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Water Quality Criteria for Colored Smokes: Solvent Yellow 33

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

November 1987

SUPPORTED BY

U.S. ARMY MEDICAL
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The available data on the environmental fate, aquatic toxicity, and mammalian toxicity of Solvent Yellow 33, a quinoline dye used in colored smoke grenades, were reviewed. The U.S. Environmental Protection Agency (USEPA) guidelines were used in an attempt to generate water quality criteria for the protection of aquatic life and its uses and of human health.

Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. During typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation is released into the aquatic environment. The primary aquatic system receiving these discharges is the Arkansas River and associated drainages.

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19. Abstract (Continued)

The results of static acute toxicity tests indicate that Solvent Yellow 33 is not lethal to fish and aquatic invertebrates at solubility limits ranging from 0.089 mg/L at 12°C to 0.18 mg/L at 22°C. Toxicity tests with the green alga Selenastrum capricornutum show that Solvent Yellow 33 significantly reduces algal growth (measured as cell density and biomass) at the solubility limit of 0.20 mg/L. However, additional tests with a series of concentrations above and below solubility should be performed to accurately determine the toxicity of Solvent Yellow 33 in freshwater aquatic organisms. Consequently, according to the USEPA guidelines, data are currently unavailable to establish the Criterion Maximum Concentration and the Criterion Continuous Concentration for the protection of aquatic life and its uses.

The induction of delayed contact hypersensitivity in individuals who use commercial products containing Solvent Yellow 33 (D&C Yellow No. 11) is the only biological effect in humans reported in the literature.

In laboratory animals, Solvent Yellow 33 is absorbed from the gastrointestinal tract with an efficiency of 0.58 and from the respiratory tract with an efficiency >0.99. The dye is distributed to all the major organs in the body, metabolized primarily in the liver, and excreted predominantly in feces. Solvent Yellow 33 is only mildly toxic, whether administered by the oral, inhalation, or dermal route. The acute oral LD_{50} in rats is possibly greater than 10 g/kg body weight. A single topical dose of 2 g/kg or repeated doses of 50 to 1,000 mg/kg cause mild toxic effects in skin, gastrointestinal tract, and liver. In addition, delayed contact hypersensitivity reactions are induced in guinea pigs.

The most consistent findings in laboratory animals exposed subchronically (oral and inhalation) or chronically (oral) to Solvent Yellow 33 are pigment deposition in hepatocytes, bile duct epithelial cells and renal tubules, and the induction of bile duct hyperplasia. Inhalation exposure also affects the respiratory tract. The no-observed-effect level (NOEL) for a 4-week inhalation exposure is 51 mg/m^3, and the no-observed-adverse-effect level (NOAEL) for a 90-day exposure is 10 mg/m^3.

Solvent Yellow 33 is mutagenic in Salmonella typhimurium with and without S9 activation. The dye is mutagenic and clastogenic in mouse lymphoma cells with and without S9 activation, but the dye is more active without S9. Solvent Yellow 33 is not carcinogenic in the Mouse Lung Tumor Bioassay.

A criterion for the protection of human health could not be calculated according to USEPA guidelines. Nevertheless, after converting the NOEL obtained from a subchronic inhalation study to an oral equivalent dose, the acceptable daily intake for a 70-kg person is 2.8 μg/day, and for a 10-kg child it is 0.41 μg/day.

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WATER QUALITY CRITERIA FOR COLORED SMOKES: SOLVENT YELLOW 33

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

Chemical Effects Information Task Group
Information Research and Analysis Section
Health and Safety Research Division

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EXECUTIVE SUMMARY

Solvent Yellow 33 is an oil soluble quinoline dye that is used by the military in yellow and green smoke grenades, which are deployed for communication. The dye that has been certified and approved for use in drugs and cosmetics is known as D&C Yellow No. 11. Solvent Yellow 33 is prepared by the condensation of quinaldine with phthalic anhydride at 190 to 220°C in the presence of zinc chloride.

The environmental release of Solvent Yellow 33 and its combustion products may occur during manufacturing, during formulation and loading of smoke grenades, or upon detonation of grenades during training and testing operations. Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. It is reported that during typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation is released into the aquatic environment. The primary aquatic system receiving discharges from the arsenal is the Arkansas River and associated drainages. Prior to the installation of a pollution abatement facility in 1979, contamination to this system from untreated pyrotechnic wastes was reported as significant. The low water solubility of Solvent Yellow 33 indicates that the dye released into aquatic systems will either occur as a suspensoid in the water column or be deposited in the bottom sediments. No information is currently available concerning the environmental degradation or transformation of this dye.

The results of static acute toxicity tests indicate that Solvent Yellow 33 is not lethal to fish and aquatic invertebrates at its solubility limits ranging from 0.089 mg/L at 12°C to 0.18 mg/L at 22°C. However, since aquatic organisms may be exposed to concentrations above solubility, additional tests should be performed in order to determine a possible low-effect level and establish a Criterion Maximum Concentration for the dye. Due to the possible deposition of the dye in aquatic sediments, toxicity studies with burrowing mayflies are recommended.

Toxicity tests with the green alga Selenastrum capricornutum show that Solvent Yellow 33 significantly reduces algal growth at solubility limits of 0.20 mg/L. Cell density is reduced by 68 percent and biomass is reduced by 75 percent from the controls. Additional testing with a series of concentrations above and below solubility is needed to calculate EC_{50} values in order to determine a Final Plant Value according to USEPA guidelines. Data required by the USEPA guidelines to calculate Final Chronic and Final Residue Values are currently unavailable. Therefore, a Criterion Continuous Concentration cannot be established for Solvent Yellow 33.

Solvent Yellow 33, administered orally, is absorbed from the gastro-intestinal tract with an efficiency of 0.58. After a single or repeated exposures by inhalation the dye is also rapidly and efficiently absorbed into the blood (efficiency >0.99). The dye is distributed to all the major organs of the body, metabolized primarily in the liver, with some metabolism taking place in the kidney, and excreted in the urine and

feces. The primary route of excretion is in feces; five to ten times more of the dye is excreted in feces than in urine.

Solvent Yellow 33 is only mildly toxic, whether administered by oral, inhalation, or dermal routes. The acute oral LD_{50} in rats is >5 g/kg body weight and possibly >10 g/kg. A single dose of 2 g/kg applied to the skin causes minimal to mild hyperkeratosis and mild gastrointestinal effects. Repeated doses of 50 to 1,000 mg/kg cause hyperkeratosis, acanthosis, and adnexal hyperplasia of the skin, gastrointestinal effects, and fatty changes in the liver. Solvent Yellow 33 is essentially nonirritating to the skin and is only minimally irritating to the eyes. A single inhalation exposure to approximately 1,000 mg/m^3 is not toxic, whereas repeated exposures of 1,290 mg/m^3 cause hypertrophy and hyperplasia of the epithelium in the nasal cavity and inflammation of the naso-lacrimal duct and naso-vomer organ.

Solvent Yellow 33 causes delayed contact hypersensitivity reactions in guinea pigs and humans. The no-observed-adverse-effect level (NOAEL) in guinea pigs is 1 ppm for an induction dose and 0.1 ppm for a challenge dose; the NOAEL in humans is 0.5 ppm for a challenge dose, but humans may be sensitive to challenge doses as low as 1×10^{-4} ppm. Therefore, Solvent Yellow 33 is a strong skin sensitizer.

No data were found on subchronic and chronic toxicity in humans. Subchronic (oral and inhalation) and chronic (oral) exposure of laboratory animals to Solvent Yellow 33 is consistently associated with pigment deposition in hepatocytes, bile duct epithelial cells and renal tubules and the induction of bile duct hyperplasia.

Rats exposed to aerosols of Solvent Yellow 33 at a concentration of 230 mg/m^3 for 4 weeks develop changes in the lungs suggestive of emphysema and inflammation. The lowest-observed-effect level (LOEL) for a 4-week inhalation exposure in rats is $\geq$230 mg/m^3, and the no-observed-effect level (NOEL) is 51 mg/m^3. A 90-day exposure to 100 mg/m^3, however, does not cause emphysematous or inflammatory changes in the lungs. In addition to pigment deposition, the most prevalent findings are accumulation of foamy alveolar macrophages and hyperplasia of Type II cells in the lungs. The NOAEL for a 90-day inhalation exposure to aerosols of Solvent Yellow 33 is 10 mg/m^3.

Solvent Yellow 33 induces mutations in Salmonella typhimurium with and without S9 activation. Mutations and chromosome damage are induced in mouse lymphoma cells; the lowest doses giving positive responses were 12 $\mu g/mL$ with S9 activation and 2 $\mu g/mL$ without S9 activation. Solvent Yellow 33 is both mutagenic and clastogenic in mouse lymphoma cells. Sister chromatid exchanges are not induced in mouse bone marrow cells in vivo nor in mouse lymphoma cells in vitro. Solvent Yellow 33 is not carcinogenic in the Mouse Lung Tumor Bioassay. No data on genotoxicity and carcinogenicity in humans were found.

No data on developmental and reproductive toxicity in laboratory animals and humans were found.

Data to determine a bioconcentration factor were not available; therefore, USEPA guidelines could not be used to calculate a water quality criterion for the protection of human health. Nevertheless, an acceptable daily intake (ADI) was calculated after selecting an NOEL from the 90-day subchronic inhalation study (1 mg/m^3) and converting this dose to an equivalent oral dose. Using an uncertainty factor of 1,000, the ADI was 2.8 μg/day for a 70-kg person and 0.41 μg/day for a 10-kg child. Additional research was recommended to fill data gaps.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

1. INTRODUCTION

Solvent Yellow 33 is an oil soluble quinoline dye. The chemical for the dye exists as three tautomeric structures in equilibrium between resonance forms: (a) 2-(2-quinolyl)-1,3-indandione (CAS No. 83-08-9); (b) 3-hydroxy-2-(2-quinolinyl)-1H-inden-1-one (CAS No.5662-02-2); and (c) 2-(2(1H)-quinolinylidene)-1H-indene-1,3-(2H)-dione (CAS No. 5662-03-3) (Chemical Abstracts Service Registry File 1987, E.J. Weber 1987, USEPA, personal communication). Based on chemical principles the main tautomeric structure in solution is structure (b) (E.J. Weber 1987, USEPA, personal communication). The major military use is in M18 colored smoke grenades that are deployed as a means of communication. To eliminate potential health and environmental hazards associated with the production and use of Vat Yellow 4 and benzanthrone, Solvent Yellow 33 replaced these dyes in yellow and green smoke grenades (Smith and Stewart 1982). Solvent Yellow 33, certified and approved by the U.S. Food and Drug Administration (USFDA) for use in externally applied drugs and cosmetics, is known as D&C Yellow No. 11 (USFDA 1984). Solvent Yellow 33 is also used in spirit lacquers, polystyrenes, polycarbonates, polyamide and acrylic resins, and occasionally in hydrocarbon solvents (Colour Index 1971). The name Solvent Yellow 33 is used throughout this document to refer to both the dye used by the military and that certified and approved by the USFDA.

The pyrotechnic composition of colored smoke grenades consists of the dye mixture, oxidizer, fuel, coolant, and diatomaceous earth as a binder. Each grenade contains approximately 352 g of the dye mixture, which is formulated at Aberdeen Proving Ground, Maryland (Smith and Stewart 1982). The cooling agent is used to prevent excessive decomposition of the organic dye due to heat produced by the fuel. Upon detonation of the grenade, heat from the burning fuel causes the dye to volatilize and the vapor to condense outside the pyrotechnic, thereby producing smoke. The burning time is adjusted by the proportion of fuel and oxidizer and by the use of the cooling agent (Cichowicz and Wentsel 1983).

The production and use of yellow and green smoke grenades could result in environmental contamination and human exposure to Solvent Yellow 33 and its combustion products. Consequently, the objective of this report is to review the available literature concerning the environmental fate, aquatic toxicity, and mammalian toxicity of Solvent Yellow 33 in order to generate water quality criteria for the protection of aquatic life and its uses and of human health. These criteria are derived using current U.S. Environmental Protection Agency (USEPA) guidelines summarized in the appendixes.

1.1 PHYSICAL AND CHEMICAL PROPERTIES

Solvent Yellow 33 is relatively insoluble in water. Fisher et al. (1985) used high-performance liquid chromatographic (HPLC) methods to determine the solubility of technical grade Solvent Yellow 33 in diluent freshwater. The diluent water had a mean pH of 7.6, alkalinity of

156 mg/L as $CaCO_3$, and hardness of 180 mg/L as $CaCO_3$. The 24-hr solubility of Solvent Yellow 33 at specific temperatures was 0.09 ± 0.02 mg/L at 12°C, 0.13 ± 0.02 mg/L at 17°C, and 0.17 ± 0.01 mg/L at 22°C. Further HPLC studies determined that these concentrations of Solvent Yellow 33 were stable for 48 hr in diluent water (Fisher et al. 1985).

Other physical and chemical properties of Solvent Yellow 33 are as follows:

CAS registry No.:	8003-22-3
Color index (CI) No.:	47000 (Colour Index 1971)
Chemical name:	C.I. Solvent Yellow 33 (8CI) (9CI) (MEDLARS[RTECS] 1987)
Synonyms, trade names:	D and C Yellow No. 11, Quinoline Yellow SS, Arlosol Yellow S, Chinoline Yellow ZSS, Waxoline Yellow T, NitroFast Yellow SL, Oil Yellow SIS, Petrol Yellow C, Quinoline Yellow Spirit Soluble, Quinoline Yellow Base (MEDLARS[RTECS] 1987)
Structural formula:	No structural formula for the dye.
Molecular formula:	$C_{18}H_{10}NO_2$
Molecular weight:	273 (Henderson et al. 1985a)
Physical state:	Bright, greenish-yellow solid
Melting point (°C):	>160; sublimes above 160 (Colour Index 1971); 236 (Krien 1984)
Boiling point (°C):	467 (Krien 1984)
Solubility:	Soluble in methanol, ethanol, petroleum jelly, toluene, stearic acid, oleic acid, mineral oil, mineral wax, ethyl ether, acetone, butyl acetate (Zuckerman and Senackerib 1979); soluble in lipids (Bjorkner and Magnusson 1981)
Octanol-water partition coefficient (log k_p)	3.0 - 3.40 (G.L. Baughman 1987, USEPA, personal communication)
Absorption λmax (nm):	439 (Aldrich 1984)

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

Solvent Yellow 33 is prepared by the condensation of quinaldine with phthalic anhydride at 190 to 220°C in the presence of zinc chloride (Zuckerman and Senackerib 1979, Bjorkner and Niklasson 1983).

As of June 30, 1976, 2.42 metric tons of Solvent Yellow 33 (as D&C Yellow 11) were certified for sale annually in the United States (Zuckerman and Senackerib 1979). In 1977, 2.23 tons of Solvent Yellow 33 (as D&C Yellow No. 11) were used in the United States. The dye is used in over 300 cosmetic preparations (Rapaport 1984).

Yellow and green smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas, using the Glatt Mixing Process, which started production in 1984. A fluidized bed granulator combines the three operations of mixing, granulation, and drying. This technique reduces cost, improves efficiency, and provides better engineering controls for material containment, thereby reducing worker exposure to dust and the pollutant discharge of acetone (Garcia et al. 1982). The formulation of the yellow smoke grenade is as follows: 42 percent Solvent Yellow 33, 21 percent magnesium carbonate (coolant), 22 percent potassium chlorate (oxidizer), and 15 percent powdered sugar (fuel). The formulation of the green smoke grenade is as follows: 12.5 percent Solvent Yellow 33, 29.5 percent Solvent Green 3, 17.0 percent magnesium carbonate, 24.5 percent potassium chlorate, and 16.5 percent powdered sugar (Smith and Stewart 1982).

Major and minor components of colored smoke mixtures can be separated and identified by various methods, depending on the solubility and volatility of the major compounds. These techniques include thin layer chromatography, liquid chromatography, combined gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, and fluorescence spectrometry (Rubin and Buchanan 1983).

Several investigators have used HPLC to analyze Solvent Yellow 33. Ohnishi et al. (1977) used high-speed liquid chromatography to separate coal tar dyes, including Solvent Yellow 33, on an irregularly shaped porous silica gel column (LiChrosorb SI 100) by isocratic elution with chloroform and n-hexane mixtures. An HPLC analysis conducted by Sato et al. (1984) determined that samples of Solvent Yellow 33 consisted of >98 percent 2-(2-quinolyl)-1,3-indandione.

Fisher et al. (1985) used reverse-phase HPLC (C_{18} column) with an isocratic 10 percent distilled water:90 percent methanol mobile phase to measure concentrations in Solvent Yellow 33 for toxicity tests. The retention time for the major component of Solvent Yellow 33 ranged from 6.80 to 7.01 min with one minor contaminant (not identified) eluted at 8.42 min. The detection limit was 0.08 mg/L.

Moore et al. (1984) and Muni et al. (1986) analyzed Solvent Yellow 33 by reverse-phase HPLC (gradient of 90:10 methanol:water up to 100 percent

methanol in 10 min, 1 mL/min flow rate, ultraviolet detection at 254 nm). The major component was 2-(2'-quinolyl)-1,3-indandione (93.1 percent) with minor components of phthalic acid/anhydride (<1.8 percent) and quinaldine (<0.4 percent). Solvent Yellow 33 was purified by recrystallizing three times with ethyl acetate. HPLC analysis of the purified dye indicated <0.1 percent impurities (Moore et al. 1984).

Fadil and McSharry (1979) extracted and separated Solvent Yellow 33 from tablet-coating formulations. The formulation was treated with phosphoric acid, dissolved in methanol, and made alkaline with ammonium hydroxide. The solution was then centrifuged and the supernate was analyzed by thin layer chromatography on silica gel plates using ethyl acetate:methanol:water:concentrated ammonium hydroxide (150:40:35:5) as the solvent system.

Bertocchi et al. (1980) used flameless atomic absorption spectrometry to determine that 1.2-g samples of Solvent Yellow 33 contained 0.26 ppm of mercury.

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

No information was found in the literature concerning the abiotic effects of Solvent Yellow 33.

2.2 ENVIRONMENTAL FATE

2.2.1 Sources and Transport

Solvent Yellow 33 may be released into the environment during manufacture of the dye, during formulation and loading of the colored smoke grenade, or during training and testing operations. Kitchens et al. (1978) reported that during typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation is released into the aquatic environment. One grenade production line uses approximately 6,000 lb of smoke formulation to produce 8,000 grenades in an 8-hr shift; consequently, without pollution abatement, a minimum of 60 lb/day may be discharged into receiving waters. Combustion products resulting from detonation of the grenades can enter the aquatic environment as fallout, through runoff, or by leaching from soils (Cichowicz and Wentsel 1983).

Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. Four main aquatic systems within the arsenal grounds drain into the Arkansas River, which fronts the Arsenal for approximately 6 miles. These include Eastwood Bayou, which originates off the installation, and Triplett Creek, Yellow Creek with associated drainages, and McGregor Reach, which originate on the installation. An aquifer also occurs below the arsenal (Kitchens et al. 1978). The pyrotechnic complex is located just southwest of Yellow Lake. A pollution abatement facility was installed in 1979 that would be expected to reduce the effluent discharges to these streams (Fortner et al. 1979, as reported in Kitchens et al. 1978); however, no data is available concerning current waste loading. Prior to 1979, untreated pyrotechnic wastes were discharged directly into receiving aquatic systems that flow into the Arkansas River, indicating that past contamination was significant (Kitchens et al. 1978). Pinkham et al. (1977, as reported in Kitchens et al. 1978) reported contamination, including pyrotechnic residues and smoke mixtures, within Yellow Lake and within a munitions test area on the Arkansas River.

2.2.2 Degradation and Transformation

No specific information was found in the literature concerning the physical, chemical, or biological degradation and/or transformation of Solvent Yellow 33. The dye exhibits low water solubility and negligible volatility indicating that dispersal should be minimal. However, it

should occur primarily in a particulate form in aquatic systems either as a suspensoid or it will settle out and be deposited in the bottom sediment.

Deiner (1982) stated that colored smokes disseminated by grenades were degraded by oxidation. No information was available on the composition of the combustion products resulting from detonation of the grenades.

2.3 SUMMARY

The production and use of Solvent Yellow 33 may result in the release of the dye and its combustion products to the environment. The primary aquatic systems receiving wastewaters from the production of yellow and green smoke grenades at the Pine Bluff Arsenal, Arkansas, are the Arkansas River and associated drainages. Past contamination of these systems by pyrotechnic residues was reported as significant; however, wastewater treatment begun in 1979 should reduce effluent discharges to acceptable levels. No information was available concerning the degradation or transformation of Solvent Yellow 33 and/or its combustion products.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY IN ANIMALS

Fisher et al. (1987) studied the acute toxicity of technical grade formulations of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 (30:70 ratio) mixture in eight freshwater species of fish and invertebrates. Fish species tested were Pimephales promelas (fathead minnow), Ictalurus punctatus (channel catfish), Lepomis macrochirus (bluegill), and Salmo gairdneri (rainbow trout). Invertebrate species tested were Daphnia magna (water flea), Gammarus pseudolimnaeus (amphipod), Hexagenia bilineata (mayfly larvae), and Paratanytarsus parthenogeneticus (midge larvae). All species were tested at the aqueous solubility limit of the dye at various test temperatures as determined by HPLC analysis (Fisher et al. 1985). The solubility of Solvent Yellow 33 was 0.09 ± 0.009 mg/L (mean ± S.E.) at 12°C, 0.12 ± 0.009 mg/L at 0.17°C, and 0.16 ± 0.031 mg/L at 22°C. The Solvent Green 3 component in the yellow/green smoke mixture was not detected in test solutions by HPLC analysis at a detection limit of 0.08 mg/L. Using a C-18 Sep-Pak cartridge, the investigators increased the sensitivity of the HPLC to a detection limit of 0.002 mg/L, but were still unable to detect the Solvent Green 3 component of the dye mixture. With this method the solubility of the Solvent Yellow 33 component of the dye mixture was 0.076 ± 0.004 mg/L at 12°C. The authors, therefore, designated the solubility limit of the Solvent Green 3 component as "less than the detection limit," i.e., <0.08 mg/L or <0.002 mg/L. Dye concentrations were measured at the beginning and end of each test. Static (96 hr for fish; 48 hr for invertebrates) acute bioassays were performed according to ASTM (1980) methods on two replicates per treatment with ten organisms per replicate.

Temperature, pH, and total hardness remained relatively constant during testing. However, dissolved oxygen (DO) decreased during testing of bluegill and rainbow trout with both Solvent Yellow 33 alone and with Solvent Yellow 33/Solvent Green 3 mixture. In tests with bluegill, DO decreased from 8.5 mg/L at the start of the test to 4.0 mg/L at the end. In tests with rainbow trout, DO decreased from 9.2 to 7.3 mg/L with Solvent Yellow 33 alone and from 9.4 to 7.1 mg/L with the Solvent Yellow 33/Solvent Green 3 mixture.

Range-finding tests with Daphnia magna and Paratanytarsus parthenogeneticus indicated no toxicity at the solubility limits of Solvent Yellow 33. Results of the static acute tests with fish and invertebrates are given in Table 1. No mortality was observed in any fish or invertebrate species tested with Solvent Yellow 33 at solubility limits ranging from 0.089 mg/L to 0.18 mg/L.

The dye mixture solution, which contained 0.076 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3, caused 50 percent mortality

TABLE 1. ACUTE TOXICITY OF SOLVENT YELLOW 33 (SY) AND SOLVENT YELLOW 33/SOLVENT GREEN 3 (SY/SG) MIXTURE IN EIGHT FRESHWATER AQUATIC SPECIES[a]

Species	Age/Mean Size	Test Temperature (°C)	Test Concentration (mg/L)		% Mortality	
			SY	SY/SG	SY	SY/SG
Daphnia magna	<24 hr	22 ± 2	0.17	0.18/ <0.08	0	0
Gammarus pseudolimnaeus	Early young	17 ± 2	0.12	0.10/ <0.08	0	0
Hexagenia bilineata	Late instar	22 ± 2	0.18	0.17/ <0.08	0	0
Paratanytarsus parthenogeneticus	2nd-3rd instar	22 ± 2	0.17	0.18/ <0.08	0	0
Pimephales promelas	Length 10.3 mm Weight 8.0 mg	22 ± 2	0.18	0.17/ <0.08	0	0
Ictalurus punctatus	Length 14.2 mm Weight 30.1 mg	22 ± 2	0.11	0.11/ <0.08	0	0
Lepomis macrochirus	Length 18.3 mm Weight 108 mg	22 ± 2	0.12	0.12/ <0.08	0	0
Salmo gairdneri	Length 33.8 mm Weight 149 mg	12 ± 2	0.089	0.076/ <0.002	0	50

a. Adapted from Fisher et al. (1987).

in two separate 96-hr static acute tests with rainbow trout. No mortality in rainbow trout was observed in tests with 0.089 mg/L of Solvent Yellow 33 alone or with a 50 percent dilution of the dye mixture solution, which contained 0.055 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3. Due to the uncertainty concerning the actual concentration of Solvent Green 3 in the test solution, this test should be repeated using known concentrations of purified Solvent Green 3 obtained by dissolving the dye in an appropriate solvent and diluting this stock solution to the desired concentrations.

3.2 CHRONIC TOXICITY IN ANIMALS

No information was found in the literature concerning the chronic toxicity of Solvent Yellow 33 in aquatic organisms.

3.3 TOXICITY IN MICROORGANISMS AND PLANTS

Fisher et al. (1987) studied the effect of technical grade formulations of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 (30:70 ratio) mixture on the growth of the green alga Selenastrum capricornutum. Tests were conducted at 24 ± 2°C with stock solutions at the solubility limits (0.20 ± 0.013 mg/L Solvent Yellow 33; 0.198 mg/L Solvent Yellow 33/<0.002 mg/L Solvent Green 3). As explained in Section 3.1, the Solvent Green 3 component of the mixture was not detected in solution by HPLC analysis with a detection limit of 0.002 mg/L. A sterile assay medium was inoculated with cells in log growth (8-day-old stock cultures). Cell density (cells/mL) and biomass (chlorophyll a content expressed as μg/L) were measured at 0, 24, 48, 72, 96, and 120 hr.

Data analysis methods used in this study determined that an algistatic effect has occurred if, after the 5-day growth period, cell counts do not increase significantly from the initial inoculum level (Fisher et al. 1987). After five days of growth, cell densities in both treatment groups were significantly greater than at the time of inoculation; consequently, according to Fisher et al. (1987), an algistatic effect was not observed. Nevertheless, after the 5-day exposure period, Solvent Yellow 33 alone significantly reduced cell density by 68 percent and biomass by 75 percent from the control level (Table 2, Figures 1 and 2). Because the dye was tested at only one concentration, an EC_{50} value could not be calculated.

The Solvent Yellow 33/Solvent Green 3 mixture significantly reduced cell density by 98 percent and biomass by 99 percent from the control level (Table 2, Figures 1 and 2). In order to accurately interpret this data, algal toxicity tests should be performed with known concentrations of purified Solvent Green 3 obtained by dissolving the dye in an appropriate solvent and diluting this stock solution to the desired concentration.

TABLE 2. DENSITY AND BIOMASS OF Selenastrum Capricornutum EXPOSED TO SOLVENT YELLOW 33 AND SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE[a]

Treatment	Density (cells/mL)						Biomass (µg/L chlorophyll a)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Solvent Yellow 33												
Solubility Limit	4,303	22,090	84,536	152,516	287,159	386,159	1.08	4.80	10.12	15.55	18.56	27.12
Control	4,729	21,251	90,124	275,548	538,326	1,220,126	0.62	3.61	14.35	29.95	61.67	110.35
Solvent Yellow 33/ Solvent Green 3												
Solubility Limit	5,204	11,984	11,111	12,505	14,896	17,220	0.51	1.63	1.41	0.51	0.85	0.51
Control	5,201	20,274	60,609	224,780	585,180	831,847	0.74	5.76	24.35	51.54	98.88	88.76

a. Adapted from Fisher et al. (1987).

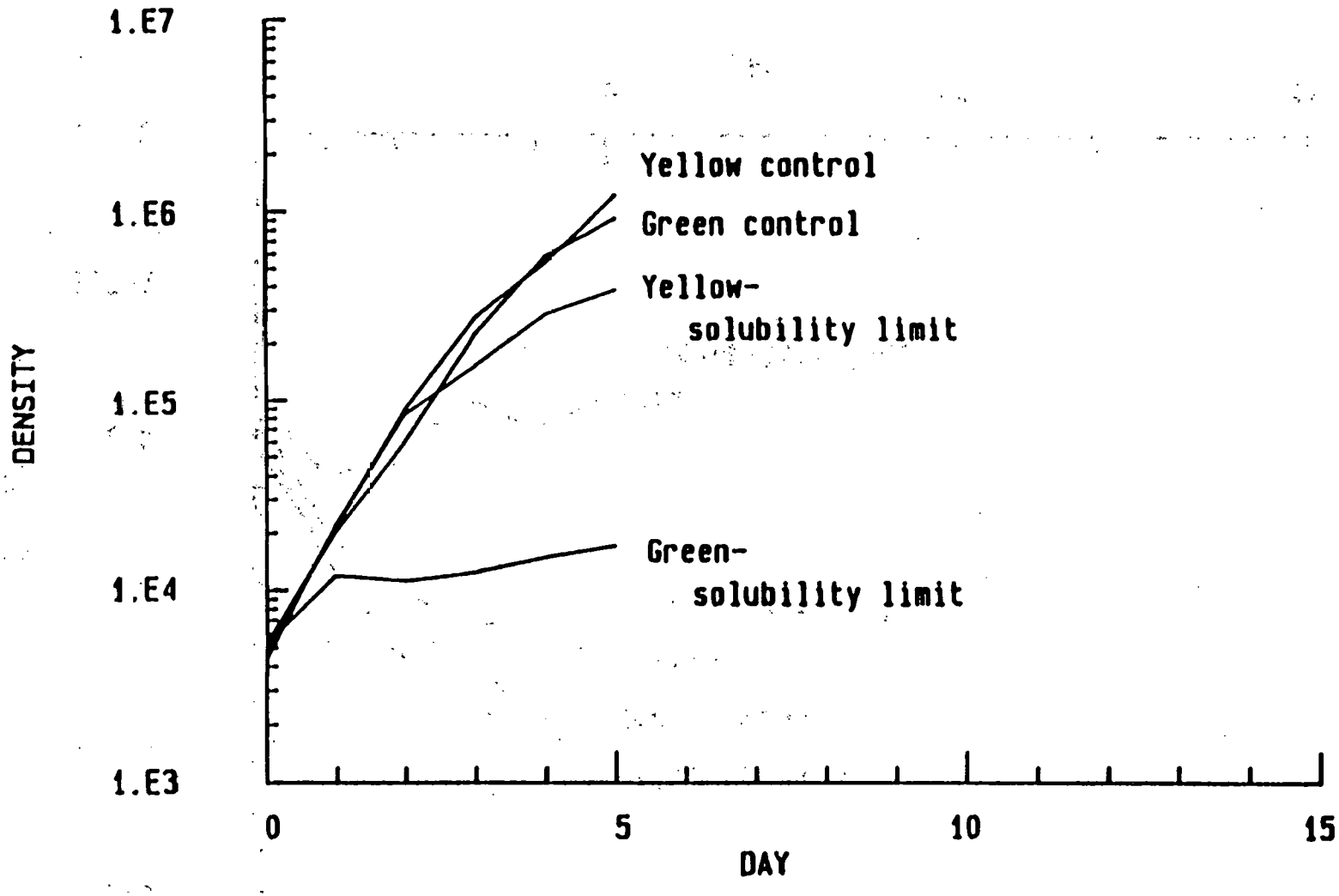

Figure 1. The effects of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture at solubility limits on the growth of Selenastrum capricornutum as measured by density (cells/mL). From Fisher et al. (1987).

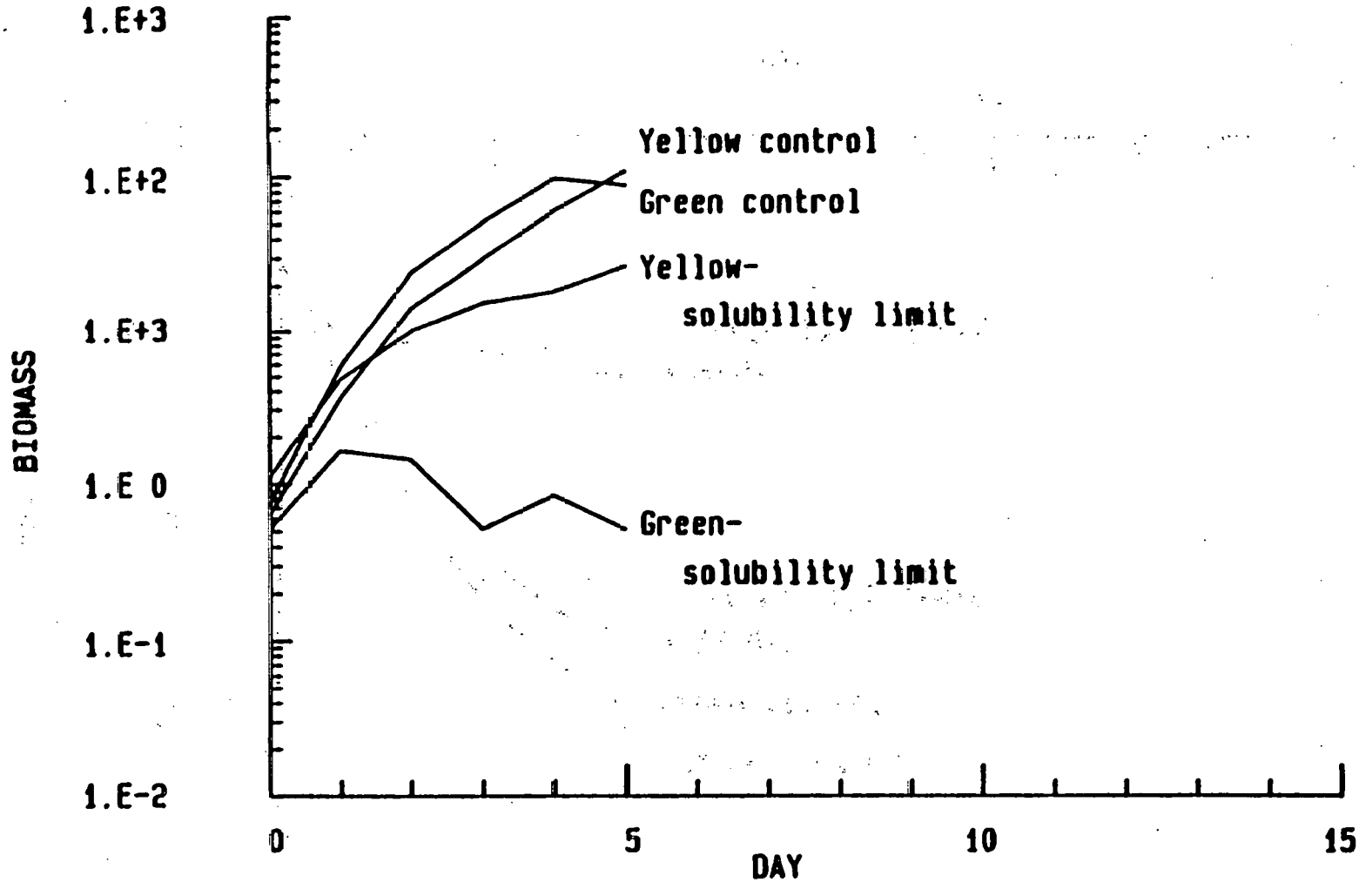

Figure 2. The effects of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture at solubility limits on the growth of Selenastrum capricornutum as measured by biomass (μg/L chlorophyll a). From Fisher et al. (1987).

3.4 BIOACCUMULATION

No information was found in the literature concerning the bioaccumulation of Solvent Yellow 33 by aquatic organisms. However, the calculated octanol-water partition coefficient for the dye is 3.0 to 3.4 (Baughman, G.L. 1987, USEDA, personal communication). The value was calculated by the substituent approach of Leo et al. (1971) based on computations used in the computer program CLOGP. Therefore, according to O'Bryan and Ross (1986), Solvent Yellow 33 would be expected to moderately bioaccumulate, with estimated bioconcentration factors of ≥100 and <200.

3.5 OTHER DATA

Little et al. (1974) investigated the acute toxicity of selected commercial dyes in Pimephales promelas (fathead minnow) and found that pH may affect toxicity by influencing the degree of ionization and the site of action of the dye within the organism. Consequently, if the dye is discharged along with acidic or alkaline substances, the toxic effect may be altered.

3.6 SUMMARY

The results of static acute toxicity tests in fish and invertebrates indicate that Solvent Yellow 33 is not lethal in aquatic organisms at solubility limits ranging from 0.089 mg/L at 12°C to 0.18 mg/L at 22°C. Algal toxicity tests with Selenastrum capricornutum indicate that 0.20 mg/L of Solvent Yellow 33 (solubility limit at 24°C) significantly reduces cell density by 68 percent and biomass by 75 percent from the control level.

Based on the calculated log K_p value, Solvent Yellow 33 would be expected to moderately bioaccumulate.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

4.1.1 Animal Data

4.1.1.1 Uptake, Absorption, and Distribution

Female Porton mice, Wistar rats, and Dunkin-Hartley guinea pigs exposed to a smoke mixture containing Solvent Yellow 33 (13 percent), Disperse Red 9 (16 percent), and Solvent Green 3 (19 percent) did not retain Solvent Yellow 33 in their lungs under the following exposure conditions: (1) 595 mg/m^3 for 30 min with sacrifices at 80 min and 1, 3, 7, 10, 14, and 21 days; (2) 500 mg/m^3 for 1 hr/day for 5 days with sacrifices at 1 day and 2, 4, 6, and 8 weeks; and (3) 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), and 1012.4 mg/m^3 (high dose for mice and rats) or 1162.1 mg/m^3 (high dose for guinea pigs), 5 days/week for 20 weeks (100 exposures) with sacrifices at 40 weeks (some mice) or 71 weeks after initiation of exposure (Marrs 1983, Marrs et al. 1984).

A detailed study of the pharmacokinetics of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture was reported by Henderson et al. (1985a) and Medinsky et al. (1986). A radioactive tracer, [^{14}C]-2-(2'quinolyl)-1,3-indandione ([^{14}C]-Solvent Yellow 33), was synthesized from [^{14}C]-phthalic acid and quinaldine. The final product was 95 percent pure, with a specific activity after recrystallization of 160 μCi/mg. A Wright Dust Feeder, which was connected to the exposure chamber, was used to generate aerosols from a mixture of 30 mg of [^{14}C]-Solvent Yellow 33 and 210 mg of unlabeled Solvent Yellow 33. The aerosol concentration generated within the chamber was 43 ± 6 mg/m^3 (mean ± S.E.), and the mass median aerodynamic diameter (MMAD) of the particles was 3.4 μm with a geometric standard deviation of 1.7.

To generate Solvent Yellow 33/Solvent Green 3 aerosols, [^{14}C]-Solvent Yellow 33 was mixed and precipitated with unlabeled Solvent Yellow 33 and Solvent Green 3; the final specific activity was 5.4 μCi/μmole; the ratio of yellow to total dye was 0.38. The aerosols were generated by a modified Trost-Jet Mill. The concentration in the chamber was 246 ± 16 mg/m^3 (mean ± S.E.); the MMAD was 2.6 μm with a geometric standard deviation of 1.7. The concentration of Solvent Yellow 33 in the aerosol mixture was 93 mg/m^3, and by subtraction, the concentration of Solvent Green 3 was 154 mg/m^3.

Deposition or whole-body retention of [^{14}C]-Solvent Yellow 33 was evaluated by exposing Fischer 344 male rats in plethysmographic tubes to Solvent Yellow 33 alone or to Solvent Yellow 33/Solvent Green 3 mixture for 60 min. The animals were sacrificed within 2 min after exposure, and the amount of radioactivity remaining in the whole depilated carcass was measured. The results are summarized in Table 3. The quantity of

TABLE 3. DEPOSITION OF [^{14}C]-SOLVENT YELLOW 33 IN RATS EXPOSED TO SOLVENT YELLOW 33 (SY) ALONE OR TO SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a,b]

Dye	Aerosol Concentration (mg/m^3)	Volume Inhaled (L)	SY Inhaled (nmol)	SY Deposited[c] (nmol)	Percent Deposited[d]
SY	43	10 ± 0.8	1,580 ± 40[e]	660 ± 140	41 ± 6
SY/SG	93/154	9.6 ± 1.3	3,180 ± 40	850 ± 270	25 ± 6

a. Adapted from Henderson et al. 1985a; Medinsky et al. 1986.
b. Values are mean ± S.E.
c. Based on ^{14}C measured in the depilated carcass of rats sacrificed immediately after exposure.
d. Percent of the inhaled dye that was deposited in the lungs.
e. $p < 0.05$, SY vs. SY/SG by one-way analysis of variance.

[^{14}C]-Solvent Yellow 33 inhaled was two times greater ($p < 0.05$) in animals exposed to Solvent Yellow 33/Solvent Green 3 mixture than in animals exposed to Solvent Yellow 33 alone, reflecting the difference in the concentration of Solvent Yellow 33 in the aerosols. Nevertheless, the quantity of [^{14}C]-Solvent Yellow 33 deposited or retained in the carcass was not significantly different between the two exposure groups. Henderson et al. (1985a) suggested that the smaller fractional deposition of Solvent Yellow 33 in the Solvent Yellow 33/Solvent Green 3 mixture was due to the smaller size of the green dye particles. They also suggested that the larger fractional deposition of Solvent Yellow 33 alone was due to the increased deposition of the larger yellow dye particles in the upper respiratory tract.

Distribution and the total amount of radioactivity found in whole tissues 1 hr after exposure to either Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture are presented in Table 4 (Henderson et al. 1985a). The total radioactivity found in all tissues combined was 206.73 nmol in animals exposed to Solvent Yellow 33 alone and 459.23 nmol in animals exposed to Solvent Yellow 33/Solvent Green 3 mixture. Based on the values from the plethysmographic study (Table 3), 31 or 54 percent of the radioactivity deposited in the lungs after exposure to Solvent Yellow 33 alone or Solvent Yellow 33/Solvent Green 3 mixture, respectively, was distributed to the tissues and organs listed in Table 4. Henderson et al. (1985a) proposed that the radioactivity not found in these tissues was associated with the contents of the gastrointestinal tract and was transported there by mucociliary clearance from the upper respiratory tract.

TABLE 4. DISTRIBUTION OF [^{14}C]-SOLVENT YELLOW 33 IN RATS 1 hr AFTER EXPOSURE TO SOLVENT YELLOW 33 (SY) OR SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a,b]

Tissue	SY [^{14}C]-SY Equivalents[c] (nmol)	SY/SG [^{14}C]-SY Equivalents[c] (nmol)
Liver	58 ± 28	93 ± 12
Skin (ear)[d]	41 ± 9.2	110 ± 40
Muscle[d]	24 ± 7.7	50 ± 10
Lung	21 ± 6.7	19 ± 1.2
Fat[c]	20 ± 4.0	34 ± 0.3
Turbinates	11 ± 9	7.5 ± 3.9
Kidney	9.0 ± 1.8	15 ± 0.65
Blood[d]	6.6 ± 1.1	14 ± 2
Bone (femur)[d]	4.7 ± 1.4	76 ± 2
Intestines[d,e]	3.9 ± 1.5	17 ± 8
Stomach[d]	3.1 ± 1.7	11 ± 3.5
Testes	1.3 ± 0.4	4 ± 0.69
Larynx/trachea	0.90 ± 0.20	4.3 ± 1.3
Brain	0.69 ± 0.12	2.3 ± 0.08
Heart	0.59 ± 0.22	0.93 ± 0.33
Urinary bladder	0.36 ± 0.02	0.14 ± 0.07
Spleen	0.26 ± 0.07	0.45 ± 0.08
Thymus	0.19 ± 0.08	0.29 ± 0.10
Adrenal	0.08 ± 0.04	0.26 ± 0.05
Thyroid	0.04 ± 0.02	0.03 ± 0.01
Lymph nodes	0.02 ± 0.003	0.03 ± 0.01

a. Henderson et al. 1985a.
b. Values are mean ± S.E.
c. Values based on radioactivity in the whole tissue.
d. Data for tissue estimated using values for tissue weights published by Dutcher et al. (1985, as reported by Henderson et al. 1985a).
e. Contents not included.

Elimination or clearance of Solvent Yellow 33 from selected tissues (lung, liver, kidney, stomach, spleen, and blood) was studied in rats exposed to aerosols of Solvent Yellow 33 alone or to Solvent Yellow 33/ Solvent Green 3 mixture for 60 min and sacrificed at predetermined times up to about 72 hr after exposure (Henderson et al. 1985a, Medinsky et al. 1986). The data presented in Table 5 show that Solvent Yellow 33 was cleared in two phases. Initially, clearance of Solvent Yellow 33 from the tissues was rapid, indicating that a short-term component was present (component A). The half-time of clearance of the short-term component ranged from 2 to 8 hr whether the animals were exposed to Solvent Yellow 33 alone or to Solvent Yellow 33/Solvent Green 3 mixture. The apparent rate constants of elimination of component A ranged from 0.09 to 0.35 hr^{-1}. A fraction of the radioactivity was cleared at a slower rate, indicating that a long-term component (component B) was also present.

TABLE 5. CLEARANCE OF [^{14}C]-SOLVENT YELLOW 33 FROM RAT TISSUES AFTER EXPOSURE TO SOLVENT YELLOW 33 (SY) ALONE OR SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a,b]

Tissue	Exposure	Component A[c] (nmol/g)	$T_{1/2}$[d] (hr)	Component B[e] (nmol/g)
Lung	SY	19 ± 7	2	1.0 ± 0.2
	SY/SG	10 ± 4	4	2.7 ± 0.3
Liver	SY	4 ± 0.8	8	0.67 ± 0.22
	SY/SG	5 + ?	7	1.5 ± 0.3
Kidney	SY	4 ± 1	3	1.1 ± 0.2
	SY/SG	4 ± 1	6	2.3 ± 0.3
Stomach	SY	1 ± 0.8	3	0.08 ± 0.03
	SY/SG	8 ± 3	4	0.4 ± 0.1
Blood	SY	0.5 ± 0.2	5	0.15 ± 0.03
	SY/SG	0.5 ± 0.2	8	0.45 ± 0.07
Spleen	SY	0.3 ± 0.2	3	0.15 ± 0.02
	SY/SG	0.3 ± 0.2	5	0.31 ± 0.03

a. Adapted from Henderson et al. 1985a; Medinsky et al. 1986.
b. Values are mean ± S. D.
c. Short-term component.
d. The half-time of elimination of component A.
e. Long-term component.

Component B was only 0.04 to 0.3 percent of the total radioactivity deposited (Medinsky et al. 1986). Because the half-times of component B were longer than the duration of the experiment, the rate constants of elimination of component B could not be determined. Henderson et al. (1985a) concluded that the small fraction of radioactivity associated with the component B in the lung, the short half-time of elimination of Solvent Yellow 33 from the lungs, along with the rapid appearance of radioactivity in other tissues demonstrated that Solvent Yellow 33 was rapidly cleared from the lungs.

Henderson et al. (1984, 1985b) and Sun et al. (1987) reported that Solvent Yellow 33 was also rapidly cleared from the lungs after repeated exposures to Solvent Yellow 33. Male and female Fischer 344 rats were exposed to aerosols of Solvent Yellow 33, 6 hr/day, 5 days/week, for 4 weeks at concentrations of 10 ± 5, 51 ± 10, or 230 ± 30 mg/m^3 (mean ± S.D.) (Henderson et al. 1984). Lungs from three males and three females were analyzed for the quantity of dye retained approximately 16 hr after termination of exposure. The results are presented in Table 6. Based on an estimate of 10 percent deposition of inhaled dye and a minute volume of 200 mL/min, Henderson et al. (1984) estimated that 1.8 mg/day was deposited in the lungs of animals exposed to 230 mg/m^3. Therefore, only 0.23 and 0.11 percent of the dye deposited after each exposure was retained in the lungs of male and female rats, respectively. They also demonstrated that only a small fraction of Solvent Yellow 33 was retained after exposure to aerosols of Solvent Yellow 33/Solvent Green 3 mixture. The quantity of Solvent Yellow 33 retained in lungs of rats exposed to the dye at concentrations of 1.0 ± 0.2, 10.8 ± 1.8, or 100 ± 17 mg/m^3 (mean ± S.D.) for 13 weeks (90 days) is also shown in Table 6 (Henderson et al. 1985b, Sun et al. 1987). Deposition was estimated at 720 μg/day in animals exposed to 100 mg/m^3, and retention was calculated as 0.18 percent of the quantity deposited each day in both male and female rats. Solvent Yellow 33 was not detected in lungs of rats exposed to Solvent Yellow 33/Solvent Green 3 mixture for 90 days.

4.1.1.2 Excretion

Urine and feces were collected 4, 8, 12, 18, 24, 32, 44, 56, and 70 hr after exposure of Fischer 344 rats to aerosols of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture (both containing [^{14}C]-Solvent Yellow 33) for 60 min (Henderson et al. 1985a). The cumulative excretion of radioactivity is presented in Table 7. The results show that over 70 percent of the radioactivity deposited in the lungs was recovered in feces, 14 to 15 percent was recovered in urine, 0.5 to 1.8 percent was exhaled as CO_2, and 8 to 12 percent remained in the body. The apparent rate constant for urinary excretion was 0.069 to 0.070 hr^{-1} with a half-time of 10 hr; the apparent rate constant for fecal excretion was 0.047 to 0.051 hr^{-1} with a half-time of 14 to 15 hr. The data showed that Solvent

TABLE 6. SOLVENT YELLOW 33 RETAINED IN LUNGS 16 hr AFTER REPEATED EXPOSURES TO AEROSOLS OF SOLVENT YELLOW 33 (SY) OR TO SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a]

Exposure/Sex	Aerosol Conc. (mg/m^3)	Lung Content[b]
4 Weeks		
SY		
Male	10	0.24 ± 0.10[c]
Female		0.17 ± 0.01
Male	51	0.90 ± 0.1
Female		1.3 ± 0.6
Male	230	4.1 ± 1.0
Female		1.9 ± 0.2
SY/SG		
Male	11	<1[d]
Female		<1
Male	49	<5
Female		<5
Male	210	<10
Female		<10
13 Weeks		
SY		
Male	1.0	0.05 ± 0.030[c]
Female		0.03 ± 0.01
Male	10.8	0.20 ± 0.10
Female		0.10 ± 0.04
Male	100	1.3 ± 0.3
Female		1.3 ± 0.2

a. Adapted from Henderson et al. 1984, 1985b; Sun et al. 1987.
b. Values are Mean ± S.E.; n = 3.
c. μg SY/lung.
d. μg SY/SG per g of lung.

TABLE 7. CUMULATIVE EXCRETION OF [^{14}C]-SOLVENT YELLOW 33 EQUIVALENTS 70 hr AFTER A 1-hr EXPOSURE TO SOLVENT YELLOW 33 (SY) OR TO SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a,b]

Exposure (mg/m^3)	Exhaled CO_2 (nmol)	Urine (nmol)	Feces (nmol)	Body[c] (nmol)
SY, 43	14 ± 1 (1.8)	110 ± 14 (14)	610 ± 75 (77)	61 ± 11 (8)
SY/SG, 93/154	10 ± 3 (0.5)	290 ± 40 (15)	1460 ± 230(73)	230 ± 45 (12)

a. Adapted from Henderson et al. 1985a.
b. Value are mean ± S.E.; numbers in parentheses are percentages of the total recovered material that was excreted or remained in the body.
c. Body includes pelt, carcass, and tissues.

Yellow 33 was rapidly excreted from the body. Henderson et al. (1985a) suggested that fecal excretion was via bile and by direct passage of the dye through the gastrointestinal tract following mucociliary clearance tract could also be absorbed into the blood, and subsequently excreted via bile or in urine.

Henderson et al. (1985a) compared the excretion pathways of [^{14}C]-Solvent Yellow 33 (5 μCi, 655 nmol/rat) administered to rats by gavage or by intratracheal instillation. The animals were placed in metabolism cages, and urine, feces, and expired CO_2 were collected for 94 hr. Of the dose administered by gavage or intratracheal instillation, 88 or 78 percent, respectively, was excreted in the feces; 8 or 15 percent, respectively, was excreted in urine; only two percent was exhaled as $^{14}CO_2$, and 1 to 2 percent remained in the body. The urinary:fecal ratio of excretion was 14:77 after inhalation, 15:78 after intratracheal instillation, and 8:88 after gavage. According to their calculations, only 58 percent of the radioactivity was absorbed from the gastrointestinal tract. If Henderson et al. (1985a) had measured the radioactivity in the stomach contents after exposing rats in the plethysmographic tubes, then the quantity of the dye available for absorption from the gastrointestinal tract could have been determined also.

Muni et al. (1986) observed external color changes in Fischer 344 albino rats administered a single dose of 5,000 mg/kg of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture by gavage. The color changes, which first appeared on day 2 after dosing, were observed throughout a 14-day observation period. The males were light green and the females were yellow. In the absence of vomiting, this observation indicates that the dyes may be excreted through the skin. Henderson et

al. (1984, 1985a,b) did not report external color changes in Fischer 344 rats exposed once or repeatedly by inhalation to aerosols of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture at concentrations ranging from 1 to 246 mg/m^3.

4.1.1.3 Metabolism

The previous sections showed that Solvent Yellow 33 is absorbed from the respiratory and gastrointestinal tracts, distributed to almost all tissues in the body, and eliminated primarily by fecal excretion. Excretion by exhalation of small amounts of $^{14}CO_2$ indicated that Solvent Yellow 33 is indeed metabolized. Henderson et al. (1985a) performed more extensive studies to determine if the radioactivity recovered from tissues or excreted in feces and urine was metabolized or unmetabolized [^{14}C]-Solvent Yellow 33. Lung, liver, and kidneys were taken from animals 60 min after exposure to Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture. Feces collected from 24 to 48 hr were pooled and urine was collected during the first 24 hr. Acetonitrile extracts of tissues and feces and ethyl acetate extracts of urine were analyzed by HPLC for unmetabolized and metabolized Solvent Yellow 33. Extracts of the tissues and feces and unextracted urine were also analyzed for glucuronide or sulfate conjugates of Solvent Yellow 33 or its metabolites.

More than 95 percent of the radioactivity was extracted from lung, kidney, and liver; approximately 50 percent from feces; and approximately 25 percent from urine. The proportion of radioactivity associated with unmetabolized [^{14}C]-Solvent Yellow 33 is presented in Table 8. More than

TABLE 8. PERCENT OF RADIOACTIVITY ASSOCIATED WITH UNMETABOLIZED SOLVENT YELLOW 33 IN RATS EXPOSED TO AEROSOLS OF SOLVENT YELLOW 33 (SY) OR SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a,b]

Exposure	Lung	Liver	Kidney	Feces	Urine
Extract only					
SY	95 ± 0.8	50 ± 9.3	16 ± 4	40 ± 7	13 ± 2
SY/SG	91 ± 0.04	73 ± 2	34 ± 2	31 ± 3	12 ± 2
Whole sample					
SY	94 ± 0.08	48 ± 9	15 ± 4	22 ± 6	3 ± 0.4
SY/SG	91 ± 0.04	71 ± 0.9	34 ± 2	15 ± 3	3 ± 1

a. Adapted from Henderson et al. 1985a.
b. Values are mean ± S.E.

90 percent of the radioactivity recovered from lung was unmetabolized [^{14}C]-Solvent Yellow 33, whereas only 15 to 22 percent of that recovered from feces and 3 percent of that recovered from urine was unmetabolized. The quantity of unmetabolized Solvent Yellow 33 recovered from liver and kidney was intermediate to that of lung and excretory products. Henderson et al. (1985a) concluded that Solvent Yellow 33 was rapidly absorbed from the lungs, extensively metabolized in the liver, and excreted in urine and feces. They also reported that some metabolism may also take place in the kidney.

HPLC profiles revealed that the metabolites in the kidney were qualitatively different from those of the other samples. Studies to determine if urinary metabolites were conjugates of glucuronide or sulfate showed no evidence of conjugation.

4.1.2 Human Data

No data on pharmacokinetics of Solvent Yellow 33 were found.

4.2 ACUTE TOXICITY

4.2.1 Animal Data

4.2.1.1 Oral, Dermal, and Ocular Toxicity

The data found in the literature show that Solvent Yellow 33 is a compound with very low acute toxicity. Muni et al. (1986) administered Solvent Yellow 33 (93.1 percent pure) suspended in corn oil to five male and five female Fischer 344 albino rats by gavage. The dose was 5 g/kg body weight. The animals were observed for 14 days after dosing. One male with a small stomach containing a solid granular material, intestines containing a yellow gel and a cecum filled with a green solid material, died due to the toxic effects of the compound. One additional male and one female died due to experimental error. Although three of the surviving females had a yellow liquid in their intestines at necropsy, gross internal lesions were not observed. All surviving animals gained weight during the observation period. Mild diarrhea, which disappeared within 24 hr, was observed on the day of dosing in one animal. In addition, on day 2 the fur of all animals was yellow, and the fur and tail were yellow by day 4. At the end of the observation period, all the males were light green and the females were yellow.

Five male and five female rats were treated identically with of Solvent Yellow 33/Solvent Green 3 mixture (24.1 percent:70.9 percent) at a dose of 5 g/kg (Muni et al. 1986). At the end of the 14-day observation period all animals showed a net weight gain or only an insignificant weight loss. There were no deaths or gross internal lesions. As with

Solvent Yellow 33 alone, external color changes were also noted; the males were light green and the females were yellow.

Muni et al. (1986) did not perform tests to determine the oral LD_{50} for Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture. They concluded, however, that the LD_{50} in rats was >5 g/kg for both dyes, but another report showed that the oral LD_{50} for Solvent Yellow 33 in rats was >10 g/kg (Hazelton Laboratories, Inc. 1962a). In dogs the oral LD_{50} was >1 g/kg (Hazelton Laboratories, Inc. 1962b). Thus, as shown by these studies, Solvent Yellow 33 has a low acute oral toxicity.

Gershbein (1982) reported that Solvent Yellow 33 (D&C Yellow No. 11) had a significant effect on the liver weights in rats exposed to the dye in their diets. A diet containing 0.15 percent of Solvent Yellow 33 fed to both intact and partially hepatectomized male Sprague-Dawley rats (12 to 15 per group) for 10 days caused significant increases in liver weights ($p < 0.01$). In partially hepatectomized rats, a dietary concentration of 0.060 percent caused only insignificant increases in liver weights. Intact animals were not exposed to the lower concentration. The increased liver weights were not accompanied by histopathological lesions.

In acute dermal toxicity tests, Solvent Yellow 33 applied to the skin of rabbits had low systemic effects (Muni et al. 1986). Solvent Yellow 33 absorbed to a saline moistened pad was applied at 2 g/kg to the shaved and abraded skin of five male and five female New Zealand rabbits. The rabbits were treated for 24 hr and observed for 14 days. The only toxic effect observed was a mild to moderate transient diarrhea in two females. Although body weights fluctuated during observation, a net weight loss was observed in only one animal. No gross visible lesions were observed in the five males and in two females; a gaseous cecum without formed feces in the colon, mottled kidneys, and a raised white hepatic lesion measuring 1 cm^2 were observed in one each of the three remaining females. Histopathological examination of treated and untreated skin of two male and two female rabbits revealed minimal to mild hyperkeratosis of the treated skin. According to Muni et al. (1986) this lesion was due to increased metabolism and maturation of keratinocytes.

In contrast to Solvent Yellow 33 alone, application of Solvent Yellow 33/Solvent Green 3 mixture at a dose of 2 g/kg to five male and five female rabbits did not cause significant lesions in the skin. There were, however, mild diarrhea in one female, fluctuations in body weights without net weight loss, and no gross internal lesions (Muni et al. 1986).

A multiple dose dermal toxicity study was conducted with Solvent Yellow 33 (Muni et al. 1986). Five male and five female rabbits were treated with a dose of 50, 200, or 1,000 mg/kg applied to the skin for 6 hr/day, 5 days/week, for 2 weeks. Food consumption and body weights were evaluated at 3- to 4-day intervals and toxic signs, pharmacologic signs, and dermal irritation were evaluated daily; gross necropsy and histopathological examination of all animals dying and sacrificed at termination were performed. A control group treated with vehicle only was not included in this study.

At the 50-mg/kg dose, two male rabbits died during the experiment. The death of one animal was due to an accident. The other animal, which died on day 10, showed gastrointestinal damage involving the duodenum, colon, and cecum. This animal also lost approximately 500 g of body weight and consumed significantly less food prior to death. All of the female rabbits survived, but one suffered a net body weight loss of approximately 100 g. Hyperkeratosis was observed in four males, whereas hyperkeratosis, acanthosis, and adnexal hyperplasia were observed in all females. Signs of dermal irritation (very slight erythema) were occasionally observed.

No deaths related to treatment were found in rabbits exposed to a dose of 200 mg/kg. Although weight gain fluctuated, at the end of the 14-day observation period, all animals weighed more than at the beginning of the test. A mild nasal discharge was observed in one male, and a mild to moderate diarrhea was observed in three males and one female. Histopathological examination of the treated skin showed mild to moderate hyperkeratosis in all rabbits, acanthosis in one male and four females, and adnexal hyperplasia in one female. As with the 50-mg/kg dose, dermal irritation was limited to occasional very slight erythema but no edema. Mild to marked fatty changes in the liver were observed in four male rabbits.

The 1,000-mg/kg dose caused no deaths, but weight gain fluctuated by as much as 200 g; the final weight was equal to or exceeded that at the beginning of the test. Toxic effects included mild diarrhea in three males and nasal discharge in one male and one female. All animals displayed moderate hyperkeratosis and acanthosis and mild adnexal hyperplasia. There was no increase in the incidence or severity of dermal irritation. Again, fatty changes in the liver were observed in four males but in no females.

According to Muni et al. (1986), skin lesions consisted of thickening of the epidermal prickle cell layer, the stratum corneum, and the accessory cell of the dermis. They also stated that the severity of the skin lesions was not affected by dose, but the incidence of skin lesions increased with dose.

In a study to test for primary dermal irritation, 500 mg of Solvent Yellow 33 was applied to two abraded and two unabraded sites on six rabbits for 24 hr. Evaluation of the test site immediately after removal of the dye revealed only barely perceptible erythema that was resolved by 72 hr. The Primary Irritation Score was 0.02, indicating that Solvent Yellow 33 was practically nonirritating. The results of a similar test using Solvent Yellow 33/Solvent Green 3 mixture produced a score of 0.08.

The eye irritation test using 100 mg of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 powder placed in one eye of each of three rabbits showed that Solvent Yellow 33 was minimally irritating and that Solvent Yellow 33/Solvent Green 3 mixture was nonirritating to rabbit eyes.

4.2.1.2 Delayed Contact Hypersensitivity

Solvent Yellow 33, also known as D&C Yellow No. 11, is approved by the USFDA for use as a color additive in externally applied drugs and cosmetics (USFDA 1984). Because Solvent Yellow 33 (D&C Yellow No. 11) has been shown to cause contact hypersensitivity in humans (Section 4.2.1.3), studies in laboratory animals were conducted to study this reaction. Guinea pigs, the preferred animal model, were used in all tests.

Using the modified Buehler method, Lamson et al. (1982) applied 50 percent Solvent Yellow 33 in 95 percent ethanol with a 24-hr occluded patch to Hartley Strain female guinea pigs. The animals were treated once weekly for 3 consecutive weeks (induction phase), rested for 2 weeks, and challenged with another 24-hr occluded patch containing 1, 3, or 10 percent Solvent Yellow 33 in 95 percent ethanol. Twenty-four hours after the patches were removed, the treated areas were depilated and evaluated on a scale of 0 to 4 for erythema and edema. The lowest score of 0 indicates no reaction, and the highest score of 4 indicates strong erythema (beet red), with or without edema, eschar formation, or skin damage. Control animals were treated with 95 percent ethanol (Lamson et al. 1982).

Lamson et al. (1982) noted that the induction dose of Solvent Yellow 33 was minimally irritating. The skin sensitization reaction elicited by the challenge was statistically significant only for the 10 percent concentration; 11 of 13 animals (85 percent) responded with a score of 1 (barely perceptible erythema). Only 15 to 20 percent of the controls responded with a score of 1. Thus, Solvent Yellow 33, under the conditions of the test, was a weak sensitizer. Another group of guinea pigs were challenged with 1.0, 10, and 20 percent solution of bar soap containing 0.015 percent Solvent Yellow 33. The solutions caused irritation but not sensitization (Lamson et al. 1982).

The Freund's Adjuvant method of inducing a dermal sensitization reaction in guinea pigs was employed by Palazzolo and DiPasquale (1983) and by Sato et al. (1984). This method is more sensitive than the modified Buehler (Buehler 1965, as reported by Lamson et al. 1982).

Palazzolo and DiPasquale (1983) injected 0.1 mL of Complete Freund's Adjuvant containing 5, 25, or 50 μg of Solvent Yellow 33, or with 6 μg of 2,4-dinitrochlorobenzene (known sensitizer) into the footpad of Hartley Strain female guinea pigs (20 per group). Each group was immediately given an intradermal injection of dye or known sensitizer, and vehicle controls were given an injection of peanut oil alone. The animals were allowed to rest for 2 weeks and then challenged with an intradermal injection of the same compounds in the shaved flanks. Vehicle controls were challenged with the known sensitizer, Solvent Yellow 33, or peanut oil. The reactions were evaluated 4, 24, 48, and 72 hr after challenge; skin specimens were taken 72 hr after challenge and evaluated histologically. The scores were based on the product of the area of induration and a numerical value (ranging from 0 to 9) corresponding to the severity of erythema, edema, and necrosis. The scores for treatment groups were

calculated by subtracting the difference of the initial and challenge score of vehicle controls and then subtracting this value from the difference of the initial and challenge score of the treated groups.

The results based on the group mean score showed no response at 4 hr for animals treated with the dye and a positive response at 24 hr for the 50-μg group, with a maximum response at 48 hr. Analysis of the frequency of the sensitization response revealed that, at 24 and 48 hr, 100 percent of the animals in the 50-μg group reacted positively to the dye; fewer animals in the 5- and 25-μg groups reacted. Thus, the intensity of the response and the frequency of positive responses showed statistically significant linear dose-response relationships. According to Palazzolo and DiPasquale (1983) the 5-μg dose was approaching a no-observed-effect level (NOEL).

Histological examination of skin specimens showed that the inflammatory response in treated animals, as indicated by the infiltration of mononuclear cells, was qualitatively similar to that of vehicle controls, but more severe. The inflammatory responses in the 5- and 25-μg groups were less severe than in the 50-μg group. Necrotic lesions, sometimes involving the epithelial and dermal layers, were observed in 40 percent of the 50-μg group, whereas no necrotic lesions were observed in the 5- and 25-μg groups (Palazzolo and DiPasquale 1983).

The severity and frequency of the responses led Palazzolo and DiPasquale (1983) to conclude that Solvent Yellow 33 is a fairly strong sensitizer. They further stated that 50 μg should be considered a strong sensitizer, 25 μg a moderate sensitizer, and 5 μg a weak sensitizer.

The sensitization potential of Solvent Yellow 33 was confirmed by Sato et al. (1984), who also used Complete Freund's Adjuvant to induce dermal sensitization in guinea pigs. Sato et al. (1984) used four different commercial grade samples of Solvent Yellow 33 and a purified dye preparation. Complete Freund's Adjuvant was injected intradermally around a shaved area of the shoulder region. The skin was abraded and patches containing the dye dissolved in acetone were applied for 24 hr; abrasion and treatment were repeated on two consecutive days. On the 9th day, the animals were again treated for 48 hr. On the 21st day, the animals were challenged by applying the dye directly to a shaved area of the flank. The test sites were evaluated 24 and 48 hr after challenge; erythema and edema were scored separately on a scale of 1 to 4 and 1 to 3, respectively, which would produce an overall maximum score of 7.

The results presented in Table 9 showed that animals induced with 1,000 ppm of the four commercial dyes and purified Solvent Yellow 33 gave mean responses of 4 or more at a challenge concentration of 1,000 ppm. The mean responses showed a dose-response relationship with a minimal response observed at 1 ppm. In another test, Sato et al. (1984) varied the induction concentration (1 to 1,000 ppm) and the challenge concentration (0.1 to 1,000 ppm) of the purified dye. A dose-response relationship was observed in the induction stage.

TABLE 9. RESPONSE OF GUINEA PIGS SENSITIZED WITH COMMERCIAL GRADE SOLVENT YELLOW 33 AND WITH PURIFIED SOLVENT YELLOW 33 (SY)[a]

Induction Sample/Conc.	Challenge Concentration	Fractional Response[b]	Mean Response
No. 1, 1,000 ppm	1,000	10/10	4.0
	100	10/10	2.6
	10	8/10	1.4
	1	1/10	0.1
	0.1	0/10	0
No. 2, 1,000 ppm	1,000	10/10	4.4
	100	10/10	3.1
	10	7/10	1.6
	1	0/10	0.2
	0.1	0/10	0
No. 3, 1,000 ppm	1,000	10/10	4.0
	100	10/10	2.5
	10	8/10	1.4
	1	1/10	0
	0.1	0/10	0
No. 4, 1,000 ppm	1,000	10/10	4.1
	100	10/10	2.8
	10	8/10	1.5
	1	1/10	0.2
	0.1	0/10	0
Purified SY, 1,000 ppm	1,000	30/30	4.2
	100	30/30	2.9
	10	23/30	1.5
	1	5/30	0.2
	0.1	5/30	0

a. Adapted from Sato et al. 1984.
b. Number of animals responding per number of animals treated.

A no-observed-adverse-effect level (NOAEL) was observed at the 1-ppm dose of purified Solvent Yellow 33 for the induction stage and at the 0.1-ppm dose of the commercial dye for the challenge stage.

4.2.1.3 Inhalation Toxicity

Only one study on the acute effects of inhaling Solvent Yellow 33 was found in the literature. In this study animals were exposed to dye aerosols rather than to products of combustion as one would encounter after detonation of a smoke grenade. Henderson et al. (1985a) exposed specific pathogen-free male and female Fischer 344 rats to aerosols of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture generated by a Jet-O-Mizer air jet mill.

The dyes were 93 to 95 percent pure; major contaminants of Solvent Yellow 33 included phthalic acid, phthalic anhydride, and quinaldine. The same contaminants were in the mixture in addition to quinazarin and p-toluidine. Three animals of each sex were used for single exposures and six animals of each sex were used for multiple exposures. The conditions of exposure are described in Table 10. Control animals were not included in this test. After exposure, the animals were observed for 14 days for mortality and signs of toxicity. All animals were weighed 7 and 14 days after completion of exposure; only animals exposed repeatedly to the aerosols were subjected to gross necropsy, and selected tissues were submitted for histopathological examination. All animals survived to the end of the test without overt signs of toxicity. One week after exposure, a slight, 3 to 7 percent, decrease in body weight was observed in all groups, but body weights either returned to normal or exceeded pre-exposure weights by the end of the test.

TABLE 10. CONDITIONS FOR ACUTE INHALATION EXPOSURE TO AEROSOLS OF SOLVENT YELLOW 33 (SY) AND SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE (SY/SG)[a]

Test Material	Duration of Exposure (hr)	Dye Mass Concentration		Particle Size, MMAD
		Mean ± S.E. (mg/m^3)	Coefficient of Variation (%)	Mean ± S.E. (μm)
SY	1	1,000 ± 30	14	5.1 ± 0.4
	6	1,040 ± 30	21	5.7 ± 0.5
	6/day, 5 days	1,290 ± 20	20	5.6 ± 0.2
SY/SG	1	1,600 ± 50	16	5.0 ± 0.1
	6	1,440 ± 60	20	5.5 ± 0.2
	6/day, 5 days	1,490 ± 70	44	5.4 ± 0.3

a. Adapted from Henderson et al. (1985a).

In animals exposed repeatedly to Solvent Yellow 33, nasal congestion was the only gross condition observed. No significant histopathological lesions were found in the lungs or olfactory epithelium. Macrophages containing pigment were found in the tracheobronchial nodes, in the submucosa of the upper trachea, and in the respiratory epithelium. The following lesions were considered to be compound-related: hypertrophy and hyperplasia of goblet cells in the respiratory epithelium of the nasal cavity, chronic nonsuppurative inflammation of the naso-lacrimal duct, and serous inflammation of the respiratory epithelium in the naso-vomer organ.

In animals exposed repeatedly to the mixture, nasal congestion was less severe than in animals exposed to Solvent Yellow 33 alone. Compound-related histopathological lesions included slight to severe hyperplasia of the respiratory epithelium of the nasal cavity, serous inflammation of the naso-vomer organ with degenerative changes in the olfactory epithelium, and slight chronic nonsuppurative inflammation of the epithelium of the naso-lacrimal duct. In contrast to Solvent Yellow 33, the mixture also caused congestion in the lungs of all animals and focal alveolar histiocytosis in the lungs of almost all animals. Macrophages containing pigment were found in the tracheobronchial lymph nodes.

Henderson et al (1985a) considered the lesions found in animals exposed repeatedly to aerosols of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture to be minor in nature. Therefore, they concluded that the dyes have a low order of acute toxicity when inhaled by rats.

4.2.2 Human Data

The data on toxic effects of Solvent Yellow 33 (D&C Yellow No. 11) in humans involve exposure to the dye that has been certified and approved by the USFDA for use in externally applied drugs and cosmetics (USFDA 1984). Solvent Yellow 33 is used in approximately 300 commercial products, and delayed contact hypersensitivity has been documented in some individuals using products containing this dye.

Calnan (1975) described a case in which a 43-year-old female developed soreness at the angle of the mouth, along with swelling of the mouth, face, and eyelids. Patch tests with all the patient's cosmetic products produced a positive reaction to a lipstick that contained Solvent Yellow 33, D&C Red No. 17, and other ingredients. Subsequent patch tests using each ingredient separately showed that only Solvent Yellow 33 produced a positive reaction. Calnan (1981) also described a 24-year-old female who developed dermatitis of the eyelids after using an eye cream containing Solvent Yellow 33. Patch tests with the ingredients of the eye cream produced positive reactions to several ingredients including Solvent Yellow 33. The concentration of Solvent Yellow 33 in the test was 0.004 percent dissolved in 0.1 percent petroleum. Jordan (1981) and Weaver

(1983a,b) described patients who developed contact dermatitis after using soap containing Solvent Yellow 33, and Larsen (1975) described a patient who developed dermatitis on the face after using a rouge cosmetic containing Solvent Yellow 33.

Patch tests with Solvent Yellow 33 have shown that some individuals react strongly to this dye. The repeat insult patch test is used most often. The subjects receive five to ten exposures to the dye at regular intervals (induction phase), followed by a rest period of 10 to 14 days, and then a final exposure lasting 48 hr (challenge). Results are usually read 48 and 72 hr after initiation of challenge.

Rapaport (1980) reported that 14 of 56 subjects patch tested with 20 percent Solvent Yellow 33 in petroleum showed a strong positive reaction when challenged or during the 9th or 10th induction patch. Two years later 9 of the 14 positive subjects were rechallenged with 20 percent Solvent Yellow 33 in petroleum; 3 showed a positive reaction after a first 48-hr patch, and 2 more were positive after the second 48-hr patch (Rapaport 1984). Jordan (1981) reported that 9 of 149 subjects were sensitized by an oil-based cosmetic containing 16.4 ppm of Solvent Yellow 33, and Björkner and Magnusson (1981) reported that 4 of 88 subjects showed positive reactions to 1 percent Solvent Yellow 33 in polyethylene glycol.

The nine subjects rechallenged in the study by Rapaport (1984) were later tested with various cosmetics containing approximately 0.001 percent Solvent Yellow 33. The cosmetics, including hand creams, soaps, bath oils, and body and facial moisturizers, were applied by the subjects to the appropriate areas of their body twice a day for 1 month. Each subject used four to six different preparations. None of the subjects reacted to the preparations. The authors suggested that contact dermatitis was not induced in these individuals with previous positive patch tests because of the less than adequate contact time with the dye. Nevertheless, a cosmetic containing Solvent Yellow 33 can induce a positive reaction in only 2 days (Larsen 1975) and a soap can induce a reaction in less than 1 week (Jordan 1981, Weaver 1983a).

Kita et al. (1984) used Duhring chambers to apply 0.5 percent Solvent Yellow 33 to sites on the arm of 35 subjects pretreated with sodium lauryl sulfate. The dye was applied five times for 48 hr each time. After a 10-day rest, the sites were challenged with different concentrations of the dye. The reactions were evaluated 48 and 72 hr after challenge and scored on a scale of 0 to 5. A score of 0 to 0.5 was doubtful, 1 was weak positive, 2 was strong positive, and 3 was extreme. The results are presented in Table 11. At a challenge concentration of 1,000 ppm (0.1 percent), 75 percent of the subjects responded with a mean intensity rated between weak and strong positive. A doubtful reaction was observed in patients challenged with 1 ppm, and a weak reaction was observed in those challenged with 5 to 50 ppm. A biopsy taken from a strong positive site revealed a typical eczematous response of contact sensitization. Kita et al. (1984) concluded that Solvent Yellow 33 is a potent contact sensitizer. The NOAEL was 0.5 ppm.

TABLE 11. RESPONSE OF HUMAN SUBJECTS SENSITIZED WITH SOLVENT YELLOW 33[a,b]

Challenge Concentration (ppm)	48 hr		72 hr	
	SF[c]	MI[c]	SF	MI
1,000	12/20	1.2	15/20	1.6
100	9/10	1.0	9/10	1.2
50	3/10	0.6	4/10	0.9
10	2/10	0.5	2/10	0.7
5	2/10	0.4	2/10	0.6
1	1/10	0.2	1/10	0.3
0.5	0/10	0.1	0/10	0.1
0.1	0/10	0	0/10	0

a. Adapted from Kita et al. 1984.
b. Induction concentration = 0.5 percent in petroleum.
c. SF = sensitization frequency; MI = mean intensity of response.

Björkner and Magnusson (1981) described a patient with a prior history of severe dermatitis who initially did not react within 72 hr to a patch test with 1 percent Solvent Yellow 33. A "flareup," however, was observed 14 days later. The patient reacted to a subsequent patch test with 0.00001 percent but not to 0.000001 percent Solvent Yellow 33 in polyethylene glycol.

Björkner and Niklasson (1983) patch tested this patient with Solvent Yellow 33 dissolved in ethanol and with D&C Yellow No. 10 or purified D&C Yellow No. 10 dissolved in water. The patient reacted to all three preparations; the lowest concentrations that induced a positive reaction are as follows: Solvent Yellow 33 at 1×10^{-8} percent (0.8×10^{-12} g); D&C Yellow No. 10 at 5×10^{-3} percent (0.5×10^{-6} g); and purified D&C Yellow No. 10 down to at least 2×10^{-2} percent (may have responded to lower concentration, but the patient refused further testing).

By HPLC analysis, the detection limit of Solvent Yellow 33 was 1.6×10^{-9} g, which is 2,000 times higher than the lowest concentration giving a positive response (Björkner and Niklasson 1983). Because the patient was sensitive to concentrations of Solvent Yellow 33 below the detection limit of the HPLC system, it is possible that the D&C Yellow No. 10 was contaminated with sufficient Solvent Yellow 33 to induce a positive reaction. After additional evaluations of the response of the patient to the dyes, Björkner and Niklasson (1983) concluded that cross-reactivity between Solvent Yellow 33 and D&C Solvent Yellow No. 10 was possible.

Other investigators reported that subjects tested with D&C Yellow No. 10 did not respond (Weaver 1983a,b, Kita et al. 1984). Weaver (1983a,b) attributed the lack of response to differences in physical and chemical

characteristics: Solvent Yellow 33 is insoluble in water, whereas D&C Yellow No. 10 is relatively soluble in water; Solvent Yellow 33 is not ionized in organic solvents, whereas D&C Yellow No. 10 is ionized in organic solvents. Weaver (1983a,b) further suggested that these characteristics would cause a decrease in the penetration of D&C Yellow No. 10 in skin, and consequently, a decrease in its allergic potential.

The USFDA has approved D&C Yellow No. 10 for use in a wider variety of products than Solvent Yellow 33. D&C Yellow No. 10 is not restricted for external use; it can be used in coloring drugs in amounts not to exceed 10 mg/day and in lipstick and other cosmetics in amounts not to exceed 1.0 percent of the finished products (USFDA 1984). If there is cross-reactivity between the Solvent Yellow 33 and D&C Yellow No. 10, then the use of products containing D&C Yellow No. 10 may exacerbate the hypersensitivity response to Solvent Yellow 33.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Data

Several studies on the subchronic or chronic administration of Solvent Yellow 33 were available. In a range-finding study performed by Hazelton Laboratories, Inc. (1962c), rats were fed a diet containing 0.1, 0.23, 0.55, 1.29, or 3.0 percent Solvent Yellow 33 for six weeks. A significant decrease in body weight gain was noted in animals receiving the 3 percent diet, and increased relative liver weights were noted in animals fed 0.55, 1.29, and 3.0 percent diets. Pigment was deposited in periportal hepatocytes and in the renal convoluted tubules. Proliferation in the bile duct epithelium was increased. Pigment was also deposited in periportal hepatocytes and bile duct epithelial cells in dogs that received Solvent Yellow 33 for 90 days (Hazelton Laboratories, Inc. 1962d). Increased proliferation of bile duct epithelial cells was also noted. The dogs received a variable dose that ranged from 1 to 3 percent in the diet; the 2- and 3-percent diets were changed to capsule administration of 630 mg/kg/day and 946 mg/kg/day, respectively, because the dogs refused to eat the test diets.

In another study, male and female rats were fed 0, 0.03, 0.1, 0.3, or 1.0 percent Solvent Yellow 33 in their diets for 1 year (Hazelton Laboratories, Inc. 1967a). The control groups consisted of 80 animals per sex, and the treated groups consisted of 25 animals per sex per dose. The animals were observed daily for mortality and clinical signs of toxicity. Body weights and food consumption were recorded weekly up to the 26th week and biweekly thereafter. Hematology tests and urinalyses were performed on 5 animals per sex per dose at 30, 90, 180, and 365 days. All animals that died during the study or killed at termination were subjected to gross necropsy, and tissues were submitted for histopathological examination.

The weight normalized doses decreased throughout the study. The consumption of compound in animals fed the 1-percent diet ranged from 1,120 mg/kg/day during week 1 to 398 mg/kg/day during week 50 in males and from 1,230 mg/kg/day during week 2 to 517 mg/kg/day during week 50 in females. In animals exposed to the other doses, the reductions were just as severe, and the difference between males and females was also noted.

Statistically significant reductions in mean terminal body weights were observed in both male and female rats given the highest dose; body weight was reduced in males by 10 percent and in females by 18 percent. Food consumption fluctuated throughout the study in control and treated animals, but fluctuation in food consumption could not be related to changes in weight gain. Relative liver weights were higher in males given the 0.3- and 1.0-percent diets and in females given the 1.0-percent diet. Statistically significant changes in hematology values were noted, but were not related to dose. The results of the urinalyses show that the treated rats were similar to controls.

Gross pathology and histopathology evaluations showed consistent changes in the liver and kidneys in both male and female rats. These changes were related to the deposition of pigment on the outer surfaces and within cells of these organs. Pigment was observed histologically in periportal hepatocytes and phagocytes and in the epithelial cells of the proximal convoluted tubules of the kidneys in all dose groups. The kidneys in females were more severely affected than kidneys in males. This difference may be a reflection of the higher weight normalized doses in female rats. Pigment was also observed in the bile duct epithelial cells in all animals examined (except one female in the high-dose group). The bile duct epithelium was also hyperplastic, with the incidence of hyperplasia increasing with dose. This study did not show a NOEL because pigment was deposited in the bile duct epithelial cells and in kidneys in animals of all dose groups.

In a similar study, dogs were given 0.03, 0.2, or 1.0 percent Solvent Yellow 33 in their diets for one year (Hazelton Laboratories, Inc. 1967b). The 0.03-percent diet was continued for 1 year, the 0.2-percent diet was continued as 50 mg/kg/day in gelatin capsules after 179 days, and the 1.0-percent diet was continued as 250 mg/kg/day after 24 days. Histopathological evaluations showed changes similar to those observed in rats. Pigment was deposited in liver (periportal hepatocytes) and kidneys (epithelium of the proximal convoluted tubules) at all dose levels. The degree of deposition increased from minimal to slight in the low-dose groups, slight to moderate in the intermediate-dose groups, and moderate to severe in the high-dose groups. The bile ducts were hyperplastic, but the authors did not report pigment deposition in bile duct epithelial cells.

Solvent Yellow 33 at 0.1 and 1.0 percent in both hydrophilic ointment or white petroleum bases applied topically to abraded (15 times) or unabraded (65 times) skin did not affect the skin or internal organs (Hazelton Laboratories, Inc. 1965). Swiss-Webster mice treated topically

with 1 percent Solvent Yellow 33 in benzene for 95 weeks did not exhibit effects not also observed in vehicle controls (Hazelton Laboratories, Inc. 1967c).

In other subchronic and chronic toxicity studies, animals were exposed to Solvent Yellow 33 by inhalation. In a 4-week inhalation toxicity test Henderson et al. (1984) exposed male and female Fischer 344 rats to aerosols of Solvent Yellow 33 for 6 hr/day, 5 days/week. The mean measured aerosol concentrations were 10 ± 5 mg/m^3 (low dose), 51 ± 10 mg/m^3 (medium dose), and 230 ± 30 mg/m^3 (high dose) (mean ± S.D.). The particle sizes, expressed as MMAD, were 3.2 ± 0.3 μm, 3.5 ± 0.5 μm, and 4.4 ± 0.7 μm, respectively. A control group was included but was not described.

The animals were observed for clinical signs of toxicity before exposure, 2 weeks after initiation of exposure, and after termination of exposure. Body weights and respiratory function were measured before and after termination of exposure; lung biochemistry, hematology tests, serum chemistry tests, and histopathological evaluations were performed after termination of exposure (Henderson et al. 1984).

Clinical observations in controls and in animals exposed to all concentrations revealed no gross adverse effects of the dye. Body weight measurements showed that both male and female rats exposed to the high dose gained significantly less weight than controls. Weight gain during exposure was as follows: control males, 22 g; low-dose males, 23 g; medium-dose males, 19 g; high-dose males, 5 g; control females, 10 g; low-dose females, 13 g; medium-dose females, 11 g; high-dose females, 0 g. The high-dose males weighed 9.7 percent less than control males and high-dose females weighed 5.7 percent less than control females (Henderson et al. 1984).

Parameters of respiratory function were measured or calculated for 16 control and 16 high-dose animals. Dynamic and quasi-static lung compliance were greater in exposed animals, but total lung capacity was not significantly altered except when normalized against body weight. The functional residual capacity and forced vital capacity were also significantly larger in exposed animals. The absolute expiratory rates were not significantly altered, but they were significantly lower when normalized against the forced vital capacity. Henderson et al. (1984) summarized the pulmonary effects of exposure to aerosols of Solvent Yellow 33 as decreased lung elastic recoil and increased resting lung volume, with a slight forced airflow obstruction. They concluded that the changes were indicative of mild emphysema. Histopathological examination of the tissues of the respiratory tract, however, showed no evidence of emphysema (Henderson et al. 1984).

Lung biochemistry was evaluated by analysis of bronchoalveolar lavage (BAL) fluid and lung tissue. Lactate dehydrogenase (LDH), ß-glucuronidase, acid and alkaline phosphatases, glutathione reductase, acid proteinase, protein content, macrophages, and neutrophils were analyzed in animals from all exposure groups. Alkaline phosphatase activity was

significantly decreased in all exposure groups. Because alkaline phosphatase activity in concurrent controls was higher than in historical controls, the apparent decrease in activity in exposed animals may have been artifactual and of no physiological significance. Acid proteinase activity in BAL fluid was unchanged in high-dose animals, but acid proteinase activity in lung tissue was significantly elevated. The greatest increase was associated with cathepsin B, the activity inhibited by leupeptin. According to Henderson et al. (1984), an increase in acid proteinase activity is indicative of an inflammatory response, but an inflammatory reaction was not confirmed by significant increases in the numbers of neutrophils and macrophages.

Hematology and serum chemistry tests were performed on blood taken from six males and six females from controls and from each exposure group. The hematology parameters were not affected by Solvent Yellow 33. Modest, but statistically significant increases were found in the total CO_2, alkaline phosphatase, inorganic phosphorus, cholesterol, and glucose. Alkaline phosphatase activity in the low-dose group was significantly decreased. The physiological significance of these results was not apparent (Henderson et al. 1984).

This study by Henderson et al. (1984) showed that, for the most part, a 4-week exposure to Solvent Yellow 33 aerosols caused only minimal toxic effects in the respiratory tract and no physiologically significant toxic effects in systemic organs. Based on these results, Henderson et al. (1984) concluded that the lowest-observed-effect level (LOEL) for Solvent Yellow 33 was $\geq$230 mg/m^3 and the NOEL was 51 mg/m^3.

Male and female Fischer 344 rats were also exposed to aerosols of Solvent Yellow 33/Solvent Green 3 mixture (approximately 30 percent Solvent Yellow 33 and 70 percent Solvent Green 3) using a protocol identical to that for Solvent Yellow 33 (Henderson et al. 1984). The mean measured aerosol concentrations were 11 $\pm$ 5 (low dose), 49 $\pm$ 11 (medium dose), and 210 $\pm$ 50 mg/m^3 (high dose), with particle sizes (MMAD) of 3.2 $\pm$ 0.4, 3.7 $\pm$ 0.5, and 4.9 $\pm$ 0.6 μm, respectively.

No adverse gross clinical effects were observed. Male and female animals exposed to the high dose gained significantly less weight than did controls. As with Solvent Yellow 33 alone, the differences in weight gain were slight, resulting in only a 6.5 and 7.4 percent decrease in males and females, respectively. Both male and female rats exposed to the medium and low doses gained slightly more weight than controls.

Sixteen control and 16 high-dose animals were subjected to respiratory function tests. In contrast to animals exposed to Solvent Yellow 33 alone, quasi-static lung compliance, functional residual capacity, and forced vital capacity were not significantly altered by the Solvent Yellow 33/Solvent Green 3 mixture. Absolute expiratory rates were significantly decreased, but unlike animals exposed to the Solvent Yellow 33 alone, the expiratory rates normalized against the forced vital capacity were not significantly altered. Other parameters significantly altered by exposure to the dye mixture were as follows: vital capacity normalized against

total lung capacity (increased); residual volume, both absolute and normalized against total lung capacity (decreased); and diffusing capacity normalized against body weight or alveolar volume (decreased). Henderson et al. (1984) concluded that the dye mixture caused a trend toward smaller lung volume, reduction in gas exchange efficiency, and a slight airflow obstruction, but only in animals exposed to the highest dose.

Evaluation of lung biochemistry by analysis of BAL fluid showed that the following parameters were significantly elevated in high-dose rats: LDH, ß-glucuronidase, alkaline phosphatase, glutathione reductase, glutathione peroxidase, acid proteinase, protein content, macrophages, and neutrophils. Almost all of the acid proteinase activity was associated with cathepsin D, the activity resistant to inhibition by leupeptin. Protein content and neutrophils were elevated in medium-dose rats; macrophages and neutrophils were elevated in low-dose rats. Henderson et al. (1984) suggested that the elevation of enzymes in BAL fluid, along with the increases in macrophages and neutrophils, were symptomatic of an inflammatory response in the high-dose animals and a mild inflammatory response in the medium-dose animals. They further suggested that the high level of cathepsin D, along with the more modest increase in cathepsin B, indicated that the cleanup of lung particles and cellular debris was more important than turnover of pulmonary architecture.

Acid proteinase activity was elevated in lung tissue of animals exposed to the high dose of Solvent Yellow 33/Solvent Green 3 mixture. The activity was resistant to leupeptin, indicating that it was cathepsin D; cathepsin B was not elevated. The neutral proteinases (plasminogen and cathepsin 6-polymorphonuclear leucocyte elastase) were moderately increased. According to Henderson et al. (1984), these results were also indicative of an inflammatory response.

Hematology tests in 12 control rats and 12 rats exposed to each concentration revealed no changes. Serum chemistry tests showed that serum alkaline phosphatase activity, total bilirubin, creatinine, and inorganic phosphorus were elevated in exposed animals. Cholesterol and glucose were elevated, but not significantly. The absence of histopathological changes in the liver, however, indicated that these changes were not physiologically significant.

Histopathological evaluation of animals exposed to the highest dose showed a mild reaction around the terminal airways of the lungs that consisted of minimal to slight proliferation of foamy alveolar macrophages and minimal to slight hyperplasia of Type II pulmonary epithelial cells. This reaction was observed more often in males than in females and was even observed in some medium-dose animals. Reticuloendothelial cells with lymphoid hyperplasia were observed in the tracheobronchial lymph nodes, suggesting that even in the absence of phagocytized particles, the dye had moved into the lymph nodes. A yellowish-brown pigment was found below the respiratory epithelium of the nasal septum and turbinates, but not in the larynx, trachea, or bronchi. No other exposure-related lesions were observed (Henderson et al. 1984).

From these studies, Henderson et al. (1984) concluded that the LOEL for aerosols of Solvent Yellow 33/Solvent Green 3 mixture was ≥50 mg/m^3; the NOEL was 11 mg/m^3.

In a 90-day subchronic study, Henderson et al. (1985b) exposed male and female Fischer 344 rats to aerosols of Solvent Yellow 33, 6 hr/day, 5 days/week for 13 weeks. A total of 392 rats (196 each male and female) were entered into four exposure groups with target concentrations of 0, 1, 10, and 100 mg/m^3. The highest concentration was expected to cause minimal toxicity, and the lowest concentration was the lowest that could be maintained. The mean measured aerosol concentrations were 1.0 ± 0.2 mg/m^3 (low dose), 10.8 ± 1.8 mg/m^3 medium dose), and 100 ± 17 mg/m^3 (high dose), with particle sizes (MMAD) of 2.1 ± 0.1, 2.9 ± 0.3, and 4.0 ± 0.4 m, respectively. After termination of exposure, 64 animals of each sex, representing the four exposure groups, were observed for an additional 30 days. Evaluations of toxicity were performed as described for the 4-week exposure.

No gross clinical signs of toxicity or mortality were observed during exposure or during the 30-day observation period. Animals exposed to the high dose gained weight at a slower rate than controls. The decrease in weight gain was first observed during the 5th week of exposure. At termination of exposure, total weight gain in each group was as follows: control males, 70 g; low-dose males, 71 g; medium-dose males, 70 g; high-dose males, 57 g; control females, 19 g; low-dose females, 14 g; medium-dose females, 17 g; high-dose females, 8 g. The high-dose males weighed 4.1 percent less than control males, and high-dose females weighed 5.4 percent less than control females. Although the differences in weights of the high-dose groups were statistically significant, physiological significance was doubtful. By the end of the 30-day recovery period, the weight of the high-dose males was not different from that of control males, but the weight of high-dose females remained significantly less (3.5 percent) than that of control females (Henderson et al. 1985b).

Respiratory function was measured in eight male and eight female rats of each exposure group. Measurements taken prior to exposure, at the end of the 90-day exposure period, and at the end of the 30-day recovery period included 37 variables designed to evaluate ventilation, lung mechanics, gas distribution, and gas exchange. Exposure to Solvent Yellow 33 had almost no effect on respiratory function. The only variables signficantly altered were carbon monoxide diffusing capacity normalized against alveolar volume in high-dose animals at the end of the 90-day exposure period, and forced expiratory flow rate at 10 percent of forced vital capacity normalized against forced vital capacity in high-dose animals at the end of the 30-day recovery period. Therefore, in contrast to the 4-week exposure, emphysematous changes were not observed, and the 90-day exposure to Solvent Yellow 33 had very little effect on respiratory function (Henderson et al. 1985b).

Analysis of BAL fluid showed only a slight increase in macrophages in the low- and high-dose groups at the end of the 90-day exposure and in the high-dose group at the end of the recovery period. All other parameters

(LDH, acid phosphatase, ß-glucuronidase, protein content, and neutrophils) were similar to those in controls. In addition, BAL fluid and lung proteinase activities were also unchanged after exposure to Solvent Yellow 33. These results indicate that the dye did not induce an inflammatory reaction in the lungs (Henderson et al. 1985b).

Hematology tests performed on blood taken from rats at the end of exposure and at the end of recovery showed that none of the parameters were affected by Solvent Yellow 33. Serum chemistry tests revealed that immediately after exposure, alkaline phosphatase activity was significantly decreased in high-dose animals; serum glutamic pyruvic transaminase (SGPT) was decreased in medium- and high-dose animals; and bilirubin and cholesterol were increased in high-dose animals. Although the changes were statistically significant, it is doubtful that they were physiologically significant. Serum chemistry values were normal at the end of the recovery period (Henderson et al. 1985b).

For histopathological evaluation, ten rats of each sex from each exposure group were sacrificed immediately after exposure and at the end of the 30-day recovery period. Exposure-related lesions were usually associated with the deposition of pigment in various organs or tissues.

In all animals of the high-exposure group that were killed immediately after termination of exposure, pigment was deposited in the submucosa of the nasal cavity at levels III and IV, in the cortical tubules in the kidney, and in the bile duct epithelium or in the hepatocytes adjacent to the bile duct in the liver. Lung lesions consisted of minimal focal accumulation of foamy macrophages (containing pigment) in alveoli adjacent to bronchioles in only one male exposed to the high dose. This lesion was accompanied by minimal hyperplasia of Type II cells.

In animals of the medium-dose group, minimal submucosal pigment was deposited at level III in three males and two females and at level IV in five males and seven females. Minimal pigment deposition was noted in the liver of two female rats. Pigment deposition in the kidney was not increased above control levels. Exposure-related lesions were not found in the lungs. Exposure-related lesions were not found in animals exposed to the lowest concentration (Henderson et al. 1985b).

Ten animals of each sex in each exposure group were killed after a 30-day recovery period. The types and incidence of microscopic lesions in high-dose animals were similar to those in animals killed immediately after exposure; the lesions in the nasal cavity and kidney, however, were less severe, but in the liver and lungs, they were comparable to those observed immediately after exposure. In medium-dose animals, pigment was deposited at level III in two males and four females and at level IV in seven males and eight females. Pigment deposition in kidney was comparable to control. Exposure-related lesions were not found in the liver and lung of medium-dose animals. No exposure-related lesions were found in low-dose animals (Henderson et al. 1985b).

Microscopic lesions observed in the liver and kidney after inhalation exposure to Solvent Yellow 33 are similar to those observed after oral exposure, indicating that inhalation and oral exposure affect the same systemic organs. Bile duct hyperplasia, however, was not a significant lesion in rats exposed by inhalation.

Henderson et al. (1985b) analyzed tissue sections to determine if the pigment observed in the tissues was Solvent Yellow 33 (or a metabolite) or a natural constituent of the tissues. Sections of liver, kidney, and lung were stained with Prussian Blue (iron), periodic acid Schiff (PAS), and Hall's stain (bile). A large portion of the pigment did not stain, prompting Henderson et al. (1985b) to conclude that the pigment was Solvent Yellow 33 or a metabolite.

In the medium-dose animals, the effects of inhaling aerosols of Solvent Yellow 33 were either reversible or were not considered to be adverse. Henderson et al. (1985b) concluded that in the 90-day exposure test, 10 mg/m^3 was the NOAEL.

Male and female Fischer 344 rats were also exposed to aerosols of Solvent Yellow 33/Solvent Green 3 mixture using the same protocol as described for Solvent Yellow 33 alone. Concentrations were 0 (control), 1.1 ± 0.5 (low dose), 10.2 ± 3.1 (medium dose), and 101 ± 23 mg/m^3 (high dose) with particle sizes (MMAD) of 2.8 ± 0.4, 3.0 ± 0.2, and 4.2 ± 0.4 μm, respectively.

Clinical observations 6 weeks after initiation of exposure, at termination of exposure, and after a 30-day recovery showed no signs of gross toxicity and no mortality. Weight gain during exposure was as follows: control males, 69 g; low-dose males, 72 g; medium-dose males, 62 g; high-dose males, 50 g; control females, 39 g; low-dose females, 30 g; medium-dose females, 33 g; high-dose females, 20 g. Immediately after termination of exposure, high-dose males weighed 8.0 percent less than control males and high-dose females weighed 9.2 percent less than control females. At the end of the 30-day recovery period, the body weights of high-dose male rats remained significantly lower than control males, whereas the body weights of high-dose female rats were normal.

Respiratory function was measured as in animals exposed to Solvent Yellow 33 alone. There were no significant differences between values of absolute functions in control and exposed animals. Because the body weights of high-dose animals was lower than control, there was a trend for variables normalized against weight to be higher than in control animals, but the only variable significantly higher was carbon monoxide diffusing capacity normalized against body weight. After 30 days of recovery, the only variable significantly affected by exposure was a lower carbon monoxide diffusing capacity normalized against alveolar volume. These results demonstrated that the 90-day exposure to aerosols of Solvent Yellow 33/Solvent Green 3 mixture had very little effect on respiratory function in rats (Henderson et al. 1985b).

Lung biochemistry was evaluated by analysis of BAL fluid 6 weeks after initiation of exposure, at termination of exposure, and after 30 days of recovery. In contrast to animals exposed to Solvent Yellow 33 alone, LDH, ß-glucuronidase, protein content, the number of macrophages, and the number of neutrophils were significantly affected by exposure to Solvent Yellow 33/Solvent Green 3 mixture (Table 12). The effects, which were noted only in high-dose animals killed 6 weeks after initiation of exposure, did not become progressively worse, but became less severe with continued treatment and recovery. Acid proteinase was not elevated in BAL fluid. Acid proteinase activity, however, was significantly elevated in the lung tissue of rats exposed to the high dose and killed immediately after termination of exposure. The level of activity decreased during recovery but remained significantly higher than in control animals. These

TABLE 12. ANALYSIS OF BRONCHOALVEOLAR LAVAGE (BAL) FLUID IN RATS EXPOSED TO 101 mg/m^3 OF SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE[a]

Parameter	Exposure	Sacrifice Week[b] 6	13	17
LDH[c] (mIU/g)[b]	Control	490 ± 40	390 ± 30	370 ± 20
	Exposed	1210 ± 50[d]	930 ± 50[d]	780 ± 40[d]
Acid Phosphatase (mIU/g)	Control	9.7 ± 0.8	9.2 ± 0.7	7.9 ± 0.6
	Exposed	16.1 ± 2.2	11.5 ± 1.0	7.4 ± 0.6
ß-Glucuronidase (mIU/g)	Control	1.5 ± 0.2	2.2 ± 0.8	0.9 ± 0.1
	Exposed	7.2 ± 0.3[d]	5.7 ± 0.8[d]	3.4 ± 0.5[d]
Protein (mg/mL)	Control	1.5 ± 0.2	1.1 ± 0.2	0.9 ± 0.1
	Exposed	3.4 ± 0.3[d]	3.2 ± 0.6[d]	2.0 ± 0.2[d]
Macrophages (10^3 cells/g)	Control	730 ± 60	600 ± 60	450 ± 40
	Exposed	770 ± 110	1000 ± 160[d]	580 ± 70
Neutrophils (10^3 cells/g)	Control	5 ± 2	0 ± 0	7 ± 3
	Exposed	1300 ± 130[d]	470 ± 100[d]	290 ± 50[d]

a. Adapted from Henderson et al. 1985b.
b. Values represent total amounts of material recovered in BAL divided by the net weight of the lung in g; Mean ± S.E.
c. LDH - lactate dehydrogenase.
d. $p \leq 0.05$, by Bonferroni pairwise comparison of means.

changes were indicative of an inflammatory reaction that had not cleared up by the end of the recovery period. Henderson et al. (1985b) attributed the inflammation to Solvent Green 3 in the mixture and not to Solvent Yellow 33.

Serum chemistry and hematology tests revealed that alkaline phosphatase activity was significantly decreased and cholesterol, glucose, inorganic phosphorus, total protein, and albumin were significantly increased in rats exposed to the high dose. Glucose, inorganic phosphorus, total protein, and albumin were elevated in medium-dose animals, and glucose, total protein, and albumin were elevated in low-dose animals. Because blood urea nitrogen (BUN), SGPT, and creatinine levels were normal, indicating no damage to the kidneys and liver, Henderson et al. (1985b) concluded that these changes were not clinically significant. All serum chemistry parameters returned to normal by the end of recovery, indicating that the changes were reversible.

Histopathological evaluation of animals exposed to Solvent Yellow 33/Solvent Green 3 mixture showed changes similar to those observed after exposure to Solvent Yellow 33 alone. In almost all high-dose animals, pigment was deposited in the submucosa of the nasal epithelium, with the heaviest deposit at level III and level IV; pigment was also observed in the cortical tubules in the kidneys and in the bile duct epithelium or in hepatocytes adjacent to the bile duct in all high-dose animals. In the lungs of all high-dose animals, lesions consisted of slight to moderate accumulation of foamy alveolar macrophages (containing pigment) accompanied by slight to moderate hyperplasia of Type II cells. In the tracheobronchial lymph nodes, reticuloendothelial cell hyperplasia (containing pigment) accompanied by moderately severe lymphoid hyperplasia was observed.

In medium-dose animals, pigment was deposited in the submucosa at level III in one male and four females and at level IV in four females. Minimal lesions in the lungs were observed in three male and three female rats. Reticuloendothelial cell hyperplasia with pigment deposition was observed in two male and two female rats, and lymphoid hyperplasia was observed in one male. No exposure-related lesions were observed in low-dose animals.

After the 30-day recovery period, the lung lesions were slightly less severe than those observed immediately after exposure in high-dose animals. Pigment deposition in the nasal cavity and in cortical tubules in the kidney was less severe, but was unchanged in the liver. In the tracheobronchial lymph nodes, reticuloendothelial cell hyperplasia was more severe, and lymphoid hyperplasia was unchanged.

In medium-dose animals, minimal lung lesions were present in two male and two female rats; pigment deposition was noted in the the nasal cavity at level III in six males and five females and at level IV in two males and six females. Pigment in the kidney was comparable to control, but pigment was absent in the liver. In the tracheobronchial lymph nodes, lymphoid hyperplasia was observed in one male, but reticuloendothelial

cell hyperplasia was absent in all animals. No exposure-related lesions were observed in the low-dose animals (Henderson et al. 1985b).

Because exposure-related microscopic lesions were observed in animals exposed to aerosols of Solvent Yellow 33/Solvent Green 3 mixture at the medium concentration (10 mg/m^3) but not at the low concentration, Henderson et al. (1985b) concluded that the NOAEL was 1 mg/m^3.

Marrs et al. (1984) described the toxic effects resulting from chronic inhalation of a smoke mixture composed of 13 percent Solvent Yellow 33, 16 percent Disperse Red 9, and 19 percent Solvent Green 3. Three animal species, 400 Porton-strain SPF female mice, 200 Porton-Wistar-derived female rats, and 200 Dunkin-Hartley female guinea pigs were exposed to the combusted smoke mixture for 1 hr/day, 5 days/week for 20 weeks (100 exposures), at concentrations of 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), and 1012.4 mg/m^3 (high dose, mice, rats) or 1161.1 mg/m^3 (high dose, guinea pigs). Starting with the initiation of exposure, the animals were observed for 71 weeks for toxicity effects and then sacrificed for histopathological evaluation.

Because the animals were exposed to a mixture of dyes, toxic effects could not be attributed to Solvent Yellow 33 alone. During the treatment period, the mortality rates were low in all groups, with the exception of the guinea pigs exposed to the high dose. After 16 exposures, treatment of guinea pigs was discontinued because of a high intercurrent mortality during exposure, which was 18 percent after 4 weeks. Dose-related trends in mortality rates were not significant in mice, rats, and low- and medium-dose guinea pigs (F-test). In high-dose guinea pigs, the mortality rate at 71 weeks was 28 percent compared with 12 percent in the control group; a dose-related trend in the mortality rate was also not significant (chi-square test).

During the treatment period, mean body weights of exposed and control groups, related to chronological age, were significantly different ($p < 0.005$, Kolmogorov-Smirnov test). Terminal weights were significantly different only in rats exposed to medium and high doses. Guinea pigs exposed to the high dose lost weight rapidly during exposure, but body weights stabilized after exposure was terminated.

Organ weights were not affected by treatment, with the exception of lung weights in mice and rats. The lungs in mice exposed to the high dose weighed more than lungs in mice exposed to medium and low doses ($p < 0.05$), and the lungs in rats exposed to the high dose weighed more than those in control rats ($p < 0.001$).

Histopathological evaluation of all animals dying prior to or surviving until termination revealed changes related almost exclusively to the respiratory tract. In mice sacrificed at 40 weeks, significant dose-related trends for severe chronic pneumonia ($p < 0.001$), bronchiectasis ($p < 0.001$), and alveolitis ($p < 0.05$) were revealed. These changes were attributed to nonspecific damage caused by inhaling particulate matter, and not to specific toxic effects of the smoke mixture. At 71 weeks,

significant dose-related trends were observed for the presence of alveolar macrophages ($p < 0.001$), combined incidence of mild and severe chronic pneumonia ($p < 0.05$), and fatty livers ($p < 0.05$).

In rats evaluated at 71 weeks, significant trends were observed for the presence of submucosal lymphocytes in the larynx ($p < 0.05$) and trachea ($p < 0.01$), perivascular lymphocyte aggregates ($p < 0.001$), alveolitis ($p < 0.05$), and mild and severe foreign-body reaction characterized by the presence of alveoli packed with macrophages $p < 0.001$). According to Marrs et al. (1984), the foreign-body reaction often caused complete obliteration of alveolar spaces, which should have led to a loss of respiratory capacity and a high mortality rate; the mortality rate, however, was not affected.

In guinea pigs, a significant increase in the incidence of severe alveolitis was observed in the low- and medium-dose groups ($p < 0.05$), but not in the high-dose group, which received only 16 exposures.

The incidence of hyperplastic and neoplastic lesions in animals exposed to this mixture is discussed in Section 4.6.

4.3.2. Human Data

No data were found on the effects of chronic exposure to Solvent Yellow 33 in humans.

4.4 GENOTOXICITY

4.4.1 Animal Data

Moore et al. (1984) tested Solvent Yellow 33 (93.1 percent pure), Solvent Yellow 33/Solvent Green 3 mixture (1:2 ratio, 95.0 percent pure), and Solvent Yellow 33 purified by recrystallizing three times from ethyl acetate (99.9 percent pure) in seven strains of Salmonella typhimurium, mouse lymphoma cells, and mouse bone marrow cells. The in vitro tests were performed with and without activation with the S9 fraction from Aroclor 1254-induced rat liver.

Strains TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98 were tested in the Salmonella Reversion Assay using the standard plate incorporation method. The dyes were dissolved in DMSO (dimethylsulfoxide) and tested at the following concentrations: 0, 1, 5, 10, 30, 50, 100, 300, 500, and 1,000 μg/plate. Solvent Yellow 33/Solvent Green 3 mixture precipitated at 100 μg/plate, and Solvent Yellow 33 precipitated at 300 μg/plate, causing a narrower dose-response range and increased variations in the data (Moore et al. 1984).

The data showed that strain TA100 gave a weak positive response to all three dyes with S9 activation and a negative response without S9 activation. Strain TA104 gave a weak positive response to all three dyes with and without S9 activation. Strain TA102 gave a strong positive response to all three dyes with and without S9 activation, and strains TA1535, TA1537, TA1538, and TA98 gave negative responses to all three dyes with and without S9 activation, except for one positive response to purified Solvent Yellow 33 using TA1537. Therefore, all three dyes were mutagenic in three strains of Salmonella typhimurium (TA100, TA104, and TA102) (Moore et al. 1984).

The Mouse Lymphoma Assay, which detects mutations affecting the thymidine kinase locus, was performed with L5178Y/TK$^{+/-}$ mouse lymphoma cell line. Because solubility of the dyes in 1 percent DMSO was limited, the concentration range was narrower than is usually prescribed, and concentrations above 20 μg/mL had to be prepared in 2 percent DMSO. A positive response was indicated by a twofold increase in the mutant frequency at one or more concentrations from two separate assays and by a dose-response relationship when cell survival was greater than 10 percent (Moore et al. 1984).

The results are summarized in Tables 13 and 14. Cell survival was greater than 10 percent at all concentrations of the three dyes, with the exception of the 40-μg/mL concentration of Solvent Yellow 33/Solvent Green 3 mixture in Test 1 without activation, where survival was only 9.2 percent.

With S9 activation, Solvent Yellow 33 was mutagenic; the lowest concentration that gave a positive response was 12 μg/mL; toxicity was observed at 40 μg/mL (Table 13). Purified Solvent Yellow 33 was also mutagenic with S9 activation; the lowest concentration that gave a positive response was also 12 μg/mL in Test 1 and 10 μg/mL in Test 2 (Table 14). The Solvent Yellow 33/Solvent Green 3 mixture formed a precipitate at concentrations of 6 μg/mL or higher. With S9 a positive mutagenic response was observed only at the highest concentration tested (40 μg/L). At this concentration, however, the dye mixture contained sufficient Solvent Yellow 33 to induce the observed mutant frequency, because one third of the 40 μg/mL of the dye mixture was Solvent Yellow 33 (13.3 μg/mL). The data in Table 13 showed that with S9 activation, Solvent Yellow 33 alone is mutagenic at 12 μg/mL. Therefore, the mutagenic component in the mixture could be Solvent Yellow 33.

Solvent Yellow 33, purified Solvent Yellow 33, and Solvent Yellow 33/Solvent Green 3 mixture were more potent as mutagens in mouse lymphoma cells without S9 activation than with S9 activation (Tables 13 and 14). Without S9 activation, a clear dose response was not observed, but according to Moore et al. (1984), compounds tested near their solubility limit tend to give a plateau-type dose response, and the closely spaced doses could be considered as replicates. The lowest doses giving a positive response were Solvent Yellow 33 at 2 μg/mL and purified Solvent Yellow 33 at 1.0 μg/mL (Test 2). The authors reported that in the presence of S9, Solvent Yellow 33 is toxic at 40 μg/mL; in the absence of

TABLE 13. SUMMARY OF MOUSE LYMPHOMA CELL MUTAGENICITY TESTS WITH SOLVENT YELLOW 33 OR SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE[a]

	Total Mutant Frequency ($x10^6$)[b]					
	Solvent Yellow 33			Solvent Yellow 33/Solvent Green 3		
Concentration	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
With S9						
AAF[c]	374.7	252.2	370.8	374.7	252.2	384.8
1% DMSO	56.2	71.3	40.8	56.2	71.3	49.8
2% DMSO	58.4	68.9	NT[d]	58.4	68.9	46.0
2 μg/mL	52.0	61.1	88.4	76.4	61.4	NT
6 μg/mL	57.9	65.1	93.9	42.4	57.4	55.8
12 μg/mL	156.8	123.1	176.0	50.5	65.9	57.7
16 μg/mL	233.3	204.5	127.1	69.6	65.0	71.9
20 μg/mL	308.5	227.1	157.1	61.8	59.4	86.5
40 μg/mL[e]	Toxic	Toxic	NT	150.7	140.3	274.4
Without S9						
MMS[c]	531.9	819.8		531.9	898.7	
1% DMSO	56.6	49.6		56.6	52.1	
2% DMSO	46.6	51.3		46.6	NT	
2 μg/mL	337.7	424.4		77.9	62.7	
6 μg/mL	398.1	765.0		295.5	347.0	
12 μg/mL	329.2	547.7		443.1	314.0	
16 μg/mL	375.0	576.8		408.4	451.5	
20 μg/mL	417.7	527.4		373.6	571.1	
40 μg/mL[e]	488.1	1,033.1		381.6	NT	

a. Adapted from Moore et al. 1984.
b. Total number of mutant colonies per number of viable cells plated.
c. AAF = 2-acetylaminofluorene (40 μg/mL); MMS = methylmethanesulfonate (15 μg/mL).
d. NT = not tested; both dyes tested only 2 times without S9.
e. Because of low solubility, this concentration was prepared in 2 percent DMSO; all others were prepared in 1 percent DMSO.

TABLE 14. SUMMARY OF MOUSE LYMPHOMA CELL MUTAGENICITY TESTS WITH PURIFIED SOLVENT YELLOW 33[a]

Concentration	Total Mutant Frequency ($x10^6$)[b]			
	With S9		Without S9	
	Test 1	Test 2	Test 1	Test 2
Pos. Cont.[c]	193.1	482.8	544.3	315.8
1% DMSO	56.5	35.9	44.2	92.1
0.1 μg/mL	NT[d]	NT	41.0	72.0
0.5 μg/mL	NT	NT	77.4	118.3
1.0 μg/mL	NT	NT	NT	235.7
2.5 μg/mL	NT	49.4	525.9	235.7
5 μg/mL	44.6	41.4	493.5	277.1
10 μg/mL	99.5	93.4	836.4	347.9
12 μg/mL	145.3	116.9	NT	NT
16 μg/mL	153.1	123.8	NT	NT
20 μg/mL	117.4	120.4	943.3	326.9
24 μg/mL	191.1	183.1	NT	NT
30 μg/mL	NT	NT	425.4	316.9
40 μg/mL	NT	NT	423.8	385.5
50 μg/mL	NT	NT	390.9	349.1

a. Adapted from Moore et al. 1984.
b. Total number of mutant colonies per number of viable cells plated.
c. AAF = 2-Acetylaminofluorene (40 μg/mL); MMS = methylmethanesulfonate (15 μg/mL).
d. Not tested.

S9, the purified dye was not reported to be toxic at 50 μg/mL. Solvent Yellow 33/Solvent Green 3 mixture at 6 μg/mL gave a definite positive response without S9 activation. Precipitates were observed at concentrations of 9 μg/mL or higher, indicating that, unlike tests with S9 activation, the mixture was mutagenic at concentrations that did not produce a precipitate. It appears that the mutagenic component in the mixture is Solvent Yellow 33, because one-third of the 6 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture is Solvent Yellow 33 (2 μg/mL). A concentration of 2 μg/mL of Solvent Yellow 33 was mutagenic when tested alone and was, therefore, sufficient to produce a positive response similar to that observed with 6 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture (Table 13).

Moore et al. (1984) also analyzed the size distribution of the mutant colonies induced by 20 μg/mL of Solvent Yellow 33, 20 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture, and 10 μg/mL of purified Solvent Yellow 33 without S9 activation. Small colonies represent chromosome damage (clastogenic effects), and large colonies represent single-gene damage (mutations). A large fraction of the mutant colonies induced by the three dyes were small, suggesting that the dyes induced chromosome damage. Analysis of the gross aberration frequency showed that Solvent Yellow 33/Solvent Green 3 mixture induced 100 aberrations/100 cells at 12 to 40 μg/mL. Solvent Yellow 33 induced 100 to 140 aberrations/100 cells at concentrations of 6 to 40 μg/mL. The types of aberrations noted were chromosome breaks, translocations, and chromosome deletions. These results were confirmed by Doerr et al. (1986), who demonstrated that a dose as low as 1 μg/mL of Solvent Yellow 33 induced gross chromosome aberrations in mouse lymphoma cells. They also showed that Solvent Yellow 33 was as potent as benzo(a)pyrene.

In vivo sister chromatid exchange in male C57BL/6 mouse bone marrow cells was analyzed by Moore et al. (1984) as an in vivo test for genotoxicity. Solvent Yellow 33 in 0.1 mL of DMSO and Solvent Yellow 33/Solvent Green 3 mixture in 0.1 mL DMSO + 0.1 mL corn oil was injected intraperitoneally into three to four animals per group. Solvent Yellow 33 at doses of 5, 15, 25, or 35 mg/kg was given one or three times over 3 days. Solvent Yellow 33/Solvent Green 3 mixture was given as a single dose of 10, 20, or 40 mg/kg. Positive controls were injected with cyclophosphamide, and negative controls were injected with the appropriate vehicle. The results showed that all treatments were ineffective in inducing in vivo sister chromatid exchange in mouse bone marrow cells. The dyes were not cytotoxic, and they were not shown to be localized in bone marrow cells. Nevertheless, the authors presumed that the dyes were distributed to bone marrow cells; evidence for this conclusion was not presented.

Solvent Yellow 33 also did not induce sister chromatid exchange in mouse lymphoma cells in vitro. Moore et al. (1984) concluded that the inability to induce in vivo sister chromatid exchange in mouse bone marrow cells was due to insensitivity of the end point and not to a nongenotoxic effect of Solvent Yellow 33. The data presented in Tables 13 and 14 definitely show that Solvent Yellow 33 induced mutations in mouse lymphoma

cells in vitro. Additional studies showed that Solvent Yellow 33 also induced chromosome aberrations in mouse lymphoma cells. Therefore, at least in mouse lymphoma cells, Solvent Yellow 33 is genotoxic, and induction of sister chromatid exchange is probably an insensitive end point.

4.4.2 Human Data

No data were found on genotoxic effects of Solvent Yellow 33 in humans.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

No data were found on developmental or reproductive toxicity of laboratory animals or humans.

4.6 ONCOGENICITY

No data were found on the carcinogenicity of Solvent Yellow 33 in humans. One study on the carcinogenicity of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture in mice and another study reporting the incidence of neoplastic lesions in animals exposed to a dye mixture containing Solvent Yellow 33, Solvent Green 3, and Disperse Red 9 were found.

Stoner (1985) tested Solvent Yellow 33 (93.1 percent purity) alone and Solvent Yellow 33/Solvent Green 3 mixture (24:71 percent ratio) in a lung tumor bioassay using strain A mice. The maximum tolerated dose was established from the results obtained after injecting mice intraperitoneally with 25 mg/kg of both dyes six times over a 2-week period. Because the dose did not result in mortality or weight loss, the maximum tolerated dose was set at 25 mg/kg.

Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture was dissolved in tricaprylin and injected (intraperitoneally) at doses of 5, 12.5, or 25 mg/kg into 50 mice (25 per sex). The animals received three injections per week for 8 weeks. Untreated, vehicle-treated, and urethane-treated controls were included. The animals were killed 30 weeks after initiation of treatment and examined histologically for lung adenomas and tumors at other sites if gross lesions were observed.

During the course of the study 10 percent of the mice treated with 25 mg/kg of Solvent Yellow 33 died and 26 percent of the mice treated with 25 mg/kg of Solvent Yellow 33/Solvent Green 3 mixture died. Death was attributed to peritonitis caused by accumulation of dye in the peritoneal cavity. The incidence and multiplicity of lung tumors were not increased, and tumors at other sites were not induced by either dye. Therefore, both Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture were noncarcinogenic in the mouse lung tumor bioassay.

Slaga et al. (1985) tested 12 chemicals in the lung tumor bioassay using the same protocol as described by Stoner (1985) and found that all the chemicals were negative including benzo(a)pyrene and 4-nitroquinoline-n-oxide. The authors reported that the lung tumor bioassay "is not always an appropriate and reliable screening test for carcinogens." Smith and Witschi (1983) reported that the lung tumor bioassay correctly identified only 5 of 18 known animal or human carcinogens. They concluded that the lung tumor bioassay was not a sensitive or accurate short-term in vivo screening procedure for carcinogens.

Marrs et al. (1984) exposed mice, rats, and guinea pigs to a smoke mixture containing 16 percent Disperse Red 9, 13 percent Solvent Yellow 33, and 19 percent Solvent Green 3 for 1 hr/day, 5 days/week for 20 weeks at concentrations of 105.8 mg/m^3, 309.6 mg/m^3, and 1,012.4 mg/m^3 or 1,161.1 mg/m^3. Further details of this experiment were presented in Section 4.3.

Seventy-one weeks after initiating treatment, histopathological evaluation revealed three lesions in medium-dose and two lesions in high-dose mice classified as hepatoma A and one lesion classified as hepatoma B in low-dose mice (no significant dose-related trend). One adenocarcinoma of the breast was observed in the low- and medium-dose groups, but the incidence did not show a significant dose-related trend.

In rats killed 71 weeks after initiating exposure, one adenocarcinoma and one squamous cell carcinoma of the lungs were observed, but no significant dose-related trend was observed. In addition, two hemangiomas in the adrenal gland in the high-dose group ($p < 0.05$), one biliary hyperplastic lesion in the medium-dose and four in the high-dose groups ($p < 0.01$), and three adenocarcinomas of the breast in the high-dose group were significant for dose-related trends. The incidence of neoplastic lesions in exposed guinea pigs was not significantly different from that of controls. Because these animals were exposed to Solvent Green 3 and Disperse Red 9 in addition to Solvent Yellow 33, the induction of hyperplastic lesions could not be attributed to Solvent Yellow 33.

4.7 SUMMARY

Very few data were available on the pharmacokinetics of Solvent Yellow 33 administered orally. One study showed that within 94 hr, approximately 58 percent of Solvent Yellow 33 administered to rats by gavage was absorbed from the gastrointestinal tract. In another study, the fur of albino rats turned light green or yellow within 2 days after a single oral dose of Solvent Yellow 33 or Solvent Yellow 33/Solvent Green 3 mixture, indicating that the dye may be excreted through the skin.

A detailed study on the pharmacokinetics of Solvent Yellow 33 aerosols inhaled by rats showed that 41 percent of the dye inhaled in 1 hr is deposited in the lungs. One hour after exposure 32 percent of the Solvent Yellow 33 deposited in the lungs is absorbed and distributed to

the major organs and tissues, indicating that Solvent Yellow 33 is rapidly absorbed from the lungs and distributed to other tissues. The rapid clearance of the dye from the lungs is confirmed by the short half-time of elimination from the lungs (2 hr). Solvent Yellow 33 is also rapidly absorbed from the lung after repeated exposures; less than 0.2 percent of the quantity deposited after each exposure is retained (i.e., 99.8 percent is absorbed within 16 hr).

Solvent Yellow 33 is rapidly eliminated from the tissues and excreted from the body. Within 70 hr after inhalation exposure, 1.8 percent is exhaled as CO_2, 14 percent is excreted in urine, 77 percent is excreted in feces, and only 8 percent is retained in the body. Only 13 and 40 percent of the products excreted in urine and feces, respectively, are unmetabolized Solvent Yellow 33. Therefore, a large fraction of the dye is metabolized, probably in the liver and kidney.

The acute oral LD_{50} for Solvent Yellow 33 is >10 g/kg in rats and >1 g/kg in dogs. In rats a dietary concentration of 0.15 percent causes increased liver weight, without histopathological lesions, within 10 days. Topical application of 2 g/kg to abraded rabbit skin causes minimal to mild hyperkeratosis of the skin and mild gastrointestinal effects. Solvent Yellow 33 applied to the skin in doses of 50, 200, and 1,000 mg/kg 5 days/week for 2 weeks causes hyperkeratosis, acanthosis, and adnexal hyperplasia of the skin. The 200- and 1,000-mg/kg doses also induce fatty changes in the liver. Solvent Yellow 33 at a dose of 500 mg is essentially nonirritating to the skin, and 100 mg of the dry powder is minimally irritating to the eyes.

Although Solvent Yellow 33 is only mildly toxic to the skin, the dye is very active in inducing delayed contact hypersensitivity reactions in guinea pigs and humans. The NOAEL in guinea pigs is 1 ppm for the induction stage and 0.1 ppm for the challenge stage. Contact dermatitis is induced in humans by commercial products containing Solvent Yellow 33. The NOAEL in humans is 0.5 ppm, but very sensitive individuals may respond to a dose as low as 1×10^{-4} ppm (1×10^{-8} percent). Therefore, Solvent Yellow 33 is considered to be a strong sensitizer.

In rats exposed to aerosols of Solvent Yellow 33 by inhalation, a single 1-hr exposure at approximately 1,000 mg/m^3 causes no mortality or gross toxic effects within 14 days. Repeated 6-hr exposures at 1,290 mg/m^3 cause hypertrophy and hyperplasia of goblet cells of the respiratory epithelium in the nasal cavity, chronic nonsuppurative inflammation of the naso-lacrimal duct, and serous inflammation of the epithelium of the naso-vomer organ.

No data were found on subchronic and chronic toxicity in humans. Subchronic (oral and inhalation) and chronic (oral) exposure of laboratory animals to Solvent Yellow 33 is consistently associated with pigment deposition in hepatocytes, bile duct epithelial cells, and renal tubules and the induction of hyperplasia of the bile duct epithelium.

In rats exposed to aerosols of Solvent Yellow 33 at concentrations of 10, 51, or 230 mg/m^3 for 4 weeks, the high-dose animals gain weight at a slower rate than controls and show no gross signs of toxicity but develop changes in respiratory function suggestive of emphysema. Biochemical analysis of the lungs reveals changes suggestive of an inflammatory response. Hematology and serum chemistry changes were either absent or physiologically insignificant. The LOEL for a 4-week exposure to aerosols of Solvent Yellow 33 is ≥230 mg/m^3, and the NOEL is 51 mg/m^3.

In addition to changes in respiratory function, a 4-week exposure to aerosols of Solvent Yellow 33/Solvent Green 3 mixture at concentrations of 49 or 210 mg/m^3 causes an inflammatory reaction in the lungs, hyperplasia of Type II pulmonary epithelial cells, and hyperplasia of reticulo-endothelial and lymphoid cells in the tracheobronchial lymph nodes. The LOEL for Solvent Yellow 33/Solvent Green 3 mixture is ≥50 mg/m^3, and the NOEL is 11 mg/m^3.

In a 90-day subchronic study, rats exposed to Solvent Yellow 33 at concentrations of 1, 10.8, or 100 mg/m^3 show no statistically significant biochemical or physiological changes. Histopathological lesions are observed in the lungs, kidney, and liver in animals exposed to 100 mg/m^3. These lesions include focal accumulation of pigment-containing macrophages adjacent to bronchioles in the lungs accompanied by Type II cell hyperplasia, and pigment deposition in the submucosa of the nasal cavity. The NOAEL was observed at 10.8 mg/m^3. Solvent Yellow 33/Solvent Green 3 mixture at concentrations of 1.1, 10.2, or 101 mg/m^3 for 90 days caused an inflammatory reaction in the lungs that was attributed to Solvent Green 3 in the mixture. The NOAEL was observed at 1 mg/m^3.

No data on the genotoxicity of Solvent Yellow 33 in humans were found. Genotoxicity tests show that Solvent Yellow 33 induces mutations in three strains of Salmonella typhimurium. TA100 gave a weak positive response with S9 activation and a negative response without S9 activation; TA104 gave a weak positive response, and TA102 gave a strong positive response with and without S9 activation. Solvent Yellow 33 induced mutations and chromosome damage in mouse lymphoma cells. The dye, however, was more potent without S9 activation. The lowest concentration of the technical grade dye that induced mutations was 12 μg/mL with S9 activation and 2 μg/mL without activation. The lowest concentration of purified Solvent Yellow 33 (99.9 percent pure) that induced mutations was 10 μg/mL with activation and 1.0 μg/mL without activation. Solvent Yellow 33 did not induce sister chromatid exchange in mouse bone marrow cells in vivo or in mouse lymphoma cells in vitro.

No data on the carcinogenicity of Solvent Yellow 33 in humans were found. In the Mouse Lung Tumor Bioassay, doses of 5, 12.5, and 25 mg/kg of Solvent Yellow 33 are not carcinogenic.

No data on the developmental or reproductive toxicity of Solvent Yellow 33 in humans or laboratory animals were found.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

As of December 20, 1976, the USFDA permanently listed D&C Yellow No. 11 (Solvent Yellow 33) for use in externally applied drugs and cosmetics (USFDA 1976). The dye is subject to certification with the following specifications: (1) not >1 percent volatile matter (at 135°C), (2) not >0.4 percent ethyl alcohol-insoluble matter, (3) not >0.3 percent phthalic acid, (4) not >0.2 percent quinaldine, (5) not >5 percent subsidiary colors, (6) not >20 ppm lead (as Pb), (7) not >3 ppm arsenic (as As), (8) not >1 ppm mercury (as Hg), and (9) not <96 percent total color (USFDA 1984).

During the production of colored smoke grenades, workers are exposed to fine-powdered dusts. The U.S. Occupational Safety and Health Administration (USOSHA) standard (8-hr time-weighted average) for the levels of inert or nuisance dust in the occupational environment is 15 mg/m^3 of total dust or 5 mg/m^3 of respirable dust (USOSHA 1986). The threshold limit value for inert or nuisance dust is 10 mg/m^3 of total dust or 5 mg/m^3 of respirable dust (ACGIH 1986, ILO 1980). The federal ambient air quality standard for particulate matter is 75 $\mu g/m^3$ annual geometric mean and 260 $\mu g/m^3$ for a maximum 24-hr concentration not to be exceeded more than once per year (USEPA 1981, as reported in Cichowicz and Wentsel 1983).

The Surgeon General of the Army has established interim guidelines for the disposal of colored smokes. There should be no open burning, and personnel should not be exposed to dye components at levels above 0.2 mg/m^3 (8-hr time-weighted average) (Cichowicz and Wentsel 1983).

5.2 OCCUPATIONAL EXPOSURE

Manufacturing personnel are exposed to fine-powdered dusts through inhalation, skin, and eye contact. During training and testing operations, Army personnel are exposed to pyrolysis reaction products formed during combustion of colored smoke grenades and upon dissemination of dye vapors as condensate in the smoke cloud (Tatyrek 1965). According to Garcia et al. (1982), the levels of dust in the colored smoke grenade production facility at Pine Bluff Arsenal exceeded the limits established by USOSHA.

Henderson et al. (1985c) monitored worker inhalation exposure to Solvent Yellow 33 during normal operation of the colored smoke grenade fabrication facility at the Pine Bluff Arsenal. Field sampling was conducted in 1984 to measure the concentration and size distribution of airborne dye-containing particles. HPLC analysis of filter samples showed that 40 percent of the total airborne particulate matter was Solvent Yellow 33. Within the general vicinity of some workers, concentrations of

dye-containing aerosols ranged from 0.1 to 1.5 mg/m^3. Impactor samples indicated that 50 to 70 percent of the aerosols were of respirable size (<10 μm MMAD). The maximum concentration of respirable Solvent Yellow 33 aerosols detected outside protective acrylic curtains in the production area was <0.5 mg/m^3. The concentration of respirable Solvent Yellow 33 aerosols within the acrylic curtain ranged from 0.6 to 5.8 mg/m^3, indicating that the protective curtain reduced particle concentration by 10- to 20-fold. The highest total airborne particle concentration was 32 mg/m^3 within an acrylic curtain at a fill and press station.

5.3 PREVIOUSLY CALCULATED CRITERIA

No aquatic or human health criteria have previously been calculated for Solvent Yellow 33.

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value (FAV), whereas the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Although static acute toxicity tests with seven of the required eight freshwater aquatic species indicated that Solvent Yellow 33 is not lethal at its solubility limit, these data are insufficient to establish a CMC. As recommended by ASTM guidelines (ASTM 1980), in order to calculate an EC_{50} or LC_{50} with reasonable accuracy, acute tests should include one or more controls and a geometric series of at least five toxicant concentrations. Also, due to the limited aqueous solubility of Solvent Yellow 33 and because aquatic organisms are sometimes exposed to concentrations above solubility (ASTM 1980), the tests should be repeated in an attempt to determine a low-effect level. Stock solutions of Solvent Yellow 33 should be prepared by dissolving the dye in an appropriate solvent and diluting this stock solution to the desired series of concentrations. ASTM (1980) recommends that the concentration of solvent should not exceed 0.5 mL/L (a solvent control should also be tested) and that surfactants should not be used.

Acute tests with the green alga Selenastrum capricornutum showed that after a 5-day growth period, Solvent Yellow 33 significantly reduced cell density by 68 percent and biomass by 75 percent at the aqueous solubility concentration of 0.20 mg/L. Additional tests with a series of concentrations above and below the solubility limit are needed to determine a Final Plant Value. Stock solutions should be prepared as described above. Because data are also not available to determine the

Final Chronic and Residue Values, a CCC cannot be established for Solvent Yellow 33.

5.5 HUMAN HEALTH CRITERION

In a lung tumor bioassay in mice, Solvent Yellow 33 at doses of 5, 12.5, and 25 mg/kg (intraperitoneal, 3 times per week, 8 weeks) was not carcinogenic (Stoner 1985). The other study reporting the incidence of neoplastic lesions was inconclusive because the animals were exposed to Disperse Red 9 and Solvent Green 3 in addition to Solvent Yellow 33. No data on carcinogenicity in humans were found. Therefore, a criterion based on carcinogenicity (nonthreshold chronic toxicity) cannot be calculated.

Threshold chronic toxicity data in humans were not available. One-year feeding studies in rats (Hazelton Laboratories, Inc. 1967a) and dogs (Hazelton Laboratories, Inc. 1967b) did not establish NOELs, because pigment deposition in bile duct epithelial cells and renal tubules was observed in animals of all dose groups. In addition, the weight-normalized doses decreased significantly throughout the studies. Therefore, these studies were judged to be inadequate for calculating a criterion.

A 90-day subchronic inhalation study in rats was available (Henderson et al. 1985b). Henderson et al. (1985b) exposed rats to aerosols of Solvent Yellow 33 at concentrations of 0, 1.0, 10.8, and 100 mg/m^3, 6 hr/day, 5 days per week, for 13 weeks (90 days). An inflammatory reaction was not observed in the lungs at any dose, but focal accumulation of foamy macrophages (containing pigment) in alveoli adjacent to bronchioles accompanied by hyperplasia of Type II cells was observed in one animal at the high dose; pigment deposition in the submucosa of the nasal cavity was also observed in animals exposed to the medium and high doses. Systemic effects included pigment deposition in the bile duct epithelium, in hepatocytes adjacent to the bile duct, and in cortical tubules in the kidney in medium- and high-dose groups. No lesions in the respiratory tract or systemic organs were observed in animals exposed to the low dose. Henderson et al. (1985b) considered 10 mg/m^3 the NOAEL; the adverse effects observed, however, were in the respiratory tract. If pigment deposition in systemic organs is considered an effect, but not an adverse one, then the NOEL was 1 mg/m^3, the LOEL was 10.8 mg/m^3, and the "frank effect level" (FEL) was 100 mg/m^3.

The pharmacokinetics data for inhalation of Solvent Yellow 33 and efficiency of gastrointestinal absorption data from Henderson et al. (1985a) are used to calculate an oral dose (gavage) equivalent to an inhalation dose of 1 mg/m^3. Henderson et al. (1985b) measured the content of Solvent Yellow 33 retained in rat lungs 16 hr after the last exposure (90-day subchronic study) and found that rats exposed to 1 mg/m^3 retained 0.050 μg of the dye in their lungs. Based on an assumption of a minute volume equal to 0.2 L and 10 percent deposition in the lungs, 72 μg/day of Solvent Yellow 33 was inhaled and 7.2 μg/day was deposited. The amount of

dye deposited minus the amount retained equals the amount absorbed into the blood, which was 7.15 μg/day. For a 0.3-kg rat, the systemic dose was 23.8 μg/kg/day. Henderson et al. (1985a) also determined that the efficiency of absorption from the gastrointestinal tract was 0.58 (94 hr). Therefore, the oral dose equivalent to an inhalation dose of 1 mg/m^3 is 41 μg/kg/day. The oral dose equivalent to 10.8 mg/m^3 is 446 μg/kg/day, and the oral dose equivalent to 100 mg/m^3 is 4,131 μg/kg/day. Therefore, after conversion to oral equivalent doses, the NOEL is 41 μg/kg/day, the LOEL is 446 μg/kg/day (pigment deposition in only two animals), and the FEL is 4,131 μg/kg/day.

Due to the absence of a bioaccumulation factor, sufficient data are not available for calculating a criterion according to EPA guidelines (USEPA 1980). There are, however, sufficient data to calculate an acceptable daily intake (ADI) using an uncertainty factor of 1,000 (data taken from a 90-day subchronic study). The ADI is calculated using the following equation:

$$\text{ADI (mg/day)} = \frac{\text{70 kg (or 10 kg) x NOEL (μg/kg/day)}}{\text{uncertainty factor}} .$$

The ADI for a 70-kg adult is 2.8 μg/day, and for a 10-kg child it is 0.41 μg/day.

5.6 RESEARCH RECOMMENDATIONS

To satisfy the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data:

1. To obtain more complete information for calculating the FAV, additional acute toxicity tests following ASTM methods (ASTM 1980) as described in Section 5.4 should be performed for at least eight different families of aquatic organisms, as described by Stephan et al. (1985).

2. Chronic flow-through tests using measured concentrations for an invertebrate species, a fish species, and a sensitive freshwater species must be performed to calculate a Final Chronic Value.

3. Acute flow-through tests must be conducted using measured concentrations and following ASTM (1980) procedures as described in Section 5.4 for the three aquatic species for which chronic tests are also performed. This data will be used to calculate acute-chronic ratios.

4. Additional toxicity tests with Selenastrum capricornutum, using a series of measured concentrations above and below solubility and an end point of growth inhibition, must be conducted to calculate a Final Plant Value.

5. A definitive steady-state or 28-day bioaccumulation study must be conducted. A maximum permissible tissue concentration must be determined by conducting a chronic wildlife feeding study or a long-term wildlife field study. These data will provide information to calculate a Final Residue Value.

6. Limited environmental fate information indicates that Solvent Yellow 33 exhibits low water solubility and negligible volatility; consequently, the dye will probably occur in aquatic systems in a particulate form, either as a suspensoid or it will settle out and be deposited on bottom sediments. Based on log K_p values moderate bioaccumulation would be expected. Since burrowing organisms and bottom feeders may be exposed to the highest concentration of the dye, it is suggested that sediment bioassays be performed with Hexagenia (Insecta: Ephemeroptera) using the modified recycling apparatus described in Fremling and Mauck (1980, pp. 91-92). In addition, studies should be undertaken to determine the fate of the dye in aquatic sediments, (i.e. sorption kinetics, partitioning between sediment and water phases, potential pathways of degradion).

7. The results of the genotoxicity tests, which demonstrated that Solvent Yellow 33 is mutagenic in bacteria and mutagenic and clastogenic in mammalian cells (Moore et al. 1984) suggest that Solvent Yellow 33 may be carcinogenic. A 2-year oral (gavage) toxicity test, performed in rats and/or mice, with carcinogenicity and chronic toxicity as end points, should be given high priority. This test and those listed below should be performed according to USEPA Toxic Substances Control Act Test Guidelines (USEPA 1985). The NOEL, LOEL, and FEL calculated in Section 5.5 could be used as a basis for selecting doses.

8. The results of the genotoxicity tests (Moore et al. 1984) also suggest that Solvent Yellow 33 should be tested for possible skin tumor initiating activity using the two-stage mouse skin carcinogenicity assay as described by Slaga et al. (1985) for testing Disperse Red 9 (evaluate hazards due to skin contact, especially for workers and military personnel).

9. An additional genotoxicity test, the dominant lethal assay in mice and/or rats, should be conducted to assess the in vivo genotoxicity of Solvent Yellow 33 (to specifically evaluate germ cell mutagenicity).

10. Because Solvent Yellow 33 is efficiently absorbed from the respiratory tract (Henderson et al. 1985a), a 2-year inhalation toxicity study should also be conducted in rats. The 90-day subchronic inhalation study demonstrated that Solvent Yellow 33 has low toxic effects up to 100 mg/m^3 (Henderson et al. 1985b); therefore, rats may be able to tolerate this dose for a longer period of time. Both local and systemic organs should be evaluated for chronic toxicity and carcinogenicity (evaluate hazards due to inhalation, especially for workers and military personnel).

11. Tests to evaluate developmental and reproductive toxicity should also be performed in rats or mice.

6. REFERENCES

ACGIH. 1986. American Conference of Governmental Industrial Hygienists. Threshold Limit Values and Biological Exposure Indices for 1986-1987. ACGIH Publication Office, Cincinnati, OH.

Aldrich. 1984. Aldrich Catalog/Handbook of Fine Chemicals 1984-1985. Aldrich Chemical Company, Milwaukee, WI. 1511 pp.

ASTM. 1980. American Society for Testing and Materials. Standard practice for conducting toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM Annual Book of Standards. ASTM Designation E 729-80. ASTM, Philadelphia, PA. pp. 272-296.

Bertocchi, G., F. Marchetti, L. Benfenati, and D. Spinelli. 1980. Determination of mercury traces in dyes used for food, pharmaceuticals and cosmetics. Relata Tech. 12(25):55-57.

Björkner, B. and B. Magnusson. 1981. Patch test sensitization to D & C Yellow No. 11 and simultaneous reaction to Quinoline Yellow. Contact Dermatitis 7:1-4.

Björkner, B. and B. Niklasson. 1983. Contact allergic reaction to D & C Yellow No. 11 and Quinoline Yellow. Contact Dermatitis 9:263-268.

Buehler, E.V. 1965. Delayed contact hypersensitivity in the guinea pig. Arch. Dermatol. 91:171-175. (As reported in Lamson et al. 1982)

Calnan, C.D. 1975. F D & C Yellow 11 in lipstick. Contact Dermatitis 1:121.

Calnan, C.D. 1981. Quinazoline yellow dermatitis (D & C Yellow) in an eye cream. Contact Dermatitis 7:271.

Chemical Abstracts Service Registry File. 1987. 2-(2-Quinolyl)-1,3-indandione. American Chemical Society. Computer printout retrieved March 17, 1987.

Cichowicz, J.J. and R.S. Wentsel. 1983. Programmatic Life Cycle Environmental Assessment for Smoke/Obscurants. Vol. 5. Dye/Colored Smokes. ARCSL-EA-83010, AD A141973. U.S. Army Armament, Munitions, and Chemical Command, Chemical Research and Development Center, Aberdeen Proving Ground, MD.

Colour Index. 1971. Solvent Dyes. Colour Index, 3rd ed. Vol. 3, pp. 3563-3633. The Society of Dyers and Colourists, Bradford, Yorkshire, England.

Deiner, A. 1982. Electrochemical oxidation of smoke dyes and application to pyrotechnic dissemination. Proc. Eighth Int. Pyrotechnics Semin., July 12-18, 1982. IIT Research Institute, Chicago, IL. pp. 159-185.

Doerr, C.L., K.H. Brock, D.M. DeMarini, and M.M. Moore. 1986. Analysis of gross aberrations in mouse lymphoma cells exposed to various genotoxic agents. Environ. Mutagen. 8(Suppl. 6):25 (Abstr).

Dutcher, J.S., M.A. Medinsky, J.A. Bond, et al. 1985. Effect of vapor concentration on the disposition of inhaled 2,3-dichloropropene in Fischer-344 rats. Fundam. Appl. Toxicol. 5:in press. (As reported in Henderson et al. 1985a)

Fadil, F.A. and W.O. McSharry. 1979. Extraction and TLC separation of food, drug, and cosmetic dyes from tablet-coating formulations. J. Pharm. Sci. 68(1):97-98.

Fisher, D.J., D.T. Burton, M.J. Lenkevich, and K.R. Cooper. 1985. Analytical Methods for Determining the Concentration of Solvent Yellow 33, Solvent Green 3, Synthetic HC Smoke Combustion Products and DEGDN in Freshwater Aquatic Organisms. Final Report for Phase I, AD A167875. The Johns Hopkins University, Applied Physics Laboratory, Laurel, MD. NAVSEA N00024-85-C-5301 via ARMY MIPR85MM5505.

Fisher, D.J., D.T. Burton, and R.L. Paulson. 1987. Toxicity of DEGDN, Synthetic-HC Smoke Combustion Products, Solvent Yellow 33, and Solvent Green 3 to Freshwater Aquatic Organisms. Final Report for Phase II. The Johns Hopkins University, Applied Physics Laboratory, Laurel, MD. NAVSEA N00024-85-C-5301 via ARMY MIPR85MM5505.

Fortner, W.L., L.M. Aikman, and R.E. Shook. 1979. "Review of Draft of Final Report of Atlantic Research Corporation," SARPB-ETD, dated Jan 22, 1979. (As reported in Kitchens et al. 1978)

Fremling, C. R. and W. L. Mauck. 1980. Methods for using nymphs of burrowing mayflies (Ephemeroptera, Hexagenia) as toxicity test organisms. In A. L. Buikema, Jr. and J. Cairns, Jr., eds. Aquatic Invertebrate Bioassays, ASTM STP 715. pp. 81-97. American Society for Testing and Materials, Philadelphia, PA.

Garcia, D.J., L.M. Aikman, F.L. McIntyre, and T.E. Shook. 1982. Rapid plant scale mixing, granulation, and loading of dry materials during the manufacture of colored smoke munitions. Proc. Eighth Int. Pyrotechnics Semin., July 12-18, 1982. IIT Research Institute, Chicago, IL, pp. 250-276.

Gershbein, L.L. 1982. Action of dyes and indicators on rat-liver regeneration. Food Chem. Toxicol. 20(1):1-8.

Hazelton Laboratories, Inc. 1962a. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 130.

Hazelton Laboratories, Inc. 1962b. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 131.

Hazelton Laboratories, Inc. 1962c. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 133.

Hazelton Laboratories, Inc. 1962d. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 137.

Hazelton Laboratories, Inc. 1965. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 138.

Hazelton Laboratories, Inc. 1967a. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 142.

Hazelton Laboratories, Inc. 1967b. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 143.

Hazelton Laboratories, Inc. 1967c. Sponsor: Pharmaceutical Manufacturers Association. FDA Color Additive Master File No. 9, Entry No. 139.

Henderson, R.F., D.E. Bice, Y.S. Cheng, et al. 1984. Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions. Final Report for Phase III Studies: Four-Week Inhalation Exposoures of Rats to Dye Aerosols. AD A156698. Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.

Henderson, R.F., J.S. Dutcher, F.F. Hahn, et al. 1985a. Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions. Final Report for Phase II Studies: Range Finding and Toxicokinetics Studies of Inhaled Dye Aerosols. AD A163267. Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.

Henderson, R.F., Y.S. Cheng, F.F. Hahn, et al. 1985b. Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions. Final Report for Phase IV Studies: 90-Day Inhalation Exposures of Rats to Dye Aerosols. AD A170446. Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.

Henderson, R.F., G.J. Newton, Y.S. Cheng, B. Chen, and R.L. Hanson. 1985c. Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Muntions: Aerosol Measurements in the Workplace at Pine Bluff, Arkansas. AD A166459. Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.

ILO. 1980. International Labor Office. Occupational Exposure Limits for Airborne Toxic Substances: A Tabular Compilation of Values from Selected Countries, 2nd ed., pp. 264. International Labor Office, Geneva.

Jordan, W.P., Jr. 1981. Contact dermatitis from D & C Yellow 11 dye in a toilet bar soap. J. Am. Acad. Dermatol. 4(5):613-614.

Kita, S., T. Kobayashi, H. Kutsuna, and A.M. Kligman. 1984. Human maximization testing of D & C Yellow no. 10 and Yellow no. 11. Contact Dermatitis 11:210-213.

Kitchens, J.F., W.E. Harward, III, D.M. Lauter, R.S. Wentsel, and R.S. Valentine. 1978. Preliminary Problem Definition Study of 48 Munitions-Related Chemicals, Volume III Pyrotechnic Related Chemicals. Final Report, AD A066309. Atlantic Research Corporation, Alexandria, VA. DAMD17-77-C-7057.

Krien, G. 1984. Thermoanalytical study on smoke producing dyes. Thermochim. Acta 81:29-43.

Lamson, S.A., B.M. Kong, and S.J. DeSalva. 1982. D & C Yellow Nos. 10 and 11: delayed contact hypersensitivity in the guinea pig. Contact Dermatitis 8:200-203.

Larsen, W.G. 1975. Cosmetic dermatitis due to a dye (D & C Yellow # 11). Contact Dermatitis 1:61.

Leo, A., C. Hansch, and D. Elkins. 1971. Partition coefficients and their uses. Chem. Reviews 71:525-616.

Little, L.W., J.C. Lamb, III, M.A. Chillingworth, and W.B. Durkin. 1974. Acute toxicity of selected commercial dyes to fathead minnow and evaluation of biological treatment for reduction of toxicity. Proc. 29th Industrial Waste Conference. Lafayette, IN., May 7-9, 1974. Eng. Ext. Series No. 145:524-534.

Marrs, T.C. 1983. Pulmonary retention of dye following inhalation of coloured smoke. Br. J. Pharmacol. 80:494p. (abstr.)

Marrs, T.C., H.F. Colgrave, M. Gazzard, and R.F.R. Brown. 1984. Inhalation toxicity of a smoke containing Solvent Yellow 33, Disperse Red 9 and Solvent Green 3 in laboratory animals. Hum. Toxicol. 3(4):289-308.

Medinsky, M.A., Y.S. Cheng, S.J. Kampcik, R.F. Henderson, and J.S. Dutcher. 1986. Disposition and metabolism of ^{14}C-solvent yellow and solvent green aerosols after inhalation. Fundam. Appl. Toxicol. 7:170-178.

MEDLARS(RTECS). 1987. National Library of Medicine. Registry of Toxic Substance file. Computer printout retrieved February 5, 1987.

Moore, M., J. Allen, and L. Claxton. 1984. Mutagenic Screening of Three Dyes for Marker Grenades in the Salmonella Reversion Assay, the L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay, and In Vivo Sister Chromatid Exchange in Mice. Final Report, AD A152110. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Muni, I.A., E.B. Gordon, and J.B. Goodband. 1986. Dermal, Eye, and Oral Toxicological Evaluations. Phase II Report, AD A165408. Bioassay Systems Corp., Woburn, MA. DAMD17-82-C-2301.

O'Bryan, T. and R. Ross. 1986. Chemical Scoring System for Hazard Exposure Assessment, Draft Report. Oak Ridge National Laboratory, Oak Ridge, TN.

Ohnishi, S., Y. Nishijima, K. Kijima, and S. Kano. 1977. Chromatographic analysis of tar dyes in cosmetic products. Bunseki Kagaku 26(12):814-818.

Palazzolo, M.J. and L.C. DiPasquale. 1983. The sensitization potential of D & C Yellow No. 11 in guinea pigs. Contact Dermatitis 9:367-371.

Pinkham, C.F.A., H.D. Hertert, J.J. Fuller, D.A. Stiles, and E.G. Worthley. 1977. Terrestrial Ecological Surveys at Pine Bluff Arsenal, Pine Bluff, Arkansas. Technical Report, ARCSL-TR-77042. U.S. Army Armament Research and Development Command, Aberdeen Proving Ground, MD. (As reported in Kitchens et al. 1978)

Rapaport, M.J. 1980. Allergy to D and C yellow dye #11. Contact Dermatitis 6:364-365.

Rapaport, M.J. 1984. Allergy to yellow dyes. Arch. Dermatol. 120(4):535-536.

Rubin, I.B. and M.V. Buchanan. 1983. Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures. Chemical Characterization of Army Colored Smokes: Inventory Smoke Mixes (Red, Violet, Yellow, and Green). Final Report, ORNL/TM-8956. Oak Ridge National Laboratory, Oak Ridge, TN.

Sato, Y., H. Kutsuna, T. Kobayashi, and T. Mitsui. 1984. D & C Nos. 10 and 11: chemical composition analysis and delayed contact hypersensitivity testing in the guinea pig. Contact Dermatitis 10:30-38.

Slaga, T.J., L.L. Triplett, L.H. Smith, and H.P. Witschi. 1985. Carcinogenesis of Nitrated Toluenes and Benzenes, Skin and Lung Tumor Assays in Mice. Final Report, AD A155723, ORNL/TM-9645. Oak Ridge National Laboratory, Oak Ridge, TN.

Smith, L.H. and Witschi, H.P. 1983. The Mouse Lung Tumor Assay: A Final Report. ORNL-5961. Oak Ridge National Laboratory, Oak Ridge, TN.

Smith, M.D. and F.M. Stewart. 1982. Environmentally acceptable smoke munitions. Proc. Eighth Int. Pyrotechnics Semin., July 12-18, 1982. IIT Research Institute, Chicago, IL. pp. 623-635.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report, PB85-227049. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.

Stoner, G. 1985. Bioassay of Military Relevant Compounds for Carcinogenic Activity by the Strain A Mouse Lung Tumor Bioassay. Final Report, AD A156772. Medical College of Ohio, Toledo, OH. DAMD17-81-C-1178.

Sun, J.D., R.F. Henderson, T.C. Marshall, et al. 1987. The inhalation toxicity of two commercial dyes: Solvent Yellow 33 and Solvent Green 3. Fund. Appl. Toxicol. 8:358-371.

Tatyrek, A.F. 1965. Health Hazards of Certain Smoke Dyes in Current Use. AD 469867. Feltman Research Laboratories, Picatinny Arsenal, Dover, NJ.

USEPA. 1980. Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Quality Criteria Documents. Fed. Regist. 45:79347-79357.

USEPA. 1981. U.S. Environmental Protection Agency. 40 CFR Parts 50-52. (As reported in Cichowicz and Wentsel 1983)

USEPA. 1985. U.S. Environmental Protection Agency. Toxic Substances Control Act Test Guidelines; Final Rules. 40 CFR Parts 796, 797, 798. Fed. Regist. 50:39252-39516.

USFDA. 1976. U.S. Food and Drug Administration. Color additives. Fed. Regist. 41:51003-51009.

USFDA. 1984. U.S. Food and Drug Administration. Color Additives. 21 CFR Parts 70-82.

USOSHA. 1986. U.S. Occupational Safety and Health Administration. Toxic and hazardous substances. 29 CFR Part 1910.1000.

Weaver, J.E. 1983a. Dose response relationships in delayed hypersensitivity to quinoline dyes. Contact Dermatitis 9:309-312.

Weaver, J.E. 1983b. Sensitization potential of D & C Yellow No. 10 and D & C Yellow No. 11 dyes. J. Am. Acad. Dermatol. 9(4):605-606.

Zuckerman, S. and J. Senackerib. 1979. Colorants for foods, drugs, and cosmetics. In M. Grayson and C. Eckroth, eds. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Vol. 6, pp. 561-596. John Wiley & Sons, New York.

7. GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
ASTM	American Society for Testing and Materials
ADI	Acceptable daily intake
BAL	Bronchoalveolar lavage
BUN	Blood urea nitrogen
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
DMSO	Dimethylsulfoxide
DO	Dissolved oxygen
EC_{50}	Effective concentration causing 50 percent inhibition of algal growth
FAV	Final Acute Value
FEL	Frank effect level
HPLC	High performance liquid chromatography
ILO	International Labor Office
LD_{50}	Lethal dose causing 50 percent mortality
LDH	Lactate dehydrogenase
LOEL	Lowest observed effect level
log K_p	Octanol-water partition coefficient
MMAD	Mass median aerodynamic diameter
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PAS	Periodic Acid Schiff
SGPT	Serum glutamic pyruvic transaminase

SY	Solvent Yellow 33
SY/SG	Solvent Yellow 33/Solvent Green 3 mixture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USOSHA	United States Occupational Safety and Health Administration

APPENDIX A

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data: it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived: however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative: much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different non-ionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants: (b) FDA action levels (FDA Guidelines Manual): and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes:

   b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish):

   c. a third family in the phylum Chordata (e.g, fish or amphibian):

   d. a planktonic crustacean (e.g, cladoceran or copepod):

e. a benthic crustacean (e.g, ostracod, isopod, or amphipod):

f. an insect (e.g., mayfly, midge, stonefly):

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca): and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms < 24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96- hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was ≤ 96 hr.

   d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are

actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as:

$$FAV = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])},$$

where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young < 48 hr old, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young < 24 hr old and last for not < 21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life- cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

   c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

      NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute--Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and salt-water species.

c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are < 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

   a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

   b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.

   c. Use V = pooled acute slope as L = pooled chronic slope.

   d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

   a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

   b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

   c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

   d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

   e. Similarly normalize the values of the water quality characteristic for each species individually.

   f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

   g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as:

$$FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])},$$

where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation

factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached: (b) highest BCF obtained, if apparent steady-state was not reached: and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

   c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

   d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be

converted to a wet tissue weight basis. If a conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

   b. Calculate the geometric mean normalized BCF.

   c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

      - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

      - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as: The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) $\mu g/L$ more than once every three years on the average and if the one-hour average concentration does not exceed (4) $\mu g/L$ more than once every three years on the average.

Where,

(1) = insert freshwater or saltwater,

(2) = name of material,

(3) = insert the Criterion Continuous Concentration, and

(4) = insert the Criterion Maximum Concentration.

13. REFERENCES

ASTM Standards E 729. Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

ASTM Standards E 724. Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs.

Dixon, W.J. and M.B. Brown, eds. 1979. BMDP Biomedical Computer Programs, P-Series. University of California, Berkeley. pp. 521-539.

Erickson, R.J. and C.E. Stephan. In Preparation. Calculation of the Final Acute Value for Water Quality Criteria for Aquatic Life. U.S. Environmental Protection Agency, Duluth, MN.

FDA Administrative Guidelines Manual. Food and Drug Administration.

Hamelink, J.L., et al. 1971. A Proposal: Exchange Equilibria Control the Degree Chlorinated Hydrocarbons are Biologically Magnified in Lentic Environments. Trans. Am. Fish. Soc. 100:207-214.

Huth, E.J., et al. 1978. Council of Biological Editors Style Manual, 4th ed., p. 117.

Lunsford, C.A. and C.R. Blem. 1982. Annual Cycle of Kepone Residue in Lipid Content of the Estuarine Clam, Rangia cuneata, Estuaries 5:121-130.

Schnoor, J.L. 1982. Field Validation of Water Quality Criteria for Hydrophobic Pollutants. In: Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766, J.G. Pearson, R.B. Foster, and W.E. Bishop, eds., American Society for Testing and Methods, pp. 302-315.

Sidwell, V.D. 1981. Chemical and Nutritional Composition of Finfishes, Whales, Crustaceans, Mollusks, and Their Products. NOAA Technical Memorandum NMFS F/SEC-11, National Marine Fisheries Service.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report, PB85-227049. U.S. Environmental Protection Agency, Office of Research and Development, Washington DC. 97 pp.

U.S. Environmental Protection Agency (USEPA). 1982. Fed. Reg. 47:49234-49252, October 29, 1982.

APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

(a) Carcinogenicity, (b) Toxicity, and (c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish. Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to

be insignificant: however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980). Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCFavg = BCFsp \times \frac{3.0\%}{PLsp},$$

where BCFsp is the bioconcentration factor for an aquatic species and PLsp is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity: synergistic and antagonistic effects: and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where,

C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C\ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where,

2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X ,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q_1^* is the ratio of risk to dose: an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/-response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

And substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X}$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of cancer potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is le, and the total length of the experiment is Le, then the lifetime average exposure (Xm) is

$$Xm = \frac{l_e \times m}{L_e \times W^{2/3}}$$

4. If the duration of the experiment (Le) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of (L/Le)3 to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW ,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man: 0.05 for a 0.35-kg rat: and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption: which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t:d) = 1 - \exp(-g(d)H(t)) ,$$

where P(t:d) is the probable response for dose d and time t: g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each S as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\{ - \sum_{i=0}^{a} \alpha d^i \},$$

or as given in the EPA guidelines (USEPA 1980):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots . q_k d^k)] ,$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where Lo is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where Ni is the number of animals in the ith dose group, Xi is the number of animals in the ith dose group with a tumor response, Pi is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (X_2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of

dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported,

but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times AA}{(AO \times SF)}$$

where,

BR = daily air intake (assume 10 m3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
AA = efficiency of absorption from air,
AO = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times DE \times d \times AA \times BR \times 70\ kg}{(BWA \times AO \times SF)}$$

where,

CA = concentration in air (mg/m3),
DE = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
AA = efficiency of absorption from air,
BR = volume of air breathed (m3/day),
70 kg = standard human body weight,
BWA = body weight of experimental animals (kg),
AO = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant: however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

6. REFERENCES

Crump, K.S. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157.

Crump, K.S., and W.W. Watson. 1979. GLOBAL,79. A FORTRAN program to extrapolate dichotomous animal carcinogenicity data to low dose. National Institute of Health Science Contract No. 1-ES-2123.

International Commission on Radiological Protection. 1977. Recommendation of the ICRP, Publication No. 26. Pergamon Press, Oxford, England.

Krewski, D., K.S. Crump, J. Farmer, D.W. Gaylor, R. Howe, C. Portier, D. Salsburg, R.L. Sielken, and J. Vanryzin. 1983. A comparison of statistical methods for low-dose extrapolation utilizing time-to-tumor data. Fund. Appl. Toxicol. 3:140-160.

NAS. 1977. Drinking water and health. Safe Drinking Water Committee, Advisory Center on Toxicology, National Research Council, National Academy of Science, Washington, DC. 939 pp.

Stephan, C.E. 1980. July 3 memorandum to J. Stara, U.S. Environmental Protection Agency. (as cited in USEPA 1980).

Stokinger, M.E. and R.L. Woodward. 1958. Toxicological methods for establishing drinking water standards. J. Am. Water Works Assoc. 50:517.

USEPA. 1980. Water Quality Criteria Documents, Environmental Protection Agency. Fed. Regist. 45:79318-79357.

Veith, G.D., et al. 1980. Measuring and estimating the bioconcentration factors of chemicals in fish. J. Fish. Res. Bd. Can. 36:1040. (as cited in USEPA 1980).

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