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**Test Plan for
Sunset Yellow
CAS No. 2783-94-0**

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**Submitted to the EPA under the HPV Challenge Program by:
The International Association of Color Manufacturers/HPV Committee**

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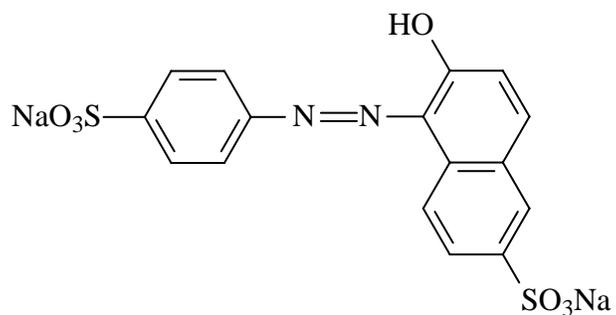
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Test Plan for Sunset Yellow

1 IDENTITY OF SUBSTANCES



Sunset Yellow

CAS No. 2783-94-0

Synonyms:

FD&C Yellow 6
C.I. Food Yellow 3

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 represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This test plan provides data for FD&C Yellow 6 (Sunset Yellow). FD&C Yellow No. 6 is a yellow powder that is freely soluble in water and is used as a food colorant in dairy products, snack foods, cereals, bakery items, confectionery products, frozen deserts and beverages, cosmetics, ingested and externally applied drugs, and dietary supplements.

FD&C Yellow No. 6 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*.

2.3 REGULATORY STATUS

FD&C Yellow 6 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is

tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

Table 1. US FDA Specifications

FD&C Yellow No. 6 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.706)

- Sum of volatile matter at 135°C (275°F) and chlorides and sulfates (calculated as sodium salts), not more than 13 percent.
 - Water-insoluble matter, not more than 0.2 percent.
- Sodium salt of 4-aminobenzenesulfonic acid, not more than 0.2 percent.
- Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid, not more than 0.3 percent.
- Disodium salt of 4,4'-(1-triazene-1,3-diyl)bis[benzenesulfonic acid], not more than 0.1 percent.
- Sum of the sodium salt of 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid, not more than 1 percent.
- Sum of the trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-,7-naphthalenedisulfonic acid and other higher sulfonated subsidiaries, not more than 5 percent.
- 4-Aminoazobenzene, not more than 50 parts per billion.
- 4-Aminophenyl, not more than 15 parts per billion.
 - Aniline, not more than 250 parts per billion
 - Azobenzene, not more than 200 parts per billion.
 - Benzidine, not more than 1 part per billion.
 - 1,3-Diphenyltriazene, not more than 40 parts per billion.
 - 1-(Phenylazo)-2-naphthalenol, not more than 10 parts per million.
 - Lead (as Pb), not more than 10 parts per million.
 - Arsenic (as As), not more than 3 parts per million.
 - Mercury (as Hg), not more than 1 part per million.
 - Total color, not less than 87 percent.

FD&C Yellow No. 6 was first listed for food use in the United States in 1929. In 1994, 994,406 kg of FD&C Yellow No. 6 dye and 283,680 kg of FD&C Yellow No. 6 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) have also evaluated the safety of FD&C Yellow No. 6 used as a coloring agent in food. An average daily intake (ADI) of 0-2.5 mg/kg bw/day was assigned by JECFA in 1982 based on the extensive human toxicological information available (see Table 2 below).

Table 2. Regulatory Approvals/Consumption Limits¹

USA	FD&C Yellow No. 6 may be safely used for coloring foods (including dietary supplements) generally in amounts consistent with good manufacturing practice, except that it may not be used to color foods for which standards of identify have been promulgated under section 401 of the act unless added color is authorized by such standards (21 CFR 74.706).
EEC	0-2.5 mg/kg (14th series, 1983)
JECFA	0-2.5 mg/kg (26th report, 1982)

Based on the long history of use of FD&C Yellow No. 6 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Yellow No. 6, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical “Right to Know” Program.

2.4 STRUCTURAL CLASSIFICATION

FD&C Yellow No. 6 is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4[(4-sulfophenyl)azo]-2,7-naphthalenesulfonic acid may be added in smaller amounts (USFDA-21 CFR 74.706). The diazo nucleus (-N=N-) contains a benzene ring substituted with a *p*-sulfonic acid group and a naphthalene ring substituted with *o*-hydroxy and *p*'-sulfonic acid groups.

¹ IACM, 2003

2.5 INDUSTRIAL PRODUCTION

FD&C Yellow No. 6 is manufactured by coupling diazotized sulfanilic acid with 2-naphthol-6-sulfonic acid. The dye is isolated as the sodium salt and dried.

2.6 PHARMACOKINETICS AND METABOLISM

The major route of metabolism for FD&C Yellow No. 6 is bacterial azo reduction in the gut. The major metabolites of FD&C Yellow No. 6 are sulfanilic acid and amino-2-naphthol-6-sulfonic acid [Honohan *et al.*, 1977].

Rats orally administered a single oral dose of 100 mg FD&C Yellow No. 6 (Sunset Yellow) excreted 0.8% of the intact dye in the feces [Radomski & Mellnger, 1962]. (¹⁴C) Sunset yellow (labeled on C-8 of naphthalenic moiety) was orally administered to female rats, and urine and bile were collected. After 96 hours, 8.5% of the 1-amino-2-naphthol-6-sulfonic acid equivalent, 37.4% of the sulfonic acid equivalent and 0.3% of intact dye were excreted in the urine; biliary excretion of sunset yellow was 1.5% [Honohan *et al.*, 1976]. In another study, female Simonsen/Sprague-Dawley rats orally administered 1 ml of an aqueous solution containing 2-25 mg of Sunset Yellow excreted 0.3 and 1.5% of the intact dye in the urine and bile, respectively, and 37% of the sulphanic acid equivalents in the urine. More than 90% of the dye was excreted in the feces [Honohan *et al.*, 1977].

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

FD&C Yellow No. 6 is a solid and decomposed without melting when heated to 390 °C [NTP, 1981]. Accordingly, the melting point of FD&C Yellow No. 6 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000].

3.1.2 Boiling Point

The boiling point of FD&C Yellow No. 6 was calculated to be 837 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Yellow No. 6 has been reported to be 1.43×10^{-22} mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Yellow No. 6 (452.37) and the estimated Henry's law constant for azo dyes of 10^{-15} atm-m³/mol it is highly unlikely that FD&C Yellow No. 6 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than 1×10^{-20} mm Hg.

3.1.4 Octanol/Water Partition Coefficients

Log K_{OW} value for FD&C Yellow No. 6 is -1.18 [KOWWIN EPI Suite, 2000]. The experimental log K_{OW} value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Yellow No. 6 is freely water soluble (190,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (10 mg/L) (Marmion, 1991, robust summary not included), it is anticipated that the log K_{OW} value for this substances would exceed 6.0.

3.1.5 Water Solubility

FD&C Yellow No. 6 has a reported water solubility of 190,000 mg/L at 2°C, 190,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Yellow No. 6 in 100% glycerol is 200,000 mg/L at 25 °C while the solubility in ethanol is reported to be 10 mg/L at 60 °C (Marmion, 1991, robust summary not included). The solubility of FD&C Yellow No. 6 in octanol is expected to be less than 1 mg/L.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

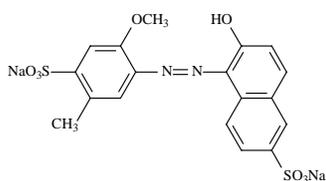
Direct and indirect photolysis experiments were conducted on the structurally related monoazo dye, FD&C Red No. 40², using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for FD&C Yellow No. 6 in hydroxyl radical reactions is 31.9 hours [AOPWIN EPI Suite, 2000].

3.2.2 Stability In Water

FD&C Yellow No. 6 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc.*) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 °C. These conditions would not typically be encountered in the environment. Therefore, FD&C Yellow No. 6 and its corresponding salts are anticipated to be stable in water.

3.2.3 Biodegradation

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



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

FD&C Yellow No. 6 was not predicted to be readily degradable by BIOWIN model calculations [BIOWIN EPI Suite, 2000].

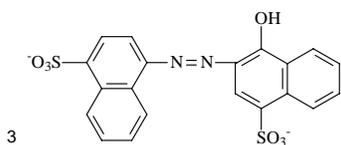
3.2.4 Fugacity

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 FD&C Yellow No. 6 is distributed completely to the water and soil compartments. Consistent with the extremely high water solubility and low log K_{OW} data, FD&C Yellow No. 6 showed no distribution to the fish compartment. These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

3.2.5 New Testing Required

None.

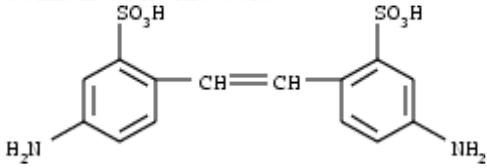
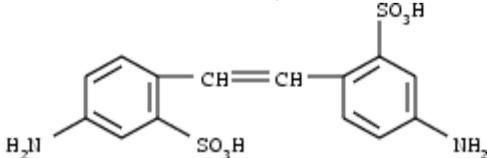


3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

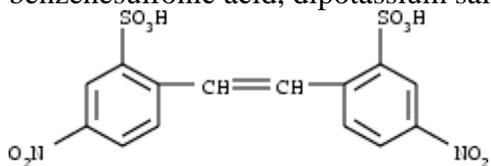
Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour LC50 for FD&C Yellow No. 6 is 6,044 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid and phenolic ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of stilbene sulfonic acid derivatives result in a 96-hour LC50 value greater than 10,000 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

Table 3

Name	Acute Toxicity to fish
2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid	48-hour LC50: 200 mg/L
	
2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt	72-hour LC50: greater than 1000 mg/L
	

• 2 11a

2,2'-(1,2-ethene-diyl)bis(5-amino)-
benzenesulfonic acid, dipotassium salt



• 2 K

96-hour LC50: greater than 10,000
mg/L

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

3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Yellow No. 6 in *daphnids* is 486.5 mg/L based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid phenolic ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. This value is consistent with calculated values.

3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour EC50 for FD&C Yellow No. 6 with green algae is 146,000 mg/L [ECOSAR EPI Suite, 2000] indicating a very

low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced measurable toxicity. Given the high-predicted value for acute toxicity to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

3.3.4 New Testing Required

None.

[HUMAN HEALTH TOXICITY

3.3.5 Acute Toxicity

The low acute oral toxicity of FD&C Yellow No. 6 is reflected by LD50 values greater than 2,000 mg/kg [Lu and Lavalley, 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 Lavalley, 1964].

In another pre-GLP acute toxicity study, groups of five male and female rats each were administered the 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 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. 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].

3.3.6 *In vitro* and *In vivo* Genotoxicity

3.3.6.1 *In vitro*

FD&C Yellow No. 6 tested negative in reverse mutation assay using TA1535, TA1537, TA98, TA100; TA92 and TA94 with and without metabolic activation [Chung *et al.*, 1981; Ishidate *et al.*, 1984; Muzzall and Cook, 1979]. In one chromosomal aberration test, FD&C Yellow No. 6 tested positive at concentrations up to 6,000 micrograms/mL without metabolic activation [Ishidate *et al.*, 1984], but tested negative in another chromosomal aberration test at a concentration up to 5,000 micrograms/mL with and without metabolic activation [Ivett *et al.*, 1989]. FD&C Yellow No. 6 gave a response judged to be equivocal in the sister chromatid exchange assay (SCE) at concentrations up to 5,000 micrograms/ml [Ivett *et al.*, 1989].

3.3.6.2 *In vivo*

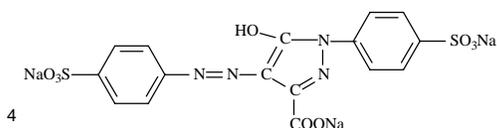
In a rodent micronucleus test, 10 ml/kg bw male rats were administered a single oral dose of 500 or 1000 mg/kg of FD&C Yellow No. 6. Bone marrow samples were taken at 24 and 48 hours later. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species. There was also no reported increase polychromatic erythrocytes [Westmoreland and Gatehouse, 1991].

In an in vivo UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw of the structurally related dye FD&C Yellow No. 5⁴ via gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested [Kornbrust and Barfknecht, 1985].

3.3.7 Repeat Dose Toxicity

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].

3.3.8 Developmental Toxicity

FD&C Yellow No. 6 was administered to 140 Charles River CD rats by gavage at dose levels of 100, 300, or 1000 mg/kg bw/day. Three negative control groups (20/group) received 0.5% methocel and one positive control group (20 rats) received 7.5% retinoic acid. All females were dosed on days 6-15 of gestation. No teratogenic effects were observed in the offspring of rats receiving up to 1000 mg/kg bw/day [International Research and Development Corporation, 1972].

3.3.9 Reproductive Toxicity

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].

New Testing Required

None.

3.4 TEST PLAN TABLE

Chemical	Physical-Chemical Properties					
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility	
Sunset Yellow CAS No. 2783-94-0	A	Calc	Calc	Calc	A	
Chemical	Environmental Fate and Pathways					
	Photodegradation	Stability in Water	Biodegradation	Fugacity		
Sunset Yellow CAS No. 2783-94-0	R, Calc	NA	R, Calc	Calc		
Chemical	Ecotoxicity					
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants			
Sunset Yellow CAS No. 2783-94-0	R, Calc	R, Calc	R, Calc			
Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity <i>In Vitro</i>	Genetic Toxicity <i>In Vivo</i>	Repeat Dose Toxicity	Reproductive Toxicity	Developmental Toxicity
Sunset Yellow CAS No. 2783-94-0	A	A	A	A	A	A

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

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