year	
Species/Strain/Supplier	Algae
Endpoint basis	
Exposure period (duration)	
Analytical monitoring	
Remarks for Test Conditions	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	EC50 at 96 hours: >500 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory	
Appropriate statistical evaluations	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt as cited in 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.

3.4 ACUTE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23

Remarks for Substance	FD&C Yellow 5
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1957
Species/Strain	Rat
Sex	Not reported
# of animals per sex per dose	Not given
Vehicle	Not given
Route of administration	Intraperitoneal
Remarks for test conditions	
Value LD50 or LC50 with confidence limits	2,000 mg/kg bw
Number of deaths at each dose level	
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany, Farbstoff Kommission (1957) Mitteilung 6.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1957
Species/Strain	Rat
Sex	Not reported

# of animals per sex per dose	Not given
Vehicle	Not given
Route of administration	Intravenous
Remarks for test conditions	
Value LD50 or LC50 with confidence limits	1,000 mg/kg bw
Number of deaths at each dose level	
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany, Farbstoff Kommission (1957) Mitteilung 6.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1964
Species/Strain	Mice
Sex	Not reported
# of animals per sex per dose	Not given
Vehicle	1% gum arabic
Route of administration	Oral
Remarks for test conditions	
Value LD50 or LC50 with confidence limits	12,750 mg/kg bw
Number of deaths at each dose level	
Remarks for results	

Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	National Institute of Hygienic Sciences of Japan. Unpublished data submitted to WHO, 1964 cited in ILSI report on FD&C Yellow 5 6/2/83.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1964
Species/Strain	Rats/Wistar
Sex	Male
# of animals per sex per dose	6
Vehicle	Water
Route of administration	Oral-Gavage
Remarks for test conditions	Wistar adult male rats were administered 2000 mg/kg bw *via* stomach tube.
Value LD50 or LC50 with confidence limits	Greater than 2000 mg/kg bw
Number of deaths at each dose level	0 deaths
Remarks for results	
Conclusion remarks	The oral LD50 for sunset yellow is greater than 2000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Lu F. and Lavalle C. (1964) The acute toxicity of some synthetic colours used in drugs and foods. Canadian Pharmaceutical Journal 9.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow

Remarks for Substance	FD&C Yellow 6; greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 10,000 mg/kg
Number of deaths at each dose level	No deaths at up to 10,000 mg/kg bw.
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6; greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No

Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 6000 mg/kg bw
Number of deaths at each dose level	No deaths at up to 6000 mg/kg bw
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6; greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water

Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	3800 mg/kg bw (2900-4600 mg/kg bw)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals convulsions. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6; greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.

Value LD50 or LC50 with confidence limits	5500 (95% C.I.: 4600-6700) mg/kg bw (Males) 4600 (95% C.I.: 3900-5300) (Females)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals, convulsions. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

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	Greater than 10,000 mg/kg bw

confidence limits	
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.

3.5 GENETIC TOXICITY

3.5.1 In vitro Genotoxicity

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	99% purity
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1988
Species/Strain	Salmonella typhimurium TA1535, TA 97, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats (with and without)
Doses/concentration levels	0-1500 micrograms/plate
Statistical Methods	Not given

Remarks for test conditions	The pre-incubation method as described by Haworth et al., 1983 was used to perform reverse mutation Ames assays in S. typhimurium strains TA97, TA98, TA100 and TA1535 with and without metabolic activation. The test chemical (0.05 ml), Salmonella culture (0.10 ml), and S-9 mix or buffer (0.5 ml) were incubated at 37 degrees Celsius without shaking for 20 minutes. The plates were incubated for two days at 37 degrees Celsius. The test substance was tested in a toxicity assay to determine the dose range for the mutagenicity assay. The test substance was tested at half-log dose intervals up to a dose producing cytotoxicity, or the dose immediately below the dose eliciting toxicity. The test substance was tested at five doses in triplicate. The experiment was repeated one week later. Positive controls without metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1535), and 4-nitro-o-phenylenediamine (TA98). The positive control with activation with all strains was 2-aminoanthracene. DMSO was used as the solvent.
Result	Negative
Cytotoxic concentration	1500 micrograms/plate
Genotoxic effects	Negative
Appropriate statistical evaluations	Not given
Remarks for results	Negative
Conclusion remarks	Sulfanilic acid tested negative for mutagenicity in S. typhimurium strains TA 1535, TA100, TA97 and TA98.
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	Zeiger E., Anderson B., Haworth S., Lawlor T., and Mortelmans K. (1988) (Salmonella Mutagenicity Tests: IV. Results from the testing of 300 chemicals. Environmental and Molecular Mutagenesis 2, 1-158.

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	Dissolved in sterile distilled water.
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1978
Species/Strain	Salmonella typhimurium TA1538

Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats (with and without)
Doses/concentration levels	500 micrograms/plate
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA1538.
Result	Negative
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assay for reverse mutation using Salmonella typhimurium TA1538.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Chung K.T., Fulk G.E., & Andrews A.W. (1978) The mutagenicity of methyl orange and metabolites produced by intestinal anaerobes. Mutation Research, 58, 375-379

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	
Method/guideline	Ames plate incorporation and liquid pre-incubation
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1981
Species/Strain	Salmonella typhimurium TA1535, TA 1537, TA1538, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats (with and without)

Doses/concentration levels	.005- 5.0 mg/plate
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA1535, TA 1537, TA1538, TA98, TA100. Plate incorporation tests were conducted according to Ames et al., with the Andrews et al. modifications. Duplicates were performed at each of the six concentrations of the test substance. Mutagenic compounds were assayed using duplicate plates. A substance was considered positive when the number of revertants above background was at least twice the value of the historical control mean or twice the value of the current control mean, whichever was greater and a dose response curve could be generated. Positive controls without metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1535), and 4-nitro-o-phenylenediamine (TA98). The positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene.
Result	Negative
Cytotoxic concentration	1000 micrograms/plate for plate-incorporation, and 500 microgramsg/ml for pre-incubation test
Genotoxic effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assay for reverse mutation using Salmonella typhimurium TA1535, TA 1537, TA1538, TA98, TA100.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Chung K.T., Fulk G.E., & Andrews A.W. (1981) Mutagenicity testing of some commonly used dyes. Applied and Environmental Microbiology 42, 641-648.

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	White powder. Purity not given

Method/guideline	Ames Salmonella/microsome mutagenesis assay
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Yes
Year	1985
Species/Strain	Salmonella typhimurium TA 100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague-Dawley and Fisher rats (with and without)
Doses/concentration levels	1.0 to 10,000 micrograms per plate
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA100. The test material was examined directly and in the presence of liver and kidney microsomal enzyme preparations from Aroclor-induced Sprague Dawley and Fisher rats. The solvent used for preparing the solution and subsequent dilutions was used as the negative control, while the positive controls were used by not identified.
Result	Negative
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion remarks	The test substance did not exhibit genotoxic activity with or without metabolic acivation in the AMES assay using SAL 100.
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	Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the AMES Salmonella Plate test. Unpublished report to IACM.

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid

Remarks for Substance	White powder. Purity not given
Method/guideline	Sister Chromatid Exchange test was carried out using a Chinese hamster ovary (CHO).
Test Type	Sister Chromatid Exchange
System of Testing	Mammalian
GLP	Yes
Year	1985
Species/Strain	Chinese hamster ovary cells (CHO)
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats (with and without)
Doses/concentration levels	167, 500, 1670 or 5000 micrograms/ml
Statistical Methods	Not given
Remarks for test conditions	Sister chromatid exchange tests were carried out using Chinese hamster ovary cells. The cultures with and without metabolic activation were harvested after 24.5 hrs in BrdU. The negative control was McCoy's 5a, while the positive control was cyclophosphamide.
Result	Negative. With activation, the test substance did not induce SCE's at concentrations up to 5000 micrograms/mL.
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion remarks	No evidence of SCE was reported.
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	Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the SCE assay in Chinese Hamster Ovary Cells. Unpublished report to IACM.

CAS Numerical	121-57-3
Substance Name	Sulfanilic acid
Remarks for Substance	White powder. Purity not given.
Method/guideline	Mouse Lymphoma Forward Mutation Assay
Test Type	Forward mutation
System of Testing	Mammalian
GLP	Yes
Year	1985
Species/Strain	Mouse lymphoma cells
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats (with and without)
Doses/concentration levels	1500 micrograms/ml to 5000 mg/ml
Statistical Methods	Not given
Remarks for test conditions	Mouse lymphoma forward mutation assays were conducted using mouse lymphoma cells. Six treatment were analyzed for mutant induction and cytotoxicity.
Result	Negative
Cytotoxic concentration	Percent relative growth with activation at 1500 micrograms/ml was 103.6% and was 71.5% at 5000 ug/ml. Wihout activation percent relative growth was 10.47% at 1500 micrograms/ml and 67.7% at 5000 micrograms/ml.
Genotoxic effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	None of the treatments induced a mutant frequency that exceeded the minimum criterion of 36.4 X 10-6. The test material was therefore considered non-mutagenic without activation up to 5000 ug/ml. With activation, the test material was considered non-mutagenic because none of the treatments induced a mutant frequency that exceeded the minimum criterion of 44.6 X 10-6. Negative control mutant frequencies were all in the expected range and te positive control compounds yielded mutant frequencies greatly in excess of the background.
Conclusion remarks	The test substance was considered non-mutagenic with and without metabolic activation
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.

References	Litton Bionetics. (1985) Mutagenicity evaluation of Sulfanilic acid in the Mouse Lymphoma Forward Mutation Assay. Unpublished report to IACM.

3.5.2 In vivo Genotoxicity

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 94% purity
Method/guideline	Mirsalis and Butterworth, 1980
Test Type	Unscheduled DNA Synthesis
GLP	Ambiguous
Year	1985
Species/Strain	Rat/Sprague Dawley
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	500 mg/kg bw
Exposure period	2 hr; 15 hr
Remarks for test conditions	Six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg acid yellow 23/kg bw *via* gavage. The control animal was administered corn oil only. Animals were killed at two time points, 2 hours and 15 hours. If negative results were obtained at time point 1 and time point 2, the *in vivo* testing was terminated and considered to be negative. If the initial test at time point 1 yielded a positive response, the test substance was retested at that time point. If another positive response was observed, the test was considered positive. Time points are the time the test substance was administered prior to the start of liver perfusion and isolation of hepatocytes. Hepatocytes from rats were isolated and cultured according to the two step *in sit*u liver perfusion model (Malansky and Williams, 1982). Viable hepatocytes (2 X 10+5) were seeded in wells and incubated for 4 hours with [H3]-thymidine (10 uCi/ml) and the test substance (prepared in either DMSO or water) according to a procedure similar to Williams, 1977. Control incubations were conducted with and without DMSO. The

	authors state that DMSO had no effect on DNA repair. DNA repair was quantified by the autoradiographic determination of incorporated [3H]-thymidine. Net nuclear grains (NNG) were determined by counting the number of grains in each nuclei and subtracting the average number of grains present in the three equal size adjacent cytoplasmic areas. Average NNG counts of 5 or more were assumed to constitute a positive response, because these differed from the control response by greater than 2 standard deviations. In the negative controls, NNG counts ranged from -0.6- to -2.8 and from -0.9 to -2.1 for no solvent and 1% DMSO incubations, respectively. The proportion of cells with greater than or equal to 5 NNG was less than or equal to 8.1% for all control incubations. Therefore NNG below zero were considered negative responses. Concentrations of dyes producing 90% or greater detachment of the hepatocytes from the coverslips were assumed to be toxic and not counted. The positive control was Solvent Yellow 3 (o-aminoazotoluene).
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Experiment 1 Dose (mg/kg bw) Time Avg NNG % >5NNG 500 2 hr -2.6 (+/-3.7) 2 15 hr -1.3 (+/-2.6) 2
Genotoxic effects	Negative
NOEL (C)/ LOEL (C)	Greater than 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	C.I. Acid Yellow 23 did not induce unscheduled DNA synthesis in an invivo assay using rat hepatocytes isolated from the livers of Sprague Dawley rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Enviromental Mutagenesis 7, 101-120.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Rodent Micronucleus Test
Test Type	Rodent Micronucleus

GLP	Ambiguous
Year	1991
Species/Strain	Rat/PVG
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	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 the test substance. Bone marrow samples were taken at 24 and 48 hours later.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	
Genotoxic effects	No significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point and in either species was reported. Additionally, there was reported increase in the % PE (polychromatic erthyrocytes).
NOEL (C)/ LOEL (C)	
Appropriate statistical evaluations?	Yes.
Remarks for results	No effects.
Conclusion remarks	
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.

3.6 REPEATED DOSE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Chronic Toxicity/Carcinogenicity Study
GLP	Yes
Year	1988

Species/Strain	Rat/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 0.1, 1.0, or 2.0% (original study) 0, 5.0% (high dose study)
Exposure period	113 weeks (males) or 114 weeks (females) (original study); 122 weeks (males) or 125 weeks (females) high-dose study
Frequency of treatment	Daily
Control Group	Yes, 2 concurrent controls (original study); 1 concurrent control (high-dose study)
Post exposure observation period	
Remarks for test conditions	In the *in utero* phase, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD & C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including

	parathyroid, trachea, urinary bladder, uterus.
NOAEL(NOEL)	5.0 % (Males: 2641 mg/kg/d and Females: 3348 mg/kg/day)
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Males: 48, 491, 984 or 2641 mg/kg/day; Females: 58, 589, 1225 or 3348 mg/kg/day
Toxic response/effects by dose level	*In utero* There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no compound-related effects on pup survival. In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	The NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/d and 3348 mg/kg/d for male and female rats, respectively, under the conditions of this study.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988a) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in rats. Fd Chem Toxic 26, 179-187.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Chronic Toxicity/Carcinogenicity Study
GLP	Yes
Year	1988
Species/Strain	Mice/Charles River CD-1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 0.5, 1.5, or 5.0%
Exposure period	104 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	
Remarks for test conditions	Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD & C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, detailed physical examinations and palpations for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus,

	thyroid gland including parathyroid, trachea, and urinary bladder.
NOAEL(NOEL)	5.0 % (8103 mg/kg/day)
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	M: 714, 2173 or 8103; F: 870, 2662 or 9735 mg/kg/day
Toxic response/effects by dose level	Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	The NOAEL of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/d was established for male and female mice under the conditions of this study.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988b) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in mice. Fd Chem Toxic 26, 189-194.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6; 91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rats/F344/N

Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	25,000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	Greater than 25,000 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in F344/N rats.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

CAS Numerical	2783-94-0
Substance Name	FD&C Yellow 6; Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week (female mice)
Remarks for test conditions	Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	12,500 ppm
LOAEL(LOEL)	25,000 ppm
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males:

	control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not considered clearly related to administration of the test material given the variability in tumour occurrence in control male B6C3F1 mice and because the incidence of these tumours was not significantly increased in the high dose male mice.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in B6C3F1 mice.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

CAS Numerical	2783-94-0
Substance Name	FD&C Yellow 6; Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rat/F344/N
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm
Exposure period	12 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week

Remarks for test conditions	Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.
NOAEL(NOEL)	6000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	12,500 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined animals at the 50,000 or 100,000 ppm intake levels.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

CAS Numerical	2783-94-0
Substance Name	FD&C Yellow 6; Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm
Exposure period	12 weeks

Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.
NOAEL(NOEL)	50,000 ppm (male); less than 6000 ppm (female)
LOAEL(LOEL)	100,000 ppm (male); 6000 ppm (female)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

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

CAS No.	25956-17-6
Substance Name	2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt
Remarks for Test Substance	FD&C Red 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.

3.7 DEVELOPMENTAL TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 92.7% purity
Method/guideline	FDA Teratology Study
Test Type	
GLP	Yes
Year	1990
Species/Strain	Rat/Osborne-Mendel (FDA strain)
Sex	Female
Route of administration	Oral-Gavage
Duration of test	19 days
Doses/concentration levels	0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day
Exposure period	19 days

Frequency of treatment	Daily
Control Group and treatment	Yes
Remarks for test conditions	Female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD & C Yellow No. 5 via gavage at dose levels of 0, 60, 100, 200, 400, 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.
NOAEL(NOEL) maternal toxicity	Greater than 1000 mg/kg bw/day
LOAEL(LOEL) maternal toxicity	Not determined
NOAEL (NOEL) developmental toxicity	Greater than 1000 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	Not determined
Actual dose received by dose level and sex	0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day
Maternal data with dose level	No unusual behavior or external findings were reported. One female at the 60 mg/kg bw/day dose level died on gestation day 13 due to gavage difficulties. The mean daily food consumption of rats administered the 1000 mg/kg bw/day dose level was significantly greater than the controls. Initial body weight and maternal weight gain during gestation did not significantly differ between treated animals and controls. Pregnancy rate was similar among all groups.
Fetal data with dose level	No dose related findings were reported on fetal viability or fetal development. The incidence of sternebral variations was similar for all groups.
Appropriate statistical evaluations?	Yes, ANOVA, Fisher's Exact Test, t-test.
Remarks for results	The authors commented that the significant increase in food consumption observed in the highest dose group without a corresponding effect on body weight indicated an effect on food utilization.
Conclusion remarks	The authors concluded that FD&C Yellow No. 5 was not developmentally toxic or teratogenic under the conditions of the study. The NOAEL's for maternal and fetal toxicity were greater than 1000 mg/kg bw/day.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Collins T., Black T.N., Brown L.H., and Bulhack P. (1990) Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. Fd. Chem. Toxic. Vol 28, pp 821-827.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Teratogenicity study
Test Type	
GLP	Ambiguous
Year	1974
Species/Strain	Rat/Charles River CD
Sex	Female
Route of administration	Oral-Gavage
Duration of test	20 days
Doses/concentration levels	0, 100, 300 or 1000 mg/kg bw/day
Exposure period	9 days
Frequency of treatment	Daily
Control Group and treatment	Yes, three negative control groups were maintained and administered 0.5% methocel, while one positive control group was maintained and administered 7.5% mg/kg bw/day of retinoic acid.
Remarks for test conditions	FD&C Yellow No. 6 was administered by gavage at dose levels of 100, 300 or 1000 mg/kg bw/day to 140 female Charles River CD rats. Three negative control groups (20/group) received the vehicle control while one control group received the positive control (7.5% mg/kg bw/day retinoic acid). All females were dosed on days 6-15 of gestation. Cesarean sections were performed on the 20th day of gestation.
NOAEL(NOEL) maternal toxicity	
LOAEL(LOEL) maternal toxicity	Not given
NOAEL (NOEL) developmental toxicity	100 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	300 mg/kg bw/day
Actual dose received by dose level and sex	Not given
Maternal data with dose level	
Fetal data with dose level	The mean weights of the offspring from the 300 and 1000 mg/kg bw/day groups were decreased when compared to the average fetus weight of the combined negative controls. There were no compound related effects on early or late resorptions, empty implantation sites, body weight or numbers of live or

	dead fetuses. No teratogenicity was observed among the offspring.
Appropriate statistical evaluations?	Not given
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	International Research and Development Corporation (1972) Teratology study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-004.

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	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
Remarks for Substance	FD&C Red No. 40
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.

3.8 REPRODUCTIVE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Lifetime Toxicity/Carcinogenicity study
Test Type	
GLP	Ambiguous
Year	1988
Species/Strain	Rats/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	114 weeks
Doses/concentration levels	0, 0.1, 1.0, or 2.0% (original study) 0, 5.0% (high dose study)
Premating Exposure period for males	2 months
Premating Exposure period for females	2 months
Frequency of treatment	Daily
Control Group and treatment	Yes.
Remarks for test conditions	In the *in utero* phase, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD & C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls

	groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents. Animals were housed individually and fed the test diet ad libitum. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.
NOAEL(NOEL)	5.0 % (Males: 2641 mg/kg/d and Females: 3348 mg/kg/day)
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Males: 48, 491, 984 or 2641 mg/kg/day Females: 58, 589, 1225 or 3348 mg/kg/d
Parental data and F1 as appropriate	In utero There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the in utero phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the in utero phases of the high-dose study. There were no compound-related effects on pup survival.
Offspring toxicity F1 and F2	In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically

	significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988a) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in rats. Fd Chem Toxic 26, 179-187.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	3-generation reproductive study
Test Type	
GLP	Ambiguous
Year	1974
Species/Strain	Rat/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	
Doses/concentration levels	5, 50, 150 or 500 mg/kg bw/day

Premating Exposure period for males	
Premating Exposure period for females	
Frequency of treatment	Daily
Control Group and treatment	Yes.
Remarks for test conditions	One hundred twenty Charles River CD rats (10 males and 20 females/group/generation) received 5, 50, 150 or 500 mg/kg bw/day of the test substance as a dietary admixture in a three-generation study. Ten males and twenty females received no compound and served as controls.
NOAEL(NOEL)	500 mg/kg bw/day
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Not given
Parental data and F1 as appropriate	
Offspring toxicity F1 and F2	
Appropriate statistical evaluations?	
Remarks for results	There were no compound related effects on fertility, gestation, pup viability or lactation indices, on reproductive organs of females, or on organ weights among parents and offspring. There were no compound related lesions in any tissue examined histologically, including kidneys and adrenal glands from parental rats or from offspring.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	International Research and Development Corporation (1974) Multi-generation reproduction study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-005.

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.