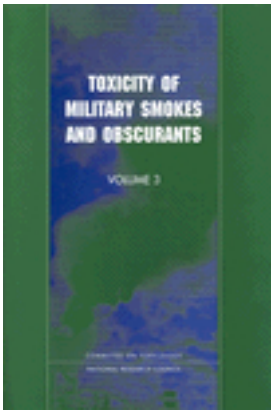

Toxicity of Military Smokes and Obscurants, Volume 3

Subcommittee on Military Smokes and Obscurants, National Research Council

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Toxicity of Military Smokes and Obscurants

Volume 3

Subcommittee on Military Smokes and Obscurants
Committee on Toxicology
Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

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Preface

THE U.S. ARMY uses smokes and obscurants to shield armed forces from view, signal friendly forces, and mark positions. Military personnel are exposed to smokes and obscurants during training exercises. The Army would like to ensure that exposures to these substances during training do not adversely affect the health of Army personnel or the public living and working near military-training facilities. To assist with this effort, the Army requested the National Research Council (NRC) to review independently the available toxicity data on certain smokes and obscurants and to recommend exposure guidance levels for each. In response, the NRC's Committee on Toxicology (COT) convened the Subcommittee on Military Smokes and Obscurants, which prepared this report.

This report (Volume 3 in the series) assesses toxicity data for seven colored smoke formulations. In Volume 1 of the series, fog oil, diesel fuel, red phosphorus, and hexachloroethane smokes were reviewed, and in Volume 2, white phosphorus, brass, titanium dioxide, and graphite smokes were reviewed.

Several individuals assisted the subcommittee by providing information on the uses and toxicity of the colored smokes addressed in this report. We gratefully acknowledge Colonel Francis L. O'Donnell, Major James Martin, Colonel David Wilder, and the Office of the Surgeon General of the U.S. Army for their interest and support of this project. We also

thank Winnifred Palmer, Sandra Thomson, and Michael Burnham of the U.S. Army for providing information to the subcommittee.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the NRC's Report Review Committee for reviewing NRC and Institute of Medicine reports. The purpose of this independent review was to provide candid and critical comments to assist the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals, who are neither officials nor employees of the NRC, for their participation in the review of this report: Michael Dorato, Lilly Research Laboratories; Andrea Hubbard, University of Connecticut; Robert Phalen, University of California, Irvine; Mary Vore, University of Kentucky; and George Rusch, Allied Signal Inc. (Review Coordinator). These individuals provided many constructive comments and suggestions. It must be emphasized, however, that responsibility for the final content of this report rests entirely with the authoring committee and the NRC.

We are grateful for the assistance of the NRC staff in the preparation of this report. The subcommittee wishes to acknowledge Kulbir Bakshi, program director of the Committee on Toxicology, and Abigail Stack, project director for this report. Other staff members contributing to this report were Paul Gilman, former executive director of the Commission on Life Sciences; James Reisa, director of the Board on Environmental Studies and Toxicology; Carol Maczka, senior program director for toxicology and risk assessment; Ruth Crossgrove, editor; and Lucy Fusco, Linda Leonard, and Christine Phillips, project assistants.

Finally, we would like to thank all the members of the subcommittee for their expertise and dedicated effort throughout the study.

Michele A. Medinsky, Ph.D.
Chair, Subcommittee On Military Smokes and Obscurants

Bailus Walker, Ph.d., M.P.H.
Chair, Committee On Toxicology

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LIST OF ABBREVATIONS

AEHA	U.S. Army Environment Hygiene Agency
ACGIH	American Conference of Governmental Industrial Hygienists
BZA	benzanthrone
CO	carbon monoxide
COT	Committee on Toxicology
DAA	1,4-diaminoanthraquinone
DBC	vat yellow 4 (dibenzochrysenedione)
DDA	1,4, diamino-2,3-dihydroanthraquinone
DMA	disperse red 11 (1,4-diamino-2-(2-quinolyl)-1,3-indandione)
DOD	U.S. Department of Defense
EEGL	emergency exposure guidance level
EPA	U.S. Environmental Protection Agency
HGPRT	hypoxanthine guanine phosphoribosyl transferase
LCt_{50}	lethal concentration multiplied by exposure time for 50% of the test animals
LD_{50}	lethal dose for 50% of the test animals
LOAEL	lowest-observed-adverse-effect level
MAA	disperse red 9 (1-(methylamino)-9,-10-anthracenedione)
MBN	solvent red 1 (- menthozbenzenazo- -napthol)
NOAEL	no-observed-adverse-effect level
NCI	National Cancer Institute
NRC	National Research Council
PTA	solvent green 3 (1,4-di-*p*-toluidino-9,10- anthraquinone)
QID	solvent yellow 33 (2-(2-quinolyl)-1,3-indandione)
REGL	repeated exposure guidance level (referred to as permissible exposure guidance level in Volume 1)
RPEGL	repeated public exposure guidance level (referred to as permissible public exposure guidance level in Volume 1)
SPEGL	short-term public emergency guidance level

Toxicity of Military Smokes and Obscurants

Volume 3

SUMMARY

A VARIETY of Smokes and obscurants have been developed and used to screen armed forces from view, signal friendly forces, and mark positions. Smokes are produced by burning or vaporizing particular products. Obscurants are anthropogenic or naturally occurring particles suspended in the air. They block or weaken transmission of particular parts of the electromagnetic spectrum, such as visible and infrared radiation or microwaves. Fog, mist, and dust are examples of natural obscurants. White phosphorus and hexachloroethane smokes are examples of anthropogenic obscurants.

The U.S. Army seeks to reduce the likelihood that exposure to smokes and obscurants during training would have adverse health effects on military personnel or civilians. To protect the health of exposed individuals, the Office of the Army Surgeon General requested that the National Research Council (NRC) independently review data on the toxicity of smokes and obscurants and recommend exposure guidance levels for military personnel in training and for the general public residing or working near military-training facilities.

The Army requested recommendations for four types of exposure guidance levels: (1) emergency exposure guidance levels (EEGLs) for a rare, emergency situation resulting in exposure of military personnel for less than 24 hr; (2) repeated exposure guidance levels (REGLs) for repeated exposure of military personnel during training exercises (referred to as permissible exposure guidance levels in Volume 1); (3)

short-term public emergency guidance levels (SPEGLs) for a rare, emergency situation potentially resulting in an exposure of the public to military-training smoke; and (4) repeated public exposure guidance levels (RPEGLs) for repeated exposures of the public residing or working near military-training facilities (referred to permissible public exposure guidance levels in Volume 1).

The NRC assigned this project to the Committee on Toxicology (COT), which convened the Subcommittee on Military Smokes and Obscurants. The subcommittee conducted a detailed evaluation of data on the toxicity of eight obscuring smokes and seven colored smokes. The results are published in three volumes. This volume, Volume 3, reviews the potential toxicity of seven colored smokes used for signaling, marking, and, in some cases, simulating exposure to chemical-warfare agents in military training. Toxicity data and exposure guidance levels for eight obscuring smokes were addressed in previous volumes: diesel fuel, fog oil, red phosphorus, and hexachloroethane were presented in Volume 1; white phosphorus, brass, titanium dioxide, and graphite were presented in Volume 2.

SUBSTANCES EVALUATED

Colored smokes are generated by deploying an M18 grenade or 40-mm cartridge containing a pyrotechnic mixture of fuel and dye. The dye mixtures originally formulated by the Army were yellow, green, red, and violet. Because of the potential health hazards of the smoke formulations and the combustion products, the Army developed new formulations for the same colors. However, grenades and cartridges containing the old smoke formulations are still in inventory. Therefore, the Army requested that the NRC evaluate the toxicity of both the old and the new smoke formulations, with the exception of the new violet-smoke formulation, which was removed from the inventory due to its acute toxicity.

Using the NRC guidelines published in 1986 and 1992 for recommending exposure guidance levels, the subcommittee evaluated the toxicity data on each of the old and new smoke formulations, the combustion products (smoke), and the individual dye components. The old smoke formulations are listed below with their dye components:

yellow smoke:	vat yellow 4 and benzanthrone
green smoke:	vat yellow 4, benzanthrone, and solvent green 3

red smoke:	disperse red 9
violet smoke:	disperse red 9 and 1,4-diamino-2,3-dihydroanthraquinone.

The new smoke formulations are listed below with their dye components:

yellow smoke:	solvent yellow 33
green smoke:	solvent yellow 33 and solvent green 3
red smoke:	solvent red 1 and disperse red 11.

Old Smoke Formulations

No studies have been conducted in animals or humans on the toxicity of the old yellow-smoke formulation or its combustion products. In one study, rats, mice, and guinea pigs were exposed to a smoke containing benzanthrone, one of the old yellow-smoke components, and a blue dye not present in the Army's old yellow-smoke formulation. Animals exposed at concentrations of 0.9 to 13.4 grams per cubic meter (g/m^3) for 1 hr exhibited injury characterized by lung necrosis, sloughing of the mucosa, edema in the alveolar space, and necrosis in the tracheobronchial tree.

No studies have been conducted on the toxicity of the old green-smoke formulation or its combustion products. In two studies rats, mice, and guinea pigs were exposed to smokes containing solvent green 3, one of the dye components of the old formulation. The smokes used in those studies also contained dye components not present in the Army's old green smoke. In one study, animals exposed to smoke containing solvent green 3 and auramine hydrochloride at concentrations of 0.6 to 12.1 g/m^3 for 1 hr exhibited injury characterized by lung necrosis, sloughing of the mucosa, and edema in the alveolar space. In the other study, animals were exposed to a smoke composed of solvent yellow 33, disperse red 9, and solvent green 3 at concentrations of 0.1 to 1.0 g/m^3 for 1 hr per day, 5 days per week for 100 days. The dye was retained in the lungs of all animals. Histological examination revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation.

Old red smoke was evaluated for acute toxic effects in several animal species. Rats, rabbits, guinea pigs, dogs, swine, and goats were exposed to old red smoke at concentrations of 1.5 to 18 g/m^3 for 10 to 240 min.

All animals showed signs of upper-respiratory-tract irritation and salivation immediately after exposure, and all had labored breathing for 7 days after the exposure. The study reported the combined mortality of the total number of animals of all the species exposed to the smoke. Only general information can be obtained from this study, because the mortality results in individual species were not given.

The toxicity of old violet smoke was evaluated in a study using rats, rabbits, guinea pigs, dogs, swine, and goats. Animals were exposed at concentrations of 1.3 to 7.8 g/m^3 for 8 to 142 min. All animals showed upper-respiratory-tract irritation and salivation. As in the study of old red smoke, only general information can be obtained from this study because the results were reported as the combined mortality of the total number of animals of all the species exposed to the smoke. The old violet-smoke formulation was tested for mutagenicity and found to be positive in the Ames assay.

New Smoke Formulations

No studies have been conducted in animals or humans on the toxicity of the new yellow-smoke formulation or its combustion products. However, two studies evaluated the toxicity of smokes containing solvent yellow 33, the major component of the new yellow-smoke formulation. The smokes used in both studies also contained dyes not present in the Army's new formulation. One study used a smoke containing solvent yellow 33, solvent green 3, and disperse red 9. In that study, histological examination of rats, mice, and guinea pigs exposed to the smoke at concentrations of 0.1 to 1.0 g/m^3 for 1 hr per day, 5 days per week for 100 days revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation. The other study evaluated the toxicity of a smoke containing solvent yellow 33 and disperse orange 11. Mice, rats, and guinea pigs were exposed at 0.11 to 1.0 g/m^3 for 1 hr per day, 5 days per week for 200 days. Toxic effects appeared to be confined to the respiratory tract. Lymphocyte infiltration in the larynx and trachea of mice and guinea pigs and dilated mucous glands in the trachea of mice and rats were reported.

The only data on the toxicity of the new green-smoke formulation are from an inhalation study on a mixture of solvent yellow 33 and solvent green 3 aerosols. The mixture was not acutely toxic; however, mild

pulmonary inflammation was observed and was attributed to solvent green 3. No-observed-adverse-effect levels for the aerosolized mixture were 50 milligrams (mg)/m^3 for a 4-week exposure and 10 mg/m^3 for a 13-week exposure. Two other studies might have some relevance in assessing the potential toxicity of new green smoke, although the smoke formulation did not have the same composition as the Army's. One study evaluated the toxicity of a smoke containing solvent yellow 33, solvent green 3, and disperse red 9. Histological examination of rats, mice, and guinea pigs exposed to the smoke at concentrations of 0.1 to 1.0 g/m^3 for 1 hr per day, 5 days per week for 100 days revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation. Another study evaluated the toxicity of a smoke composed of solvent yellow 33 and disperse orange 11. Mice, rats, and guinea pigs were exposed at 0.11 to 1.0 g/m^3 for 1 hr per day, 5 days per week for 200 days. Toxic effects appeared to be confined to the respiratory tract; lymphocyte infiltration in the larynx and trachea of mice and guinea pigs and dilated mucous glands in the trachea of mice and rats were reported.

No studies have been conducted using new red smoke. Data are available on the toxicity of an aerosolized mixture containing solvent red 1 and disperse red 11. Inhalation exposure of rats and rabbits to the aerosolized mixture resulted in nasal and lung lesions. Only minimal details of the study are available; therefore, the study's scientific merits cannot be evaluated adequately, and the information cannot be used to recommend exposure guidance levels.

Individual Dye Components

In addition to evaluating the toxicity data on the smoke formulations and the combustion products, the subcommittee reviewed toxicity data on the individual dyes used in the smoke formulations. Toxicity data for nine dyes were reviewed and summarized. Concern about the toxicity of several of them is substantial. For example, four dyes that have been demonstrated to be dermal sensitizers in humans and laboratory animals are benzanthrone, a component of the old yellow-and old green-smoke formulations; solvent yellow 33, a component of the new yellow-and new green-smoke formulations; solvent red 1, a component of the new red-smoke formulation; and disperse red 9, a component of the old red- and violet-smoke formulations. Additionally, their potential for pulmo

nary sensitization after inhalation of the smokes has not been addressed adequately and remains a source of uncertainty. Because each of the seven smokes evaluated contains one of the four dyes identified as sensitizing agents, that uncertainty applies to all smokes under consideration.

CONCLUSIONS AND RECOMMENDATIONS

The subcommittee concludes that the available toxicity data base for the combustion products of the old and new smoke formulations is inadequate for use in assessing the potential health risk of exposure to these smokes and in recommending exposure guidance levels. Review of the data identified sufficient evidence of toxicity for smoke formulations and combustion products to raise concern, particularly with regard to dermal and respiratory-tract sensitization. However, the data are too sparse to permit well-informed recommendations for exposure guidance levels. Stringent guidance levels could be arbitrarily set to protect personnel; however, the Army's current policy states that troops without protective clothing must avoid entering the smoke cloud during training. That policy should serve to protect troops until further research can be performed to provide more information for recommending exposure guidance levels.

The primary reason for the subcommittee's concern about the potential toxicity of the colored smokes is the demonstration of contact allergic dermatitis in humans and laboratory animals exposed to several of the dyes that are components of the old and new smokes. On the basis of its review and evaluation, the subcommittee concludes that additional research must be conducted on the toxicity of the colored smokes before well-informed recommendations for exposure guidance levels can be made. The subcommittee recommends that, at a minimum, acute inhalation studies be conducted in experimental animals to test the toxicity of the colored smokes. Acute toxicity studies would be most relevant for recommending emergency guidance levels such as the EEGLs and SPEGLs. Such exposures might occur during training exercises in which military personnel might be exposed for several minutes, twice per day, two to four times per year. Some military personnel, particularly instructors involved in training exercises, might be repeatedly exposed to the smokes over several years, as might a community living near a military

training facility. For those exposure scenarios, the REGL and RPEGL are the most appropriate guidance levels, and studies assessing the potential toxicity of the smokes following repeated exposure would provide the most appropriate data for setting those exposure guidance levels. Thus, the Army should also consider conducting subchronic inhalation studies in experimental animals to test the toxicity of the smokes under conditions of repeated exposure.

Both acute and repeated inhalation studies should be carried out using combusted smokes, and the particle size and combustion products should be representative of the smokes used by the Army. Toxicity testing of other smoke formulations or of individual dyes might not provide results similar to those obtained with the combusted smokes. For example, particle size, surface area, and surface characteristics of the smokes might be important determinants of toxicity. Thus, the use of smokes with surface properties different from those of the smokes used by the Army would be difficult to interpret within the context of potential exposure of military personnel.

Finally, there is concern that potential sensitization resulting from exposure to several of the dyes used in the smoke formulations might cause allergic dermatitis and respiratory-tract hypersensitivity. The subcommittee recommends that studies be conducted in animal models appropriate for assessing the sensitivity potential from dermal and inhalation exposures. Those studies should also be conducted using the combusted smokes. To ensure that such studies are designed correctly, the Army should consult with an expert panel before conducting them.

Until adequate data are available for determining exposure guidance levels, the subcommittee recommends that the Army follow its current policy on protecting military personnel from the respiratory and dermal effects of the colored smokes. In addition, the subcommittee recommends that these smokes be used only for signaling and marking and not for obscuring (the Army has developed other smokes for obscuring purposes). Because the potential toxicity of colored smokes is unknown, the subcommittee recommends that the Army avoid exposing the general public to the colored smokes.

1

Introduction

EVER SINCE since smokeless powder replaced black powder as the standard propellant for guns and firearms, the armed forces have sought methods to blanket battlefields by creating a haze similar to that created by black powder. A variety of smokes and obscurants has been developed and used in wartime operations for screening armed forces from view, deceiving the enemy, signaling friendly forces, and marking positions. To ensure defense preparedness, large quantities of smokes and obscurants also are used in military training. Obscurants are anthropogenic or naturally occurring particles suspended in air that block or weaken the transmission of particular parts of the electromagnetic spectrum, such as visible and infrared radiation or microwaves. Smokes are produced by burning or vaporizing some product. White and gray smokes are deployed in grenades to obscure vehicle locations or troop movement, and colored smokes are used to mark specific locations.

THE SUBCOMMITTEE'S TASK

In order to reduce the likelihood that exposure to smokes and obscurants during combat training would have adverse health effects on military personnel and the general public residing or working near military-training facilities, the Office of the Army Surgeon General requested the National Research Council (NRC) review the data on the toxicity of

military smokes and obscurants and recommend exposure guidance levels for military personnel during combat training and for the general public residing or working near military-training facilities.

The NRC assigned this project to the Committee on Toxicology (COT), which convened the Subcommittee on Military Smokes and Obscurants. This volume, Volume 3, reviews data for the seven colored smokes used by the Army for signaling purposes. In Volume 1 of this series, the subcommittee evaluated four obscuring smokes: fog oil, diesel fuel, red phosphorus, and hexachloroethane (NRC 1997). In Volume 2, four additional obscuring smokes were reviewed: white phosphorus, brass, titanium dioxide, and graphite (NRC 1999).

The specific task of the subcommittee was to review the health effects associated with exposure to the smokes and other obscurants and, if appropriate, to recommend four exposure guidance levels: (1) emergency exposure guidance levels (EEGLs) for a rare, emergency situation resulting in an exposure of military personnel for a period of less than 24 hr; (2) repeated exposure guidance levels (REGLs) for repeated exposure of military personnel during training (referred to as permissible exposure guidance levels in Volume 1); (3) short-term public emergency guidance levels (SPEGLs) for a rare, emergency situation potentially resulting in an acute exposure of the public to a military-training smoke; and (4) repeated public exposure guidance levels (RPEGLs) for possible repeated exposures of the public residing or working near military-training facilities (referred to as permissible public exposure guidance levels in Volume 1). It was further requested that all four guidance levels take into account developmental and reproductive toxicity in men and women and that exposures of potentially susceptible subpopulations (e.g., ill or elderly persons and children) be considered in deriving the SPEGL and RPEGL.

SMOKES REVIEWED IN THIS REPORT

The military uses colored smokes, disseminated via the M18 grenade or the M713, M715, or M716 40-millimeter (mm) cartridge, for signaling and marking and, in some cases, for training to simulate exposure to chemical-warfare agents (Rubin and Buchanan 1983). The colored-smoke formulations are incorporated into the M18 grenade and the 40-mm cartridge with a pyrotechnic mixture to deploy the smoke.

Four original (old) smoke formulations, which contain dyes—red,

yellow, green, and violet—are used in the M18 grenade and the 40-mm cartridge. Studies indicate that some of those dye materials are transformed during the combustion process to yield different chemical species, some of which are potentially hazardous (Rubin et al. 1982). To avoid potential health hazards, the Army developed four new formulations of the same colors. However, the new violet-smoke formulation was removed from the inventory because of its acute toxicity (Costa et al. 1990; AEHA 1992, 1993a,b; Lundy and Eaton 1994). Because quantities of grenades and cartridges still in inventory contain the old smoke formulations, the Army requested an evaluation of the toxicity of the four old and the three new formulations.

Composition of the Colored Smokes

Table 1-1 lists the components of the colored-smoke M18 grenades and the 40-mm cartridges.

OLD M18 GRENADES

In the old M18 grenades, the colored dyes were mixed with sulfur, potassium chlorate, and sodium bicarbonate, with optional amounts of a mixture of pure, refined kerosene and tricalcium phosphate for control of dusting and caking, respectively (Lundy and Eaton 1994). The old smoke formulations were studied extensively by Rubin et al. (1982). The mixtures were fractionated by vacuum sublimation, differential solubility, and liquid chromatography. The major components were isolated and identified by comparison with the pure dyes using a variety of instrumental techniques. A number of contaminants at minor concentrations (less than 1%) were identified by gas chromatography-mass spectroscopy. All the smoke formulations contained relatively large quantities (10-25%) of chloroform-insoluble or nonvolatile undifferentiated carbonaceous material (Lundy and Eaton 1994).

NEW M18 GRENADES

In the new M18 grenades, sugar is used as the fuel instead of sulfur, and magnesium carbonate is used as a coolant instead of sodium bicarbonate

TABLE 1-1 Components of the M18 Colored-Smoke Grenades and 40-mm Colored-Smoke Cartridges (expressed as weight percent)

	Yellow				Red				Green				Violet
	M18		40 mm		M18		40 mm		M18		40 mm		M18
Component	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Vat yellow 4	14.0		17.0						4.0		6.0		
Solvent yellow 33		42.0		42.0						12.6		12.5	
Disperse red 9					40.0		44.0						8.4
Solvent red 1						34.2		38.0					
Disperse red 11						6.8		7.0					
Solvent green 3									28.0	29.4	27.3	29.5	
Benzanthrone	24.5		24.0						8.0		11.0		
1,4-Diamino-2,3-dihydroanthraquinone													33.6
Sulfur	8.5		11.2		9.0		9.9		10.4		11.2		9.0
Sodium biocarbonate	33.0		14.7		25.0		17.7		22.6		14.5		24.0
Potassium chlorate	20.0	24.1	28.5	28.4	26.0	17.7	27.9	21.5	27.0	25.0	28.5	28.5	25.0
Magnesium carbonate		17.5		10.3		9.6		4.0		15.5		10.5	
Terephthalic acid						14.0		8.0					
Sugar		16.4		19.3		17.7		21.5		17.5		19.0	
Polyvinyl alcohol				2.0				2.0				2.0	
Stearic acid				0.5		0.5		0.5				0.5	

Source: Adapted from U.S. Army Technical Data Package (1989).

(Lundy and Eaton 1994). The new red grenade also contains terephthalic acid in addition to the dye and pyrotechnic ingredients.

The composition of the yellow, green, and red dyes in the new formulation was also changed (Lundy and Eaton 1994). The new yellow dye was reformulated to replace benzanthrone (BZA) and dibenzochrysenedione (DBC) with a single component: dye solvent yellow 33 (2-(2'quinolyl)-1,3-indandione (QID)). 1,4-Di-*p*-toluidino-9, 10-anthraquinone (PTA) is retained in the new green grenade and combined with QID instead of BZA and DBC. Thus, some of the results of chemical characterization of the smoke from the detonated old green grenades and those portions of the toxicity information obtained using the PTA fraction of the old green-smoke formulation are applicable to the new green grenade as well. The new red grenade contains solvent red 1 (-methoxybenzenazo-1-naphthol) (MBN) and disperse red 11 (1,4-diamino-2-methoxy-anthraquinone) (DMA). The smoke formulations were studied by Buchanan and Ma (1988). All the mixtures contained small amounts (0.6% to 3.7%) of insoluble residue. The minor contaminants (less than 1% of mixture), 1-*p*-toluidinoanthraquinone and aminoanthraquinone were identified in the new green-and red-smoke formulations, respectively. No minor contaminant was identified in the new yellow formulation.

Combustion Chemistry

Signaling smokes are produced by volatilizing and condensing a mixture containing an organic dye (Owens and Ward 1974). A colored-smoke munition is composed of a pyrotechnic mixture of fuel and dye; a cooling agent is sometimes added to prevent excessive decomposition of the dye. The heat produced by the fuel volatilizes the dye, which then condenses outside the munition to form the colored smoke. The fuel is formulated as a mixture of an oxidizing agent and a combustible material. The burning time can be regulated by adjusting the proportions of oxidant and combustible material and by using coolants.

Combustion Products

In separate studies, Rubin et al. (1982) and Buchanan and Ma (1988) characterized the components of the smoke produced by deploying the

old red and violet grenades and all four colors of the new M18 grenades, respectively. The grenades were detonated inside canvas tents, and samples of the generated vapors and particles were collected. Chin et al. (1983) (as cited in Lundy and Eaton 1994) studied the combustion products of the U.S. Navy red-, yellow-, and green-smoke formulations, which use the same dye constituents as the old Army smoke formulations. In this study, the grenade fill, including the dyes, the fuel, the oxidizer, the cooling agent and binders, was thermally vaporized, and the solids and vapors produced were collected on filters and traps, respectively, and analyzed by thin-layer chromatography, high-performance liquid chromatography, gas chromatography, gas chromatography-mass spectrometry, nuclear magnetic resonance, ultraviolet spectrometry, and electron dispersive X-ray analysis. The results of the Rubin et al. (1982) and Chin et al. (1983) studies were in agreement in the case of the red grenade fill, the only case of overlap in the three studies. The vast majority (90-95%) of the dyes in the colored-smoke grenades remain unchanged during the combustion process (Chin et al. 1983). Approximately 5-10 percent of those dyes undergo decomposition when the grenades are detonated. Combustion products include polynuclear aromatic hydrocarbons, polynuclear organic materials, carbon dioxide, carbon monoxide, hydrochloric acid, and water and are the result of uncombusted impurities, pyrosynthesis, and degradation of the component dyes. The amounts and types of the combustion products produced are affected by variables such as burn time, burn rate, and atmospheric conditions such as humidity. Tables 1-2 and 1-3 summarize the major combustion products.

U.S. ARMY POLICY ON USE OF COLORED SMOKES

Current Army policy regarding colored smokes states that during training, troops must avoid entering the smoke cloud (AEHA 1992, 1993a,b). If troops are required to enter the smoke plume, they must wear a chemical protective mask, long-sleeve shirts, head coverings, pants that cover the entire leg, and boots.

In addition, Army policy requires that personnel involved with production of the M18 colored-smoke grenades and exposed to the pure dyes or smoke formulations wear protective equipment, including coveralls, butyl rubber gloves, head coverings, and respiratory protection.

TABLE 1-2 Major Combustion Products of the Old Colored-Smoke M18 Grenades

Smoke Formulation	Combustion Products	Reference
Yellow	No major changes noted.	Chin et al. 1983 (as cited in Lundy and Eaton 1994)
Green	No major changes noted.	Chin et al. 1983 (as cited in Lundy and Eaton 1994)
Red	The major component, 1-methyl-aminoanthraquinone (MAA), was converted (12%) to other compounds, chiefly 1- and 2-aminoanthraquinones.	Rubin et al. 1982; Chin et al. 1983 (as cited in Lundy and Eaton 1994)
Violet	The major component, 1,4-diamino-2,3-dihydroanthraquinone (DDA), was converted (100%) to 1,4-diaminoanthraquinone. The minor component, MAA, was partially converted to 1- and 2-AA.	Rubin et al. 1982

TABLE 1-3 Major Combustion Products of the New Colored-Smoke M18 Grenades

Smoke Formulation	Combustion Products	Reference
Yellow	The dye component, 2-(2'-quinolyl)-1,3-indandione (QID), is the major component in the combusted sample. Minor combustion products include 2,3-benzacridine-1,4-dione, 2,3-benzacridine-9-one, an isomer of 2,3-benzacridine-1,4-dione, and an isomer of QID. Insoluble residue: 5.0-9.9%.	Buchanan and Ma 1988
Green	A small fraction of 1,4-di-*p*-toluidino-9,10-anthraquinone (PTA) was altered when the grenade was detonated, and QID remained relatively unchanged. New products formed (totaling about 3% of original dye weight) were tentatively identified as I-*p*-toluidinoanthraquinone and an isomer of QID. Insoluble residue: 3.4%.	Buchanan and Ma 1988
Red	Major dye components, -methoxybenzenazo-ß-naphthol (MBN) and 1,4-diamino-2-methoxyanthraquinone (DMA), were not affected to a great extent by detonating of the grenade. 2-Methoxyaniline and 2-naphthol, possible decomposition products from the combustion of MBN, were present in the combusted samples. Insoluble residue: 3.7-4.5%	Buchanan and Ma 1988

DEFINITIONS OF EXPOSURE GUIDANCE LEVELS

An EEGL is defined as a concentration of a substance in air (as a gas, vapor, or aerosol) that will permit continued performance of specific tasks during emergency exposures lasting up to 24 hr—an occurrence expected to be infrequent in the lifetime of a person (NRC 1986, 1992a). "Emergency" connotes a rare and unexpected situation with potential for significant loss of life, property, or mission accomplishment if not controlled. An EEGL, a single ceiling-exposure concentration for a specified duration, specifies and reflects the subcommittee's interpretation of available information in the context of an emergency.

An EEGL is acceptable only in an emergency, when some risks or some discomfort must be endured to prevent greater risks (such as fire, explosion, or massive release). Exposure at the EEGL might produce such effects as increased respiratory rate, headache, mild central-nervous-system effects, and respiratory-tract or eye irritation. The EEGL should prevent irreversible harm. Even though some reduction in performance is permissible, it should not prevent proper responses to the emergency (such as shutting off a valve, closing a hatch, or using a fire extinguisher). For example, in normal work situations, upper-respiratory-tract irritation or eye irritation causing discomfort would not be considered acceptable; during an emergency, it would be acceptable if it did not cause irreversible harm or seriously affect judgment or performance. The EEGL for a substance represents the subcommittee's judgment based on evaluation of experimental and epidemiological data, mechanisms of injury, and, when possible, operating conditions in which an emergency exposure might occur, as well as consideration of U.S. Department of Defense (DOD) goals and objectives. EEGLs were recommended for military use and are intended for healthy, young military personnel. Therefore, they are not directly applicable to general populations consisting of elderly, very young, and ill persons.

A SPEGL is defined as a concentration of a substance in air that is acceptable for an unpredicted, single or rare, short-term emergency exposure of the general public. The SPEGL takes into account the likely wide range of susceptibility among individuals in the general public, including potentially sensitive subgroups, such as children, the elderly, and persons with serious debilitating diseases. Effects of exposure on the developing embryo and fetus and on the reproductive capacity of men and women also are considered in setting a SPEGL.

For purposes of assessing military smokes and other obscurants for the Army, the subcommittee recommended two additional guidance levels, REGLs and RPEGLs. The subcommittee defines a REGL as the concentration of a substance in air to which healthy military personnel can be exposed repeatedly, up to a specified total exposure on a weekly basis (usually 8 hr per day, 5 days per week), for several years without experiencing adverse health effects or degradation in performance.

The subcommittee defines a RPEGL as the concentration of a substance in air to which the general public can be exposed repeatedly without experiencing adverse health effects or discomfort. RPEGLs, like SPEGLs, take into account the likely wide range of susceptibility among individuals in the general public, including potentially susceptible subpopulations (children, the elderly, and the chronically ill) and the developing embryo and fetus.

Exposure to smokes and obscurants at or below the recommended exposure guidance levels is not likely to produce adverse health effects in the general population. However, because of potential susceptibility factors including genetics, personal habits (e.g., smoking and alcohol consumption), and exposures to other chemicals, a small number of individuals might experience health effects at or below the recommended exposure guidance level.

APPROACH FOR RECOMMENDING EXPOSURE GUIDANCE LEVELS

The NRC has published guidelines for recommending EEGLs, SPEGLs, and other exposure guidance levels for continuous or repeated exposures to a variety of chemical agents (NRC 1992a,b, 1996). For purposes of assessing military smokes and other obscurants, the subcommittee recommended comparable procedures for recommending REGLs and RPEGLs. The steps in recommending exposure guidance levels are similar for EEGLs, SPEGLs, REGLs, and RPEGLs; the differences reflect attributes of the exposed populations and the duration and frequency of exposure. A detailed explanation of the approach the subcommittee used to recommend exposure guidance levels can be found in *Toxicity of Military Smokes and Obscurants, Volume 1* (NRC 1997).

The general approach for recommending EEGLs is to determine a no-observed-adverse-effect level (NOAEL) directly from laboratory experi

ments or human studies on acute (short-term) exposure or to estimate an acute-exposure NOAEL from a lowest-observed-adverse-effect level (LOAEL) with the use of a default assumption. A 15-min, 1-hr, or 6-hr EEGL is estimated from the NOAEL, depending on which exposure duration most closely matched the exposure duration associated with the NOAEL. Haber's law (the product of exposure concentration and time is a constant; C X T = k) is then used, if appropriate, to recommend EEGLs for different exposure durations.

SPEGLs are generally derived from EEGLs by applying an uncertainty factor to protect all members of the public, including susceptible subpopulations, such as the elderly, children, and the developing embryo or fetus. In the absence of specific data on variation in human susceptibility to a smoke, the subcommittee assumes that some subpopulations could be up to 10 times more susceptible than healthy military personnel. Thus, unless otherwise noted, SPEGLs are generally 10-fold lower than the EEGLs.

Data from chronic-(repeated) exposure experiments in animals or clinical observations form the basis for the REGL instead of the acute-exposure data used for the EEGL. Haber's law should not be applied to extrapolate from longer to shorter exposures.

RPEGLs for possible repeated exposures of a community near a military-training facility are calculated by dividing the REGLs recommended for military personnel by an uncertainty factor of 10 to extrapolate from healthy military personnel to a more diverse population, including potentially susceptible subpopulations.

ORGANIZATION OF THE REPORT

This volume is organized into two major chapters. Chapter 2 reviews the potential toxicity of the old formulations for yellow, green, red, and violet smokes, and Chapter 3 reviews the potential toxicity of the new formulations for yellow, green, and red smokes. In each chapter, information is presented on the physical and chemical properties, toxicokinetics, and toxicity of the formulations and on the toxicity of the component dyes. Each chapter concludes with an overall evaluation of the toxicity of the formulations, reports any existing recommended exposure limits, and presents the subcommittee's recommendations for exposure guidance levels and research. Following the two major chap

ters are nine appendixes which review the toxicity data on each of the major dye components used in the formulations.

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2

Old Smoke Formulations

YELLOW-SMOKE FORMULATION

Composition

THE MAJOR dye components of the smoke formulation are benzanthrone (BZA) (54% of the dye components) and dibenzochrysenedione (DBC) (38%) (Lundy and Eaton 1994). DBC is also referred to as vat yellow 4. In the old M18 grenades, the colored dyes are mixed with a pyrotechnic mixture containing sulfur, potassium chlorate, and sodium bicarbonate. Optional amounts of a mixture of pure, refined kerosene and tricalcium phosphate are added for control of dusting and caking, respectively.

Combustion Products

Chin et al. (1984) (as cited in Lundy and Eaton 1994) studied the combustion products of the old yellow smoke formulation and found that combustion produced no major chemical changes in the dyes. Additional information on the combustion products of the old yellow-smoke formulation is presented in Chapter 1.

Toxicokinetics

The only toxicokinetics data available for the smoke formulation is the finding that yellow particles were present in the airways of exposed mice, rats, and guinea pigs many days (number of days not specified) after a 1-hr inhalation exposure to the combusted smoke formulation (Weeks and Yevich 1963). See Appendixes A and B for information on the toxicokinetics of the component dyes.

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the old yellow smoke formulation and its combustion products. The toxicity of the component dyes is evaluated in Appendixes A and B.

No epidemiological studies have been conducted on military workers or others exposed to the smoke formulation or its combustion products. A dermal lethal dose for 50% of the test animals (LD50) in rats exposed to the smoke formulation is more than 4.6 grams per kilogram (g/kg) of body weight (Lundy and Eaton 1994). An intraperitoneal LD_{50} is 1.5 g/kg in the rat and 0.29 g/kg in the mouse.

In a study by Weeks and Yevich (1963), grenades containing an old yellow-smoke formulation were fired inside a static exposure chamber to achieve initial concentrations of particles that were high (13.4 grams per cubic meter (g/m^3)), medium (4.8 g/m^3), or low (0.9 g/m^3). The dye composition of the smoke formulation was reported to be BZA and indanthrene (a blue powder) rather than BZA and vat yellow 4. Rats (male), mice (female), and guinea pigs (both sexes) were exposed in the static chamber for 1 hr at 13.4, 4.8, or 0.9 g/m^3. Carbon monoxide (CO) concentrations reached 1% for the high-concentration group and might account for some of the toxic effects observed, including eye irritation, nasal discharge, gasping, and lethargy. Surviving animals were sacrificed 24 hr after the exposure or at later time points for up to 4 weeks after the exposure and then examined histologically.

All animals died during or soon after the exposure to the high concentration. After exposure to the medium concentration, 3 of 5 male guinea pigs and 8 of 10 male rats died. No animals died after exposure to the low concentration. All surviving animals had a retarded growth rate after the exposure; however, those exposed to the medium and low concentrations recovered and eventually gained weight at a normal rate.

Histopathological examination on animals sacrificed 24 hr after exposure indicated that the yellow-dye particles clogged the nasal passages of the animals and sometimes obliterated bronchi and bronchioles. Lungs exhibited necrosis, sloughing of the mucosa, and edema in the alveolar space. Necrosis of the epithelium of the tracheobronchial tree extended to the basement membrane and the submucosa. Gastric changes were observed with isolated areas of necrosis and hemorrhage. Those changes might be a result of ingestion of smoke components from grooming by the animals; it is unlikely that humans would be exposed by that route. At the later sacrifice times, there were signs of chronic inflammation in the nose, and the lung contained "foreign-body giant cells" that obliterated the alveoli.

Two female rhesus monkeys were exposed to the medium concentration (4.8 g/m^3). One monkey collapsed and went into convulsions 22 min into the exposure and was removed from the exposure chamber. The second monkey was exposed for 30 min. Both monkeys developed a hacking cough resulting in a yellow mucoid exudate from the nostrils. The first monkey was sacrificed 5 days after exposure and the second, 27 days after exposure. Histopathological changes consisted of pulmonary mucosal necrosis and sloughing, serocellular plugs, necrosis extending to the adventitia in some areas, submucosal polymorphonuclear infiltration, and dilation of the mucoid glands.

Toxicity of Component Dyes

One of the dyes used in the smoke formulation is BZA, a known photosensitizer and a compound that causes dermal toxicity in workers (Dacre et al. 1979). The other component, vat yellow 4, is relatively nontoxic for both noncancer (by oral and dermal route) and cancer end points. However, no inhalation toxicity studies have been completed on this chemical. For a complete review on the toxicity of the component dyes, see Appendixes A and B.

GREEN-SMOKE FORMULATION

Composition

The major dye components of the smoke formulation are BZA (24% of the dye components), 1,4-di-*p*-toluidino-9,10-anthraquinone (PTA)

(62%), and vat yellow 4 (13%) (Lundy and Eaton 1994). PTA is also called solvent green 3. In the old M18 grenades, the colored dyes are mixed with a pyrotechnic mixture containing sulfur, potassium chlorate, and sodium bicarbonate. Optional amounts of a mixture of pure, refined kerosene and tricalcium phosphate are added for control of dusting and caking, respectively.

Combustion Products

Chin et al. (1984) (as cited in Lundy and Eaton 1994) studied the combustion products of the old green smoke formulation and found that combustion produced no major chemical changes in the dyes. Additional information on the combustion products of the old green-smoke formulation is presented in Chapter 1.

Toxicokinetics

A toxicokinetics study was conducted on a mixture of solvent green 3 and solvent yellow 33 dyes (Medinsky et al. 1986). The study demonstrated that solvent green 3 cleared slowly from rat lungs after inhalation. See Chapter 3 for details of this study.

The only information on the toxicokinetics of the combustion products of the old green smoke formulation is from the studies of Weeks and Yevich (1963). The composition of the smoke formulation was not exactly that of the Army's smoke formulation, but the major dye component of the smoke formulation was solvent green 3. Animals exposed to the combustion products had green particulate matter in their nasal passages and lungs for several days after a 1-hr exposure. See Appendixes A, B, and D for information on the toxicokinetics of the component dyes.

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the old green-smoke formulation and its combustion products. The toxicity of the component dyes is evaluated in Appendixes A, B, and D.

No epidemiological studies have been conducted on military workers

or others exposed to the smoke formulation or to its combustion products. An acute oral LD_{50} of the smoke formulation in rats at a concentration of 3.1 g/kg of body weight was reported by Lundy and Eaton (1994). In a study by Weeks and Yevich (1963), grenades containing a green-smoke formulation were fired inside a static exposure chamber to achieve initial concentrations of particles that were high (12.1 g/m^3), medium (4.8 g/m^3) or low (0.6 g/m^3). The dye composition consisted of solvent green 3 (75% of the smoke formulation) and auramine hydrochloride (25%) rather than solvent green 3, BZA, and vat yellow 4, as in the Army's smoke formulation. Rats (male), mice (female), and guinea pigs (both sexes) were exposed in the static chambers for 1 hr. CO concentrations reached 1% at the end of the high-concentration exposure and might account for some of the toxic effects observed in the animals, including eye irritation, nasal discharge, gasping, and lethargy. Surviving animals were sacrificed 24 hr after exposure or at later time points for up to 4 weeks after the exposure and then examined histopathologically.

Death occurred during or soon after the exposure to the high concentration in all rats, 7 of 10 mice, and 3 of 5 female guinea pigs. No animals died after exposure to the medium concentration, and 1 of 10 rats died after exposure to the low concentration. Surviving animals in all groups had at least a slightly retarded growth rate after the exposure. Animals in the high-concentration exposure group did not recover a normal rate of weight gain; however, those in the medium-and low-concentration groups recovered and gained weight at a normal rate. Histopathological analysis on animals sacrificed 24 hr after exposure indicated that the green-dye particles clogged the nasal passages and sometime obliterated bronchi and bronchioles. Lungs exhibited necrosis, sloughing of the mucosa, and edema in the alveolar space. Gastric changes were observed with isolated areas of necrosis and hemorrhage, and few areas of ulceration were seen in the duodenum. Those gastric changes might be a result of ingestion of smoke components from grooming by the animals; it is unlikely that humans would be exposed by that route. At later sacrifice times, signs of chronic inflammation were observed in the nose, and the lung contained "foreign-body giant cells" that obliterated the alveoli.

A repeated-exposure inhalation study of the toxicity of the combustion products of a smoke formulation containing a mixture of solvent yellow 33 (13%), disperse red 9 (16%), and solvent green 3 (19%) was performed in female rats, mice, and guinea pigs (Marrs et al. 1984). The animals were exposed at concentrations of 0.1, 0.3 and 1.0 g/m^3 for 1 hr

per day, 5 days per week until they received up to 100 exposures. The animals were sacrificed 1 year after the exposure period with the exception of a group of mice killed 40 weeks after exposure. All animals had evidence of retention of dye in the lungs. In the guinea pigs, the high-dose exposure was stopped after 16 exposures because 14 of 50 animals had died from severe pulmonary congestion and alveolitis. Histologically, there were no significant effects in guinea pigs exposed at the two lower-doses except for alveolitis. There was a dose-related trend of alveolitis and chronic pneumonia in mice sacrificed 40 weeks after exposure. Mice in the high-dose group sacrificed 1 year after exposure had an increased number of macrophages and dye particles in the lungs compared with other exposure groups and controls. In rats, there was a significant dose-related trend in the occurrence of lesions related to inflammatory processes in the lung. It is important to note that the results of the Marrs et al. (1984) study could not be ascribed to solvent green 3 alone, and therefore, the relevance in quantitatively describing the toxicity of the old green-smoke formulation is questionable.

Toxicity of Component Dyes

The primary dye component of the old green-smoke formulation is solvent green 3. Although relatively nontoxic when delivered orally or dermally to animals, solvent green 3 is insoluble in the lung and accumulates there when inhaled (Sun et al. 1987). The accumulation results in an inflammatory response in the lung. One of the other dyes used in the old green-smoke formulation is BZA, a known photosensitizer and a compound that causes dermal toxicity in workers (Dacre et al. 1979). Another component, vat yellow 4, is relatively nontoxic for both noncancer end points (by oral and dermal routes) and cancer end points. However, no inhalation toxicity studies have been completed on this compound. For a complete review on the toxicity of the dye components, see Appendixes A, B, and D.

RED-SMOKE FORMULATION

Composition

The primary dye component of old red-smoke formulation is 1-methyl

aminoanthraquinone (MAA) (40% of the total components) (Rubin and Buchanan 1983). MAA is also called disperse red 9. Other components are anthraquinone (2-3%) and trace materials (less than 1%). A dark, high-molecular-weight material that is chloroform insoluble and nonvolatile is also in the mixture (Rubin and Buchanan 1983). The authors stated that the material could not be fully characterized. In the old M18 grenades, the colored dyes are mixed with a pyrotechnic mixture containing sulfur, potassium chlorate, and sodium bicarbonate. Optional amounts of a mixture of pure, refined kerosene and tricalcium phosphate are added for control of dusting and caking, respectively.

Combustion Products

Rubin et al. (1982) characterized the combustion products produced by old M18 grenades containing red-smoke formulation. The grenades were detonated inside sealed canvas tents. Samples of particulate matter and vapor generated by the smoke formulation were collected. Disperse red 9 accounted for 86% of the particulate matter in the combustion products compared with 98% in the red-smoke formulation. Approximately 10% of the disperse red 9 was converted to aminoanthraquinones (1-AA and 2-AA). The concentration of 1-AA and 2-AA increased more than 10 fold over their concentration in the old red-smoke formulation. Additional information on the combustion products of the old red-smoke formulation is presented in Chapter 1.

Toxicokinetics

No studies have been conducted on the toxicokinetics of the old red-smoke formulation or its combustion products.

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the old red-smoke formulation and its combustion products. The toxicity of the component dye is evaluated in Appendix F.

No epidemiological studies have been conducted in military workers or others exposed to the smoke formulation or its combustion products. An acute intraperitoneal LD_{50} in rats for the smoke formulation at a concentration of 1.5 g/kg of body weight was reported by Lundy and Eaton (1994).

The toxicity of the combustion products were tested in the monkey, dog, goat, swine, rabbit, rat, and guinea pig (Owens and Ward 1974). Grenades containing a red-smoke formulation were fired in test chambers exposing the animals to concentrations ranging from 1.5 to 18.0 g/m ^{3}. Exposure duration ranged from 10 to 240 min. The animals were observed over a period of 30 days. The results were presented as a Bliss analysis of the combined mortality of total number of animals of all species exposed to the combustion products. For the combustion products, the lethal concentration to 50% of the test animals multiplied by exposure time (LCT_{50}) ranged from 0.4 to 0.6 g•min/m^3. All animals showed signs of upper-respiratory irritation and salivation immediately after exposure. In the dog, swine, goat, and monkey, gagging and regurgitation of a thick red mucus were seen, and the urine was a dark red color for 24 hr immediately after exposure. Labored breathing was seen in all species for 7 days after exposure. The swine and the goat were the most resistant of all species tested. Most deaths occurred within 24 hr, and 97% of deaths occurred by day 14. Only general information could be gleaned from this study because the data were reported in such a manner that it is not possible to evaluate the results of the old red-smoke formulation on any single species.

Slaga et al. (1985) examined the initiation and promotion properties of the old red-smoke formulation in the Sencar mouse. The smoke formulation was studied for its complete carcinogenicity (administered as an initiator and a promoter to the same animals) or as an initiator only (administered as an initiator followed by 12-O-tetradecanoyl phorbol 13 acetate administration). There was no tumor response when the smoke formulation was tested as a complete carcinogen or as an initiator.

Toxicity of Component Dyes

A component of the old red-smoke formulation, disperse red 9, was reported to cause skin irritation in humans (Dacre et al. 1979) (reviewed in Appendix F). The dose and length of exposure were not reported;

however, a one-time dermal application of disperse red 9 to the abraded skin of rabbits at a concentration of 2 g/kg of body weight resulted in negligible dermal irritation, as did ocular exposure at 0.05 g/kg of body weight (Martin et al. 1983). Oral administration of disperse red 9 to dogs at up to 8 g/kg of body weight resulted in no significant differences between exposed and control animals (Sendelbach 1989). One study testing disperse red 9 for mutagenicity reported positive results (Lundy and Eaton 1994). Two other studies reported negative results (Dacre et al. 1979; Sigman et al. 1985). There is no evidence for carcinogenicity of disperse red 9 (Griswold 1968; Sigman et al. 1985). The combustion product 2-AA was found to be carcinogenic in bioassays using rats and mice (NCI 1978).

VIOLET-SMOKE FORMULATION

Composition

The major dye components of old violet-smoke formulation are 1,4-diamino-2,3-dihydroanthraquinone (DDA), significant amounts of disperse red 9, as well as an insoluble residue and a number of organic materials in trace amounts (Rubin and Buchanan 1983). The mixture is formulated to contain 80% DDA and 20% disperse red 9. DDA is easily converted to 1,4-diaminoanthraquinone (DAA) in air. In the old M18 grenades, the colored dyes are mixed with a pyrotechnic mixture containing sulfur, potassium chlorate, and sodium bicarbonate. Optional amounts of a mixture of pure, refined kerosene and tricalcium phosphate are added for control of dusting and caking, respectively.

Combustion Products

Rubin et al. (1982) characterized the combustion products produced by old M18 grenades containing violet-smoke formulation. The grenades were detonated inside canvas tents, and vapor and particle samples were collected. Upon combustion, DDA is known to convert to DAA. Disperse red 9 and AA were also detected in the combustion products along with small amounts of other organic materials, including cyanoanthraquinone, hydrocarbons, aliphatic amides, and phthalates. The inorganic materials

detected were potassium and sodium chlorides, sulfur, and trace metals. Additional information on the combustion products of old violet-smoke formulation is presented in Chapter 1.

Toxicokinetics

No studies have been conducted on the toxicokinetics of old violet-smoke formulation or its combustion products.

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the old violet-smoke formulation and its combustion products. The toxicity of the component dyes is evaluated in Appendixes H and I.

The combustion products are more significant toxicologically than the noncombusted material (the smoke formulation), because the potential mutagenicity of DAA is greater than that of DDA (Rubin et al. 1982) (see Appendix I).

No epidemiological studies have been conducted on military workers or others exposed to either the smoke formulation or its combustion products. There are no studies on the acute inhalation toxicity of the uncombusted smoke formulation. Acute inhalation studies of the combustion products disseminated from M18 munitions were conducted in the monkey, dog, goat, swine, rabbit, rat, and guinea pig (Owens and Ward 1974). The animals were exposed to concentrations ranging from 1.3 to 7.8 g/m^3 for 8 to 142 min. Exposure was followed by a 30-day observation period. The results were presented as a Bliss analysis of the combined mortality of the total number of animals of all species exposed to the combustion products. The combined LCT_{50} for the combustion products ranged from 0.21 to 0.20 $g \cdot min/m^3$. Immediately after exposure, all animals showed upper-respiratory irritation and salivation. Gagging was evident in the dog, swine, goat, and monkey. Prostration was noted in all species for 1 to 4 hr after exposure. Most deaths occurred within the first week after exposure.

Slaga et al. (1985) conducted studies of the ability of the old violet-smoke formulation to exhibit complete carcinogenicity as well as its

ability to be an initiator in the Sencar mouse. There was no tumor response to the formulation when tested as a complete carcinogen or as an initiator.

Lundy and Eaton (1994) tested the old violet-smoke formulation for mutagenicity and the formulation was positive in the Ames assay.

Toxicity of Component Dyes

No data are available on the toxic effects of DDA or DAA in humans. In animals, no data are available on DDA effects; however, positive and "marginally adequate" effects have been cited in the Ames assay (Dacre et al. 1979; Lundy and Eaton 1994). With respect to DAA, moderate eye irritation in rabbits was found at a dose of 0.5 g for a 24-hr period. DAA has a reported LD_{50} at 4.9 g/kg of body weight (RTECS 1981-82), and positive effects have been shown in the Ames assay (Lundy and Eaton 1994). For complete reviews on the toxicity of DDA and DAA, see Appendixes H and I, respectively.

OVERALL EVALUATION OF TOXICITY

There are no well-controlled inhalation toxicity studies on combustion products of old yellow-, green-, red-, and violet-smoke formulations that could provide a basis for the subcommittee to assess the potential health effects of exposure to these combustion products by military personnel or to recommend guidance levels.

The subcommittee is concerned about the toxicity of several of the component dyes. BZA, a major component of old yellow-and green-smoke formulations, is a dermal toxicant. Disperse red 9, a major component of old red-and violet-smoke formulations, also might be a dermal toxicant. Thus, even masking will not protect against the toxicity of the combustion products of these smoke formulations. Solvent green 3, a component of the old green-smoke formulation, was shown to accumulate in the lung with repeated exposures, resulting in an inflammatory response. Although DDA, a component of the old violet-smoke formulation, and DAA, a combustion product, produced positive results in the Ames assay, there is insufficient information to assess the toxicity of these compounds.

PREVIOUS RECOMMENDED EXPOSURE LIMITS

There are no previous recommended exposure limits for the combustion products of the old yellow-, green-, red-, and violet-smoke formulations. Army Health Hazard Assessment Reports (AEHA 1992, 1993a,b) on the M18 grenade and the 40 mm-cartridge components recommend masking when a smoke haze exceeds 4 hr or when passing through or operating in a smoke fog.

SUBCOMMITTEE RECOMMENDATIONS

The data base for assessing the potential toxicity of the combustion products of the old yellow-, green-, red-, and violet-smoke formulations is inadequate to make recommendations for exposure guidance levels. The BZA component of the old yellow-and green-smoke formulations is not suitable for use as a component dye because of the dermal toxicity of the dye and its photosensitization properties. The subcommittee recommends that additional research be conducted on the toxicity of the combustion products of the old red-and violet-smoke formulations if the Army is to continue to use them. At a minimum, the Army should conduct acute inhalation studies in experimental animals to test the potential toxicity of the combustion products. Acute toxicity studies would be most appropriate for recommending the emergency exposure guidance level and the short-term public emergency guidance level. Such exposures might occur during training exercises in which military personnel might be exposed for several minutes, twice per day, two to four times per year. Some military personnel, particularly instructors involved in training exercises, might be repeatedly exposed to the smokes over several years, as might a community living near a military-training facility. For those exposure scenarios, the repeated exposure guidance level and the repeated public exposure guidance level are the most appropriate exposure guidance levels, and studies assessing the potential toxicity of the smokes following repeated exposure would provide the most appropriate data for setting those guidance levels. Thus, the Army should also consider conducting subchronic inhalation studies in experimental animals to test the toxicity of the smokes under conditions of repeated exposure. Both acute and repeated inhalation studies should be carried out using combusted smokes, and the particle

size and combustion products should be representative of the smokes used by the Army. Toxicity testing of other smoke formulations or of individual dyes might not provide results similar to those obtained with the combusted smokes.

Additionally, concerns for potential sensitization resulting from exposure to disperse red 9, a component of both the old red-and violet-smoke formulations, and BZA, a component of both the old yellow-and green-smoke formulations, suggest that studies to assess contact allergic dermatitis and respiratory-tract hypersensitivity be conducted in animal models appropriate for testing hypersensitivity. Those studies should also be conducted using the combusted smokes. To ensure that such studies are designed correctly, the Army should consult with an expert panel before conducting them.

At this time, the subcommittee recommends that Army policy be followed with regard to respiratory and dermal protection from the combustion products of the old yellow-, green-, red-, and violet-smoke formulations. It also recommends that the old colored smokes grenades be used for signaling and not for obscuring. The subcommittee recommends that the Army avoid exposing the general public to the combustion products of the old yellow-, green-, red-, and violet-smoke formulations.

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3

New Smoke Formulations

YELLOW-SMOKE FORMULATION

Composition

THE MAJOR dye component of the new yellow-smoke formulation is 2(2-quinolyl)-1,3-indandione (QID) (42% of total components). This dye is also called solvent yellow 33. An insoluble residue constitutes approximately 3.7% of the formulation. In the new M18 grenades designed to replace those containing the old smoke formulation, sugar is used as the fuel instead of sulfur, and magnesium carbonate is used as a coolant instead of sodium bicarbonate.

Combustion Products

Buchanan and Ma (1988) characterized the combustion products produced by the new M18 grenades containing the new yellow-smoke formulation. The grenades were detonated inside canvas tents, and samples of the vapors and particles generated by the grenade were collected. Approximately 5% of solvent yellow 33 was converted to oxidized products tentatively identified as quinolylnapthalone and isomers of quinoylnapthoquinone. The insoluble residue constituted 5.0% to 9.9% of the combustion product, and the mass median aerodynamic diameters were 0.85 micrometer (μm) at 3 min and 1.80 μm at 30 min.

Additional information on the combustion products of the new yellow-smoke formulation is presented in Chapter 1.

Toxicokinetics

No studies have been conducted on the toxicokinetics of the new yellow-smoke formulation or its combustion products. However, toxicokinetics studies have been conducted on pure solvent yellow 33 dye. Those are summarized in Appendix C. Extrapolating the results of toxicokinetics studies conducted in animals suggests that solvent yellow 33 will be rapidly absorbed from the respiratory tract after inhalation in humans, and extensively metabolized, with metabolites excreted primarily in bile and eliminated in feces. No tissues appear to be storage depots for significant quantities of solvent yellow 33.

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the new yellow-smoke formulation and its combustion products. The toxicity of the component dye is evaluated in Appendix C.

No epidemiological studies have been conducted on military workers or others exposed to the smoke formulation or its combustion products. Likewise, no toxicity studies have been conducted in experimental animals. However, one study was conducted on the inhalation toxicity of the combustion products of a smoke formulation composed of solvent yellow 33 and disperse orange 11 (Marrs et al. 1988), and another was conducted on the inhalation toxicity of the combustion products of a smoke formulation composed of solvent green 3, solvent yellow 33, and disperse red 9 (Marrs et al. 1984). Because those smoke formulations contain solvent yellow 33, the results have some value in assessing the toxicity of the combustion products. The study on solvent yellow 33 and disperse orange 11 is discussed here. The study on the toxicity of the combustion products of the smoke formulation containing the three dyes is discussed in Chapter 2.

In the Marrs et al. (1988) study, smoke was generated by ignition of a pyrotechnic composition containing solvent yellow 33 and disperse

orange 11. Female rats, mice, and guinea pigs were exposed to freshly generated smoke for 1 hr per day, 5 days per week until they had received 200 exposures. Guinea pigs in the highest-dose group received only 75 exposures. Thick washers of the smoke components were ignited on electrically heated wire, and the resultant colored smoke was mixed using high velocity jets in a static 10-cubic-meter (m^3) chamber. Concentrations in the chamber were varied by igniting different numbers of washers. Groups of each species were exposed together, starting with exposure of the controls to air and ending with the exposure of the highest-dose group. As the concentration declined during the exposure of each test group, fresh washers of the pyrotechnic composition were ignited to maintain the smoke concentration. During each exposure, samples of the smoke were collected on glass-fiber filters for determination of concentration. Particle size was determined using an Anderson particle sizer. All animals alive at the end of the exposures were observed until they died or until the end of a 6-month observation period (17 months after the start of exposure).

Mean exposure concentrations were 0.1 grams per cubic meter (g/m^3), 0.3 g/m^3, or 1.0 g/m^3. The mass median diameter of the smoke was 0.95, 1.55, and 1.10 μm for the three dose groups, respectively. For rats, there was a significant increase in total mortality in the highest-dose group compared with controls over the total exposure period. For guinea pigs, a large number of deaths occurred in the highest-dose group within a very short period in the fourth month of the study, and thus the high-dose guinea pigs were not exposed further. An increased number of deaths during the exposure period occurred in mice of the high-dose group compared with the controls. For mice that survived to the end of the study, an increased incidence of macrophage infiltration and an increased incidence of dilated mucous glands in the trachea were observed in the high-dose group. In rats, an increased incidence in macrophages with granules was seen in the middle-and high-dose groups. Guinea pigs had a statistically significant increase in the presence of excess macrophages and peribronchial lymphocyte infiltration.

Marrs et al. (1988) noted that the large number of deaths observed in the high-dose group of guinea pigs was not unprecedented, because a similar phenomenon was observed in a previous inhalation study in which guinea pigs were exposed to the combustion products of a brown smoke-formulation containing a mixture of three dyes: solvent green 3, solvent yellow 33, and disperse red 9 (Marrs et al. 1984; reviewed in

Chapter 2). The authors commented that the lethality might be a reflection of the peculiar sensitivity of guinea pigs to the stress associated with inhalation of particles and suggested that the cause of death was due to bronchial spasms. However, solvent yellow 33 was a component of both the brown-smoke and the yellow-orange-smoke formulations. Thus, its role in the hypersensitivity of the guinea pigs to the inhaled combustion products cannot be discounted. In some cases, guinea pigs have been shown to be an appropriate animal model for study of human hypersensitivity to inhaled materials (Mauderly 1984).

Marrs et al. (1988) suggested that the combustion products of the yellow-orange-smoke formulation were clearly toxic to all test groups of the three species studied, because the relative body weights decreased during the exposure period. However, organ-specific toxicity appeared to be confined to the respiratory tract. In the surviving animals, a noteworthy and significant difference from the previously tested combustion products of the brown-smoke formulation was the absence of retained dye in the lungs of any species and the absence of sheets of packed macrophages. Those two observations were previously reported in rats exposed to the combustion products of the brown-smoke formulation containing three dyes (Marrs et al. 1984).

Marrs et al. (1988) concluded that the findings in the respiratory tract of the animals exposed to the combustion products of both the yellow-orange-smoke and the brown-smoke formulations represented the less specific effects of smoke toxicity rather than the effects of the dye components. They suggested that their findings might be common to all military smokes and were probably caused by the nondye constituents. Their findings included lymphocyte infiltration into the larynx and trachea in the mice and guinea pigs and dilated mucous glands in the trachea of mice and rats. The authors concluded that the findings could be explained on the basis of previous studies of the products of reaction between potassium chlorate and lactose, the main components of the resulting reaction being carbon dioxide, water, and potassium chloride (Marrs et al. 1988).

Toxicity of Component Dyes

The primary dye component of the new yellow-smoke formulation is solvent yellow 33, a demonstrated human contact allergen (reviewed in

Appendix C). Although most case reports and controlled studies have focused on dermal contact sensitivity, the sensitizing potential of inhaled solvent yellow 33 cannot be discounted. No studies in the appropriate animal models have been conducted to explore the potential for respiratory-tract sensitization after exposure to inhaled solvent yellow 33 particles.

GREEN-SMOKE FORMULATION

Composition

The major dye components of new green-smoke formulation are 1,4-di*p*-toluidino-9,10-anthraquinone (PTA) (29.4% of total components) and solvent yellow 33 (12.6%). PTA is also called solvent green 3. In the new M18 grenades, sugar is used as the fuel instead of sulfur, and magnesium carbonate is used as a coolant instead of sodium bicarbonate.

Combustion Products

Buchanan and Ma (1988) characterized the combustion products produced by new M18 grenades containing new green-smoke formulation. The grenades were detonated inside canvas tents, and samples of the vapor and particles generated by the grenade were collected. A small fraction (less than 1%) of solvent green 3 was altered during the combustion process, and solvent yellow 33 remained relatively unchanged upon combustion of the smoke formulation. Additional information on the combustion products of the new green-smoke formulation is presented in Chapter 1.

Toxicokinetics

No studies have been conducted on the toxicokinetics of the combustion products of the new green-smoke formulation. However, a toxicokinetics study was conducted on a mixture of solvent green 3 and solvent yellow 33 (Medinsky et al. 1986). Rats were exposed by inhalation to aerosols of a mixture of solvent green 3 and solvent yellow 33 containing trace

amounts of ^{14}C-QID. The mass median aerodynamic diameter of the particles was 2.6 µm and the inhaled concentrations of solvent yellow 33 and solvent green 3 were 0.09 g/m^3 and 0.2 g/m^3, respectively. The ratio of yellow dye to total dye was 0.38 by the weight, resulting in approximately equal molar quantities of each dye. The toxicokinetics of solvent yellow 33 in the solvent green 3 and solvent yellow 33 mixture were very similar to those observed when solvent yellow 33 was inhaled as a pure chemical (reviewed in Appendix C). Solvent yellow 33 was rapidly cleared from the lungs into the blood, rapidly metabolized, and excreted in the bile. Most of the solvent yellow 33 metabolites were eliminated in the feces. No tissues appeared to store significant quantities of solvent yellow 33 or its metabolites. In contrast, solvent green 3 was retained in the lungs during the 70-hr post-exposure period with an estimated minimum half-life of 22 days for clearance. Solvent green 3 was not detected in other tissues during that period. Increased retention of solvent green 3 is consistent with that reported by other investigators (reviewed in Appendix D).

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the new green-smoke formulation and its combustion products. The toxicity of the component dyes is evaluated in Appendixes C and D.

No epidemiological studies have been conducted on military workers or others exposed to the smoke formulation or its combustion products. Likewise, no toxicity studies have been conducted in experimental animals. However, the combustion products of a smoke formulation of solvent yellow 33 and disperse orange 11 (reviewed in the section on the new yellow-smoke formulation in this chapter) and a brown-smoke formulation composed of solvent green 3, solvent yellow 33, and disperse red 9 (reviewed in Chapter 2) were investigated. Because both smoke formulations contained solvent yellow 33, and the brown-smoke formulation also contained solvent green 3, the results have some value in assessing the toxicity of the combustion products of the new green-smoke formulation.

In both studies, organ-specific toxicity appeared to be confined to the respiratory tract. In the opinion of Marrs et al. (1988) two noteworthy

and significant differences between the two studies were the absence of retained dye in the lungs of surviving animals and the absence of sheets of packed macrophages in the study using the yellow-orange-smoke formulation and the observation of those effects in the study using the brown-smoke formulation. Toxicokinetics studies by Medinsky et al. (1986) found that solvent green 3 is retained in the lungs of animals following inhalation suggesting that the differences in the respiratory effects of the two combustion products might be due to the presence of solvent green 3 in the brown-smoke formulation.

Inhalation studies have also been conducted on a mixture of solvent yellow 33 and solvent green 3 aerosols. Sun et al. (1987) conducted 4-week and a 13-week inhalation toxicity studies of a 70:30 mixture of solvent green 3 and solvent yellow 33 in rats. The solvent yellow 33 cleared the lungs rapidly, and most of the pulmonary toxicity resulting from the exposures was attributed to the solvent green 3 which remained in the lungs with a calculated half-life of 280 days based on clearance observed during the 30-day post-exposure period. The mixture was not highly toxic, but at the highest exposure concentration (0.2 g/m^3 for the 4-week exposure and 0.1 g/m^3 for the 13-week exposure), the rats had a mild pulmonary inflammation, slight type-II cell hyperplasia, and an accumulation of vacuolated alveolar macrophages in the lungs. No other organs were affected. No effects were observed at the two lower exposure concentrations (0.01 and 0.05 g/m^3 for the 4-week exposure and 0.001 and 0.01 g/m^3 for the 13-week exposure).

Toxicity of Component Dyes

The major dye component of the new green-smoke formulation is solvent green 3, which is relatively nontoxic when delivered orally or dermally to animals. Inhaled solvent green 3 is insoluble in the lungs and accumulates there when inhaled (Sun et al. 1987). The accumulation results in an inflammatory response in the lungs. The other component in the new green-smoke formulation is solvent yellow 33. Solvent yellow 33 is a demonstrated human contact allergen (reviewed in Appendix C). Even though most case reports and controlled studies of solvent yellow 33 have focused on dermal contact sensitivity, the sensitizing potential of inhaled solvent yellow 33 cannot be discounted. No studies in the appropriate animal models have been conducted to explore the potential for

respiratory-tract sensitization after exposure to inhaled solvent yellow 33 particles.

RED-SMOKE FORMULATION

Composition

The major dye components of new red-smoke formulation are -methoxybenzenazo- -naphthol (MBN) (34.2% of total components) and 1,4-diamino-2-methoxyanthraquionone (DMA) (6.8%). MBN is also called solvent red 1 and DMA is called disperse red 11. In the new M18 grenades, sugar is used as fuel instead of sulfur, and magnesium carbonate is used as a coolant instead of sodium bicarbonate. Additional information on the combustion products of the new red-smoke formulation is presented in Chapter 1.

Combustion Products

Buchanan and Ma (1988) characterized the combustion products produced by the new M18 grenades containing new red-smoke formulation. The grenades were detonated inside canvas tents, and samples of the vapor and particles generated by the grenade were collected. A small fraction of disperse red 11 (approximately 3%) was converted during combustion (possibly to 2-methoxyaniline and 2-naphthol), and solvent red 1 remained relatively unchanged during combustion of the new red-smoke formulation.

Toxicokinetics

No studies have been conducted on the toxicokinetics of the combustion products of the new red-smoke formulation or on solvent red 1, one of the smoke components. However, one study on the retention of disperse red 11 demonstrated that, after instillation, the dye is rapidly cleared from the lungs into the blood of rats, only 3.5% of the initial dose being retained in the lungs after 24 hr (Henderson et al. 1988).

Toxicity of the Smoke Formulation and Its Combustion Products

This section reviews the toxicity of the new red-smoke formulation and its combustion products. The toxicity of the component dyes is evaluated in Appendixes E and G.

No epidemiological studies have been conducted on military workers or others exposed to the smoke formulation or its combustion products. Likewise, no toxicity studies have been conducted using the smoke formulation or its combustion products in experimental animals. Any assessment of the potential health effects following inhalation exposure to these combustion products must be made from studies conducted with either the pure individual dye components or mixtures of the dyes.

A minimal number of studies have been conducted with mixtures of solvent red 1 and disperse red 11 (Table 3-1). Although the studies do not mimic the atmosphere to which a soldier might be exposed during training exercises using the deployed smokes, they can provide some indication of the potential synergistic effects between the two dyes. Acute toxicity studies demonstrated low toxicity after either oral or dermal exposure to the dye mixture (Table 3-1). Acute studies of either eye or dermal irritation showed the red-dye mixture to be nonirritating when applied to the skin but irritating in ocular studies (Smith et al. 1986). Hypersensitivity studies with the red-dye mixture showed no pulmonary or contact hypersensitivity in mice (Sailstad et al. 1994). One mutagenicity study conducted in a bacterial assay system (Ames test) with the red-dye mixture was negative (Brooks et al. 1989).

Inhalation studies conducted after either acute or repeated exposures to the aerosolized red-dye mixture showed nasal and lung lesions (Lundy and Eaton 1994). Only minimal details of the results of those studies have been reported (Table 3-1); therefore, it is not possible to evaluate the study adequately.

Toxicity of Component Dyes

A summary of the toxicity and mutagenicity studies conducted with the two dye components of the new red-smoke formulation, disperse red 11 and solvent red 1, can be found in Appendixes G and E, respectively. In general, these dyes appear to have low acute toxicity, a mild irritation potential, and low mutagenicity. Solvent red 1 was found to produce

TABLE 3-1 Summary of Toxicity and Mutagenicity Studies Conducted with Solvent Red 1 and Disperse Red 11 Mixtures

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Acute toxicity	Rat, Fischer 344, M, F	Oral, acute, 5 g/kg; solvent red 1 to disperse red 11, 33.4:6.6 (Lot 1)	1/10 died at 14 d, LD_{50} > 5 g/kg	Smith et al. 1986
Acute toxicity	Rabbit, New Zealand White, M, F	Dermal, acute, 2 g/kg, 24 hr; solvent red 1 to disperse red 11, 33.4:6.6 (Lot 1)	0/10 died at 14 d, LD_{50} > 2 g/kg	Smith et al. 1986
Acute toxicity	Rat, Fischer 344, M, F	Inhalation, 0.1, 0.3, 1.0 g/m^3, 6 hr; mixture not specified	Nasal and lung lesions	Lundy and Eaton 1994
Subchronic toxicity	Rat, Fischer 344, M, F	Inhalation, 0.03, 0.1, 0.3 g/m^3, 6 hr/d, 5 d/wk, 13 wk; mixture not specified	Restrictive lung disorder; body-weight decrease	Lundy and Eaton 1994
Eye irritation	Rabbit, New Zealand White, M, F	0.1 g per eye; solvent red 1 to disperse red 11, 33.4:6.6 (Lot 1)	3/3 positive; redness, chemosis, discharge, iritis; no irritation 2/3 by d 7; opacity in 1/3 on d 21	Smith et al. 1986
Dermal irritation	Rabbit, New Zealand White, M, F	0.5 g/kg, clipped skin, 24 hr; solvent red 1 to disperse red 11, 33.4:6.6 (Lot 1)	Nonirritating	Smith et al. 1986
Pulmonary hypersensitivity	Mice, Balb/c, F	0.3 g/m^3, 6 h/d, 5 d/wk, 3+ wk iv challenge; solvent red 1 to disperse red 11, 87.3:8.0	Negative	Sailstad et al. 1994
Contact hypersensitivity	Mice, Balb/c, F	Local lymph-node assay: 0.0001 g/d both ears, 3 d; solvent red to disperse red 11, 87.3:8.0	Negative	Sailstad et al. 1994
Mutagenicity	*Salmonella typhimurium* (strains TA1535, TA1538, TA98, TA100) +/- S9	0-200 μg per plate; disperse red 11 to solvent red 1 to terephthalic acid, 5:25:16	Negative	Brooks et al. 1989

Abbreviations: M, male; F, female.

contact hypersensitivity in experimental animals (Sailstad et al. 1994). Disperse red 11 was negative in the same study. The sparse toxicology data base for these two dyes prevents adequate assessment of potential toxicity. Especially lacking are long-term comprehensive studies of these chemicals after inhalation exposure in which multiple end points have been examined. Additionally, conflicting results are often obtained in acute toxicity studies of these dyes. The basis for the differences in experimental results obtained by various investigators is not known. One potential explanation might be variability in the nature and extent of impurities or contaminants in the individual dye lots. The impurities, rather than the dyes, might be responsible for observed toxicity and mutagenicity.

OVERALL EVALUATION OF TOXICITY

There are no well-controlled inhalation toxicity studies on the combustion products of the new yellow-, green-, and red-smoke formulations that could provide a basis for the subcommittee to assess the potential health effects of exposure to these combustion products by military personnel or to recommend guidance levels.

The subcommittee is concerned about the toxicity of several of the dye components. For new yellow-and green-smoke formulations, the major concern is the demonstrated contact allergic dermatitis in some humans exposed to solvent yellow 33. Thus, even masking will not protect against the dermal toxicity of solvent yellow 33. Although studies have been conducted on mixtures of the new yellow, green, and red dye components, no experimental studies have been conducted on the toxicity of the combustion products of the smoke-formulations. Therefore, toxicity of the combustion products cannot be evaluated directly. For all three smoke formulations, evaluation would be limited to extrapolation from studies conducted with either the individual dye components or dye mixtures. Because of minimal data base on the toxicity of the dyes as individual components or as mixtures, it is not prudent to extrapolate results from these studies to predict the potential toxicity of the combustion products of the new smoke formulations.

PREVIOUS RECOMMENDED EXPOSURE LIMITS

There are no previous recommended exposure limits for the combustion products of the new yellow, green, and red-smoke formulations. Army

Health Hazard Assessment Reports (AEHA 1992, 1993a,b) for the M18 smoke grenade and 40-mm cartridge components recommend masking when a smoke haze exceeds 4 hr or when passing through or operating in a smoke fog.

SUBCOMMITTEE RECOMMENDATIONS

The data base is inadequate for assessing the potential toxicity of combustion products of the new yellow-, green-, and red-smoke formulations to make recommendations for exposure guidance levels. The subcommittee recommends that acute inhalation studies be conducted in experimental animals to test the toxicity of the new yellow, green, and red smokes. Acute toxicity studies would be most appropriate for recommending the emergency exposure guidance level and the short-term public emergency guidance level. Such exposures might occur during training exercises in which military personnel might be exposed for several minutes, twice per day, two to four times per year. Some military personnel, particularly instructors involved in training exercises, might be repeatedly exposed to the smokes over several years, as might a community living near a military-training facility. For those exposure scenarios, the repeated exposure guidance level and the repeated public exposure guidance level are the most appropriate guidance levels, and studies assessing the potential toxicity of the smokes following repeated exposure would provide the most appropriate data for setting those exposure guidance levels. Thus, the Army should also consider conducting subchronic inhalation studies in experimental animals to test the toxicity of the smokes under conditions of repeated exposure. Both acute and repeated inhalation studies should be carried out using combusted smokes, and the particle size and combustion products should be representative of the smokes used by the Army. Toxicity testing of other smoke formulations or of individual dyes might not provide results similar to those obtained with the combusted smokes.

Additionally, concerns for potential sensitization resulting from exposure to solvent yellow 33, a component of the new yellow-and green-smoke formulations, and solvent red 1, a component of the new red-smoke formulation, suggest that studies to assess contact allergic dermatitis and respiratory-tract hypersensitivity should be conducted in animal models appropriate for testing hypersensitivity. Those studies should also be conducted using the combusted smokes. To ensure that such

studies are designed correctly, the Army should consult with an expert panel before conducting them.

At this time, the subcommittee recommends that Army policy regarding respiratory and dermal protection from these smokes be followed. It also recommends that the colored smokes be used for signaling and not for obscuring. The subcommittee recommends that the Army avoid exposing the general public to the combustion products.

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Appendix A

Benzanthrone

BACKGROUND

ALTERNATIVE NAMES for benzanthrone (BZA) include 7H-benz(de)-anthracen-7-one, 1,9-benzanthrone, benzanthrenone, and mesobenzanthrone. BZA is a component of the old yellow-and green-dye mixtures.

TOXICOKINETICS

In rabbits administered BZA intraperitoneally at 0.2 grams per kilogram (g/kg) of body weight, 26% to 30% of the dose was excreted unchanged in the urine over a 5-day period (Pandya et al. 1976).

TOXICITY SUMMARY

Effects in Humans

BZA is reported to cause an itching and burning sensation, erythema, dermatitis, and skin pigmentation (Uebelin and Buess 1951; Singh and Zaidi 1969; Trivedi and Niyogi 1968; Schwartz et al. 1957; Schwartz 1939; Horakova and Merhaut 1966, as cited in Dacre et al. 1979). In sensitive individuals, actinic dermatitis or leukoderma can develop because of a photodynamic effect (Singh and Zaidi 1969; Trivedi and

Niyogi 1968; Schwartz et al. 1957; Schwartz 1939; Horakova and Merhaut 1966; Isaev et al. 1957; Hueper 1942, as cited in Dacre et al. 1979). Itching, precocious generalized eczema, pigmentation, and photosensitization have been observed in workers exposed to BZA (Horakova and Merhaut 1966). Systemic effects result from liver damage (Slutskii 1958), nervous-system damage (Piskunova et al. 1956), and disturbance of the autonomic-nervous-system regulatory function (Slutskii 1958). More recent studies indicate that BZA, upon exposure to light, can generate active oxygen species that might be responsible for the photocontact dermatitis caused by BZA in industrial workers exposed to this chemical (Dabestani et al. 1992). Because of the toxic nature of BZA, the U.S. Army Environmental Health Agency advised substituting BZA with a less toxic chemical in smoke mixtures (AEHA 1970).

Effects in Animals

ONE-TIME EXPOSURE

The intraperitoneal (i.p.) lethal dose for 50% of the test animals (LD_{50}) is 1.5 g/kg in rats and 0.29 g/kg in mice exposed to BZA (Lundy and Eaton 1994). BZA did not cause clinical signs of toxicity in rats given oral doses of up to 7.1 g/kg (Payne 1976). The dye was not irritating to the skin of the albino rabbit (Payne 1976) or the clipped, intact, or abraded skin of the guinea pig (Parent 1964; Weeks and Yevich 1963). There were no indications of photoallergy in guinea pigs or phototoxicity in mice or swine (Payne 1976).

White male rabbits injected i.p. at 0.2 g/kg of body weight and guinea pigs injected i.p. at 0.03 g/kg of body weight caused an inflammatory process in the urinary bladder (Pandya et al. 1976; Singh and Tripathi 1973). Intratracheal instillation of BZA (particle size, less than 5 micrograms (μg)) in guinea pigs caused a hemorrhagic edema (Singh 1971).

REPEATED EXPOSURE

Daily i.p. doses of BZA administered to rats at 0.05 g/kg of body weight led to a normocytic anemia due to hemolysis in 10-20 days (Chandra and Singh 1968). There was a significant increase in plasma fibrinogen and

a decrease in blood coagulation time (Mehrotra et al. 1975). BZA injected biweekly i.p. in rats at 0.03 g/kg of body weight for 6 months led to damage of the gametogenic function of rat testis (Singh and Khanna 1976).

CARCINOGENICITY AND MUTAGENICITY

Initial studies do not indicate that BZA is carcinogenic in mice (Parent 1964). Tests for mutagenicity in the dominant lethal mouse assay were negative (Epstein et al. 1972). BZA was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* strains in some reports (Brown and Brown 1976; Gibson et al. 1978; Bond and Gilleland 1955), but Epler (1979) reported BZA to be mutagenic in the *Salmonella* assay.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

The dermal toxicity of BZA, as reported in workers exposed to the dye, precludes any use of the dye in colored smokes unless the exposed personnel make use of effective respiratory and dermal protection.

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Appendix B

Vat Yellow 4

BACKGROUND

AN ALTERNATIVE name for vat yellow 4 is dibenzochrysenedione (DBC). Vat yellow 4 is a component of the old yellow-and green-dye mixtures.

TOXICOKINETICS

No studies have been conducted on the toxicokinetics of vat yellow 4.

TOXICITY SUMMARY

Effects in Humans

There have been no reports of humans exposed either accidentally or in controlled laboratory environments to vat yellow 4.

Effects in Animals

ONE-TIME EXPOSURE

A commercial report (Charm 1976, as cited in Dacre et al. 1979) describing acute oral and dermal toxicity tests with a vat yellow 4 paste formu

lation (unspecified concentration) indicated low acute oral and dermal toxicity in rats and rabbits. The oral lethal dose for 50% of the test animals (LD_{50}) for this paste in rats was greater than 46 grams per kilogram (g/kg) of body weight. The minimal oral LD_{50} in rabbits was 11.6 g/kg of body weight. Application of the paste to intact and abraded skin of rabbits produced no grossly discernible skin damage, and the acute dermal LD_{50} for rabbits was greater than 4.6 g/kg of body weight. Instillation of the paste into the eyes of rabbits produced minimal reversible ocular irritation.

CARCINOGENICITY AND MUTAGENICITY

Subcutaneous injection or painting of the skin of mice with vat yellow 4 for prolonged periods did not produce tumors (Kleinenberg 1939, as cited in Dacre et al. 1979). Another report of similar studies in mice indicated high mortality but no tumors in the mice (Shubik and Hartwell 1957, as cited in Dacre et al. 1979). Concentrations of vat yellow 4 used in the study were not reported by Dacre et al. (1979).

Epler (1979) reported that vat yellow 4 was positive in the Ames mutagenicity assay. However, Zeiger et al. (1987) and Sigman et al. (1985) report vat yellow 4 to be negative in the same assay. Harrington-Brock et al. (1991) report vat yellow 4 to be positive only with activation in the thymidine kinase locus and micronuclei assays in mouse lymphoma cells.

A standard cancer bioassay was conducted by the National Cancer Institute (NCI 1979) on a commercial product reported by the manufacturer to contain 18.2% vat yellow 4, 30.8% sorbitol, 5.5% dispersant (Lomar TWC), 2.7% glycerin, and 42.8% water. The test was negative for male and female rats administered vat yellow 4 at 3,500 or 7,000 parts per million (ppm) in the diet and female mice administered 12,500 or 25,000 ppm in diet but caused an increased incidence of lymphomas in male mice fed 50,000 ppm. Because the study was done on the mixture, the contribution of vat yellow 4 to the carcinogenicity is uncertain (Ashby and Tennant 1988).

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

The carcinogenic potential of vat yellow 4 is uncertain, and animal studies indicate a low toxicity via oral or dermal routes of exposure. The

toxicity of inhaled vat yellow 4 is unknown and should be investigated if the compound is to be used in colored smokes.

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Appendix C

Solvent Yellow 33

BACKGROUND

SOLVENT YELLOW 33 is also known as 2-(2-quinolyl)-1,3-indandione (QID), D&C yellow no. 11, quinazoline yellow spirit soluble, and yellow no. 204. This compound is a component of the new yellow-and green-dye mixtures.

TOXICOKINETICS

The disposition and metabolism of ^{14}C-QID were studied in male Fischer 344 (F344) rats (Medinsky et al. 1986). Rats were exposed to solvent yellow 33 by inhalation at 0.04 grams per cubic meter (g/m^3) for 6 hr. The activity median aerodynamic diameter of the particles was 3.4 micrometers (μm) with a geometric standard deviation of 1.7. At the end of exposure, greater than 90% of the radioactivity remaining in the lungs was unmetabolized solvent yellow 33. Solvent yellow 33 was rapidly cleared from the respiratory tract during and after exposure, the half-time being approximately 2 to 3 hr. Once absorbed into the blood, solvent yellow 33 was rapidly metabolized. High-pressure liquid-chromatography analysis of tissue extracts indicated that 40% to 75% of the radioactivity in liver and kidney consisted of solvent yellow 33 metabolites. The major pathway for excretion of solvent yellow 33 and solvent yellow 33 metabolites was feces, accounting for 74% of the initially

deposited material. The half-time for elimination in feces was 14 hr. Radioactivity eliminated in urine accounted for 14% of the initially deposited material, the half-time for elimination being 10 hr. Over 90% of the ^{14}C excreted in urine was in the form of solvent yellow metabolites. Very little solvent yellow was metabolized to radiolabeled carbon dioxide and exhaled. Only 10% of the initial amount of solvent yellow deposited remained in the body 72 hr after exposure.

The disposition of solvent yellow 33 was also investigated in male F344 rats administered solvent yellow 33 intravenously or by feeding (El Dareer et al. 1988). Rats were fed food dosed with solvent yellow 33 for 7 days, followed by exposure to ^{14}C-QID in the food on day 8. Rats were returned to feed containing unlabeled solvent yellow 33 on days 9 through 11. As calculated from feed consumption, the dose administered over the entire feeding period was 4.1 g per kilogram (kg) of body weight (0.144 microcuries per kilogram (μCi/kg) of body weight), 0.4 g/kg of body weight (0.146 μCi/kg of body weight), 0.04 g/kg of body weight (0.143 μCi/kg of body weight), or 0.004 g/kg of body weight (0.045 μCi/kg of body weight) for the 0.14%, 0.038%, 0.0037%, or 0.00044% diets, respectively. Urine and feces were collected daily on days 9 through 12. From 89% to 94% of the radiolabel was recovered in the feces of rats given solvent yellow 33 in their food; from 5% to 6% of the radioactivity was recovered in the urine. On day 12, the rats were euthanized and tissues were taken for analysis. Only trace amounts of radioactivity were present in the tissues 72 hr after dosing with radioactive solvent yellow 33.

In a separate study, rats were given ^{14}C-QID at 0.001 g/kg of body weight (0.116 μCi/kg of body weight) by tail-vein injection. In some studies, bile was collected from intravenously dosed rats fitted with billary cannulae. Fecal excretion accounted for 89% of the administered radioactivity, and urinary excretion represented 17% 72 hr after exposure. In rats with billary cannulae, 54% of the administered radioactivity appeared in the bile within 4 hr. Analysis of the bile revealed no intact ^{14}C-QID, but more than 10 metabolites were detected.

In summary, the results of pharmacokinetic studies of solvent yellow 33 administered to animals by inhalation, by injection, or in feed suggest that, after inhalation by humans, solvent yellow 33 will be rapidly absorbed from the respiratory tract, and extensively metabolized, with the metabolites excreted in the bile and eliminated in the feces. No tissues appear to be storage depots for significant quantities of solvent yellow 33.

TOXICITY SUMMARY

Effects in Humans

DERMAL EXPOSURES

There are numerous reports of contact dermatitis in humans exposed to solvent yellow 33. Some of the most recent case reports and clinical studies are presented in Table C-1. Solvent yellow 33 is used in soaps, shampoos, and other externally applied products. Case reports of allergic contact dermatitis due to exposure to solvent yellow 33 in cosmetics, such as eyeliner, rouge, and lipstick have been published (reviewed by Feinman and Doyle 1988). Weaver (1983a) reported that a soap containing solvent yellow 33 at 60 parts per million (ppm) elicited dermatitis in two consumers. Monk (1987) reported allergic contact dermatitis from exposure to solvent yellow 33 found in a hair cream. Reactivity was confirmed using a patch test. There is one report of a worker diagnosed with occupational allergic contact dermatitis (Noster and Hausen 1978). The individual was employed in a factory that manufactured colored smokes for use in detonators.

Controlled laboratory studies with human volunteers confirm the sensitizing potential of solvent yellow 33. Jordan (1981) describes contact dermatitis due to solvent yellow 33 in 11 of 149 volunteers who developed a sensitization reaction when tested with solvent yellow 33 concentrations of 16.4 ppm in a modified Draize test. Weaver (1983b) demonstrated sensitization in a repeated-insult patch test of Solvent yellow 33 at concentrations as low as 10 ppm but not at 5 ppm. Rapaport (1984) induced sensitization in 14 of 56 healthy volunteers using a solution containing 20% solvent yellow 33. Bjorkner and Magnusson (1981) and Bjorkner and Niklasson (1983) report sensitization in 4 of 88 normal volunteers exposed to 1% solvent yellow 33 in propylene glycol and in one patient exposed to 0.00001% of the dye. Kita et al. (1984) demonstrated sensitization to solvent yellow 33 in 15 of 20 subjects exposed to 0.5% solvent yellow 33 in petrolatum. All 15 allergic subjects reacted to challenge with solutions containing 1,000 ppm, and one reacted to challenges down to 1 ppm. Kita and colleagues concluded that solvent yellow 33 is a potent contact sensitizer because 15 of 20 subjects became sensitized using the maximization test, and almost half of those individuals reacted to challenge with 100 ppm. The authors also investigated the cross-reactivity to purified samples of D&C yellow

TABLE C-1 Summary of Human Dermal Sensitization Associated with Solvent Yellow 33

Study Population	Exposure Conditions	Response and Comments	Reference
Case report, 38-year-old male	Manufacture of smoke detonators	Contact dermatitis	Noster and Hausen 1978
Clinical study, 56 volunteers	Repeated insult patch test; 5 exposures to 20% solvent yellow 33 in petrolatum, 2 challenges	Sensitization in 15/56 (27%)	Rappaport 1984
Clinical study, 149 volunteers	Modified Draize test	11/149 (7.4%) sensitized at 16.4 ppm	Jordan 1981
Clinical study, 88 volunteers	1% solvent yellow 33 in propylene glycol	4/88 (4.5%) positive patch response at 72 hr	Bjorkner and Magnusson 1981
Case reports, 50-yr-old female, 61-yr-old male	Soap, 0.006% solvent yellow 33	Dermatitis, sensitization, vesicular dermatoses	Weaver 1983a
Clinical study, 2 patients	Repeated-insult patch test; 5, 10, and 20 ppm	Sensitization at 10 and 20 ppm; no sensitization at 5 ppm	Weaver 1983b
Clinical study, 1 patient	Patch test on upper back, various concentrations	Positive patch test to 0.00001%	Bjorkner and Niklasson 1983
Clinical study, 20 volunteers, 18-34 years old	Maximization test, 5 exposures to 0.5% solvent yellow 33 in petrolatum; 1 challenge with 1,000 to 0.1 ppm	15/20 (75%) contact sensitization	Kita et al. 1984
Case report, 50-yr-old male	Hair cream; patch test, 1% solvent yellow 33 in petrolatum	Dermatitis of scalp; sensitivity to solvent yellow 33 confirmed with patch test	Monk 1987

no. 10. Solutions containing 5% of that dye failed to sensitize human subjects. D&C yellow no. 10 is the sodium salt of solvent yellow 33 and is soluble and ionized in aqueous solutions. Weaver (1983b) attributes the differences in the sensitization potential of the two dyes to the ability of solvent yellow 33 to penetrate the skin more readily.

Effects in Animals

As outlined in Table C-2, numerous toxicity studies have been conducted on solvent yellow 33, most likely precipitated by its use in consumer products. The results of those investigations indicate that solvent yellow 33 is not acutely toxic. The studies of most relevance to the military for its intended use of solvent yellow 3 are reviewed in detail below.

INHALATION EXPOSURES

In a repeated exposure study (Henderson et al. 1985), three male and three female F344 rats per group were exposed by inhalation to concentrations of solvent yellow 33 at 1.0 g/m^3 (1 hr per day), 1.0 g/m^3 (6 hr per day), and 12.1 g/m^3 (6 hr per day) for 5 days. There were no overt signs of toxicity after any of the exposure regimens. Histological examination of the respiratory tract from rats exposed to the aerosol for 5 days showed globlet-cell hypertrophy and hyperplasia in the respiratory epithelium of the nasal cavity. There was mild inflammation of the respiratory epithelium and degeneration in the olfactory region. In the lung parenchyma, there were a few focal accumulations of alveolar macrophages centered on terminal airways.

In 4-week studies (Henderson et al. 1984), rats were exposed to solvent yellow 33 for 6 hr per day, 5 days per week. Concentrations of solvent yellow 33 were 0.01, 0.05, and 0.2 g/m^3 with a mass median aerodynamic diameter of 3.1 to 4.1 μm and a geometric standard deviation of 2.0. Animals exposed to the highest solvent yellow 33 concentration had reduced body-weight gain (8% less than controls). Mild respiratory-function changes consisting of reduced elastic recoil, increased resting lung volumes, and reduced respiratory flow rates were found at the highest concentration. Histological lesions were not observed.

In a subchronic inhalation study (Sun et al. 1987), rats were exposed 6 hr per day, 5 days per week for 13 weeks to concentrations of solvent

TABLE C-2 Summary of Toxicity Studies Conducted with Solvent Yellow 33

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Acute toxicity	Rat, Sprague-Dawley, M, F	Oral, acute, 5 g/kg	0/10 died at 14 d, LD_{50} > 5 g/kg	Manthei et al. 1983
Acute toxicity	Rat, Albino, M	Oral, acute, 0.3-10.0 g/kg	0/5 died at 7 d	Krebs 1980
Acute toxicity	Dog, M, F	Oral, acute, 0.3-10.0 g/kg	0/2 died at 7 d	Krebs 1980
Acute toxicity	Rabbit, New Zealand White, M or F	Dermal, acute, 2 g/kg for 24 hr	0/10 died at 14 d, LD_{50} > 2 g/kg	Manthei et al. 1983
Repeated toxicity	Rat, Fischer 344, M, F	Inhalation, acute, 1.0 g/m^3 (1 hr), 1.0 g/m^3 (6 hr), 12.1 g/m^3 (6 hr) for 5 d	0/6 died at 5 d; nasal lesions	Henderson et al.1985
Eye irritation	Rabbit, New Zealand White, M or F	0.1 g/eye	0/6 responded at 24, 48, 72 hr, 7 d; negative	Manthei et al. 1983
Dermal irritation	Rabbit, New Zealand White, M or F	Clipped skin, 0.05 g/kg for 24 hr	Primary irritation score 0.085; very mild irritant	Manthei et al. 1983
Sensitization	Guinea pig, Hartley, M	Injected 0.001 g/d for 22 days	Negative	Manthei et al. 1983
Sensitization	Guniea pig, Hartley, F	Induction: Injected 5, 25, 50 μg; challenge: injected 5, 25, 50 μg	Delayed-type hypersensitivity: strong (50 μg), moderate (25 μg), weak (10 μg); cellular inflammatory response	Palazzolo and DiPasquale 1983
Subchronic toxicity	Rat	Inhalation, 0.01, 0.05, 0.2 g/m^3, 6 hr/d, 5 d for 4 wk	At highest concentration, reduced body weight and respiratory effects; no histological lesions	Henderson et al. 1984
Sensitization	Guinea pig	Dermal application of 1%, 3%, 10% suspension in alcohol	Sensitizer (10% dose group)	Lamson et al. 1982

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Subchronic toxicity	Rat, Albino, M	0.1-3.0% in feed, daily for 13 wk	No gross findings; growth depression at all doses; pigment accumulation in liver and kidney	Krebs 1980
Subchronic toxicity	Rat, F344/N, M, F	500-50,000 ppm in feed, daily for 13 wk	No mortality, reduced body weights in 17,000- and 50,000-ppm groups; hepatocyte degeneration at 1,700 ppm and higher, and hyaline droplets in renal cells	Eastin et al. 1996
Subchronic toxicity	Mouse, $B6C3F_1$, M, F	500-50,000 ppm in feed, daily for 13 wk	No mortality; hepatocyte degeneration at 5,000 ppm and higher	Eastin et al. 1996
Subchronic toxicity	Rat, Fischer 344, M, F	Inhalation, 0.01, 0.05, 0.2 g/m^3, 6 hr/d, 5 d/wk for 4 wk	Mild respiratory-function changes at 0.2 g/m^3; no histopathological lesions	Sun et al. 1987
Subchronic toxicity	Rat, Fischer 344, M, F	Inhalation, 0.001, 0.01, 0.1 g/m^3, 6 hr/d, 5 d/wk for 13 wk	Foamy macrophages in lungs at 0.1 g/m^3; NOAEL = 0.01 g/m^3	Sun et al. 1987
Subchronic toxicity	Rabbits, sex/strain not specified	0.1% and 1.0% dermal application to abdominal skin; 5 d/wk, 15 or 64 total applications (total dose not specified)	Negative for skin irritation, negative hematology and urinalysis, pigmentation in liver and kidneys	Krebs 1980
Chronic toxicity	Rat, Albino, M, F	0.03-1.0% in feed, daily for 1 yr	Suppression of growth rate; extensive pigment deposition in liver and kidneys	Krebs 1980
Chronic toxicity	Dogs, M, F	Oral, 0.05 and 0.3 g/kg/d in feed, 0.03%, for 1 yr	Moderate anemia; deposition of pigment in liver and kidney; bile-duct proliferation, moderate thyroiditis	Krebs 1980
Reproductive and developmental toxicity	Rat, F344/N, F	5,000, 17,000, or 50,000 ppm in feed, from 4 wk before breeding until 4 wk after birth	No effect on fertility, gestation length, litter size, pup birth weights	Eastin et al. 1996

Abbreviations: M, male; F, female; NOAEL, no-observed-adverse-effect level.

yellow 33 aerosols of 0, 0.001, 0.01, and 0.1 g/m^3 with particle diameters of 2.1 to 4.2 μm and a geometric standard deviation of 2.0. Animals exposed to solvent yellow 33 at 0.1 g/m^3 had only a slight decrease in body-weight gain (4% less than controls) and an accumulation of foamy macrophages in the lungs. Exposure to the lower concentrations of solvent yellow 33 elicited no observed response. The no-observed-adverse-effect level (NOAEL) for these inhalation studies was 0.01 g/m^3.

DERMAL EXPOSURES

Solvent yellow 33 was tested for dermal-sensitization potential in guinea pigs by Palazzolo and DiPasquale (1983). The induction regimen consisted of a single foot-pad injection of complete Freund's adjuvant diluted with antigen followed by an interdermal injection of antigen. This regimen produced delayed-type hypersensitivity. A high sensitization frequency was observed with both 50 μ*g* of solvent yellow 33 and a positive control, dinitrochlorobenzene. The severity of reactions to solvent yellow 33 and the positive control were comparable. A dose-response profile was obtained using solvent yellow 33 at concentrations of 50, 250, and 500 micrograms per milliliter (μg/mL) and a 0.1-mL injection volume, resulting in 5, 25 and 50 μ*g* per injection. Histological evaluation of selected skin sites demonstrated a cellular inflammatory response consistent with delayed-type hypersensitivity. On the basis of the frequency and severity of the reaction scores, the authors concluded that solvent yellow 33 could be considered a fairly potent sensitizer, with a 50-μg dose considered a strong sensitizer.

Lamson et al. (1982) investigated the sensitizing potential of solvent yellow 33 by using 1%, 3%, or 10% suspensions of the dye in alcohol for topical application. Only the 10% suspension produced a significant sensitization frequency when compared with a 95%-ethanol vehicle control.

ORAL EXPOSURES

Solvent yellow 33 is often a contaminant of dye preparations of Solvent yellow no. 10. Because solvent yellow 10 has been approved for use in food, toxicity evaluations of solvent yellow 33 administered in feed have been conducted. Solvent yellow 33 (approximately 99% pure) was

administered in feed at dietary concentrations of 1,700, 17,000, and 50,000 ppm to groups of male and female F344/N rats and $B6C3F_1$ mice for 14 days or 13 weeks (Eastin et al. 1996). The toxicity results were similar for rats and mice. Solvent yellow 33 caused no deaths but reduced body-weight gain slightly in rats exposed to 17,000 or 50,000 ppm. Liver weights were increased in all exposed rats and mice. There was minimum-to-mild degeneration of the periportal portion of the liver lobules in rats fed 1,700 ppm and higher and in mice fed 5,000 ppm and higher. There was an increase in the number and size of hyaline droplets in all dosed groups of male rats.

In a study to determine the reproductive and developmental toxicity of solvent yellow 33, female rats were administered diets containing solvent yellow 33 at 5,000-50,000 ppm from 4 weeks before mating to unexposed males until 4 weeks after birth of the pups (Eastin et al. 1996). The body weights of the dams were similar to those of controls at the time of mating but were lower at parturition and weaning. Fertility, gestation days, litter size, and pup birth weights were unaffected by exposure. At weaning, body weights of pups from all dose groups (5,000, 17,000, and 50,000 ppm) were lower than weights of pups from controls. After 4 weeks of exposure to solvent yellow 33 through milk and through feed containing the same dietary concentration that the dams received, weights of the pups in the 5,000-ppm group were similar to those of controls, but the weights of the pups in the 17,000- and 50,000-ppm groups remained depressed. Microscopic evaluation showed lesions in pups of all dose groups. These lesions were similar to those described in the liver and kidney of rats in the 14-day and 13-week studies.

In summary, the results of both dietary studies with solvent yellow 33 indicate that compound-related effects occurred at all concentrations, and a no-effect level was not observed.

MUTAGENICITY STUDIES

Solvent yellow 33 has been tested for mutagenicity in a variety of assays including mouse micronucleus, L5178-Y mouse lymphoma, *Salmonella* reversion, and sister chromatid exchange using Chinese hamster ovary cells and the C57B1/6 mouse. Table C-3 summarizes studies testing solvent yellow 33 for mutagenicity.

When tested for mutagenicity in the mouse micronucleus assay and the

TABLE C-3 Summary of Mutagenicity Studies Conducted with Solvent Yellow 33

Experimental System	Exposure Conditions	End Points and Comments	Reference
Mouse, Swiss albino, male	i.p. injections, 0.001, 0.01, 0.1 g/kg, at 30 hr and 6 hr before euthanasia	No increase in micronucleated-femoral polychromatic erythrocytes	Manthei et al. 1983
Sister chromatid exchange in Chinese hamster ovary cells	0-0.04 g/mL, 3 hr	Not mutagenic or clastogenic, cytotoxic, increased sister-chromatid-exchange frequency	Brooks et al. 1989
Sister chromatid exchange in C57BI/6J mice	0.005-0.04 g/kg, i.p., 1 injection given 25-29 hr before euthanasia	Not mutagenic, no increase in the number of sister chromatid exchanges per cell	Moore et al. 1988
Salmonella typhimurium (strains TA1535, TA1537, TA 1538, TA98, TA100) ± S9	0.1-1000 µg/plate	Not mutagenic	Manthei et al. 1983
Salmonella typhimurium (strains TA 1535, TA1537, TA1538, TA98, TA100, TA102, TA104) ± S9	0-300 µg/plate	Weakly positive in TA100 +S9 and TA104 ± S9; positive in TA102 ± S9	Moore et al. 1988
Salmonella typhimurium (strains TA1535, TA1538, TA98, TA100) ± S9	0-800 µg/plate	Not mutagenic	Brooks et al. 1989
Mouse lymphoma cells	0-50 µg/mL	Positive with (`150-500 mutants/$10^6$ cells) and without (`400-1,000 mutants/10^6 cells) S9 activation	Moore et al. 1988

Abbreviations: i.p., intraperitoneal.

sister-chromatid-exchange assays, solvent yellow 33 gave negative results (Manthei et al. 1983; Moore et al. 1988; Brooks et al. 1989). Both positive and negative results have been reported in *Salmonella* reversion assays testing solvent yellow 33 for mutagenicity. The dye was nonmutagenic when assayed in *Salmonella* strains TA1535, TA1537, TA1538, and TA98 with and without metabolic activation (Manthei et al. 1983; Moore et al. 1988; Brooks et al. 1989). In one case, testing in identical strains gave conflicting results. When tested in strain TA100, solvent yellow 33 was reported to be nonmutagenic in studies by Manthei et al. (1983) and Brooks et al. (1989) but was found to be weakly mutagenic (with metabolic activation only) in a study by Moore et al. (1988). Moore et al. (1988) also reported that solvent yellow 33 gave positive results when tested in strains TA102 and TA104 with and without metabolic activation. However, because the rate of reversion in strain TA104 was less than double that in controls, the subcommittee concluded that the results were only weakly positive in this strain. Solvent yellow 33 produced positive results in the mouse lymphoma assay with and without metabolic activation (Moore et al. 1988).

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

Solvent yellow 33 is a contact allergen in some humans. Thus, even respiratory protection will not be completely effective in protecting sensitive military personnel. The respiratory sensitizing potential of solvent yellow 33 has not been adequately investigated in animals or humans. Additionally, studies testing solvent yellow 33 for mutagenicity are inconclusive.

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Appendix D

Solvent Green 3

BACKGROUND

ALTERNATIVE NAMES for solvent green 3 include 1,4-di-*p*-toluidino-9,10-anthraquinone (PTA), 1,4-bis((4-methylphenyl)amino)-9,10-anthracenedione, 1,4-di*p*-tolylamino-anthraquinone, quinizarine green G base, and D&C green no. 6. Solvent green 3 is a component of the new and old green-dye mixtures.

TOXICOKINETICS

When inhaled, solvent green 3 clears very slowly from the lung (half live, 280 days) (Sun et al. 1987). In a study on the retention of solvent green 3 in the lungs, rats were given a suspension containing the dye (Henderson et al. 1988). The authors observed that 87% of the initial dose remained in the lungs 24 hr after exposure.

TOXICITY SUMMARY

Effects in Humans

There have been no reports of humans exposed either accidentally or in controlled laboratory environments to solvent green 3.

Effects in Animals

ONE-TIME EXPOSURE

The oral lethal dose for 50% of the test animals (LD_{50}) for rats has been reported at 3 grams per kilogram (g/kg) of body weight, >10 g/kg of body weight, and >15 g/kg of body weight (reviewed in Dacre et al. 1979). The oral LD_{50} in rabbits is >10 g/kg of body weight.

Solvent green 3 produced no skin irritation when applied to intact or abraded skin of rabbits and produced "minimal" erythema in the eyes of rabbits (Dacre et al. 1979).

REPEATED EXPOSURES

No repeated-exposure studies have been conducted on solvent green 3 alone. Sun et al. (1987) conducted 4-week and 13-week inhalation toxicity studies in rats using a mixture of 70:30 solvent green 3 and solvent yellow 33, the major dye components of the new green smoke. That study is reviewed in detail in Chapter 3.

A repeated inhalation study of a combusted smoke containing a mixture of solvent green 3, solvent yellow 33, and disperse red 9 was conducted by Marrs et al (1984). That study is reviewed in Chapter 2.

CARCINOGENICITY AND MUTAGENICITY

Solvent green 3 is not mutagenic in the *Salmonella typhimurium* strains with or without activation, according to one report (Brown and Brown 1976), but Epler (1979) reported positive results in both the *S. typhimurium* assay and the thymidine kinase locus mouse lymphoma assay.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

The major health concern with the use of solvent green 3 as a component of a colored smoke is that the compound is poorly soluble in the lung and will accumulate with repeated exposures to high concentrations of the material.

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Appendix E

Solvent Red 1

BACKGROUND

ALTERNATIVE NAMES for solvent red 1 include color index sudan red G, oil soluble red G, and methoxybenzenazo-ß-naphthol (MBN). Solvent red 1 is a component of the new red-dye mixture.

TOXICOKINETICS

No studies have been conducted on the toxicokinetics of solvent red 1.

TOXICITY SUMMARY

Effects in Humans

There have been no reports of humans exposed either accidentally or in controlled laboratory environments to solvent red 1.

Effects in Animals

A minimal number of toxicity studies have been conducted with solvent red 1. Those studies are summarized in Table E-1. Acute-toxicity studies of oral exposure in rats or dermal exposure in rabbits determined the

TABLE E-1 Summary of Toxicity Studies Conducted with Solvent Red 1

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Acute toxicity	Rat, Sprague-Dawley, M, F	Oral, acute, 5 g/kg	0/10 died at 14d, LD_{50} >5 g/kg	Manthei et al. 1983
Acute toxicity	Rat, Fischer 344, M, F	Oral, acute, 5 g/kg	0/10 died at 14d, LD_{50} >5 g/kg	Smith et al. 1986
Acute toxicity	Rabbit, New Zealand White, M, F	Dermal, acute, 2 g/kg for 24 hr	0/10 died at 14d, LD_{50} >2 g/kg	Manthei et al. 1983
Acute toxicity	Rabbit, New Zealand White, M, F	Dermal, acute, 2 g/kg for 24 hr	0/10 died at 14d, LD_{50} >2 g/kg	Smith et al. 1986
Eye irritation	Rabbit, New Zealand White, M, F	0.1 g/eye	0/6 responded at 24 hr, 48 hr, 72 hr, 7 d; negative	Manthei et al. 1983
Eye irritation	Rabbit, New Zealand White, M, F	0.1 g/eye	3/3 positive; redness, chemosis, discharge, iritis; no irritation by d 7	Smith et al. 1986
Dermal irritation	Rabbit, New Zealand White, M, F	Clipped skin, 0.5 g/kg for 24 hr	24, 72 hr; 6/6 erythema; primary irritation score 2.08; moderate irritant	Manthei et al. 1983
Dermal irritation	Rabbit, New Zealand White, M, F	Clipped skin, 0.05 g/kg for 24 hr	Nonirritant	Smith et al. 1986
Sensitization	Guinea pig, Hartley, M	Injection 0.0001g/d for 22 d	Negative	Manthei et al. 1983
Contact hypersensitivity	Mice, Balb/c, F	Mouse ear-swelling test: 0.00005 g/d shaved back, 2 d); challenge on d 5	Contact sensitizer	Sailstead et al. 1994
Contact hypersensitivity	Mice, Balb/c, F	Local lymph node assay: 0.0005 g/d both ears, 3 d)	Weak contact sensitizer	Sailstead et al. 1994

Abbreviations: M, male; F, female.

lethal dose for 50% of the test animals (LD_{50}) to be greater than 5 grams per kilogram (g/kg) of body weight after oral administration and greater than 2 g/kg of body weight after dermal administration. Those doses (5 g/kg and 2 g/kg) were the highest doses used (Manthei et al. 1983; Smith et al. 1986).

Results regarding the eye-irritation potential of solvent red 1 are conflicting. Manthei et al. (1983) reported that this chemical did not cause ocular irritation in rabbits; however, Smith et al. (1986) reported positive results in the same species. Conflicting results were also obtained for the dermal-irritation potential of solvent red 1. Manthei et al. (1983) suggested that it was moderately irritating, and Smith et al. (1986) suggested that it was nonirritating. The dose used in the Smith et al. (1986) study was 10 times higher than that in the Manthei et al. (1983) study. Studies using either an eye-swelling test or a local lymphoid assay to evaluate contact hypersensitivity in mice exposed to solvent red 1 showed that this chemical was a contact sensitizer (Sailstad et al. 1994).

Mutagenicity

Mutagenicity tests conducted with solvent red 1 are summarized in Table E-2. Mammalian studies have yielded conflicting results. For example, in vivo mutagenicity studies using Swiss albino mice showed no increase in micronucleated femoral polychromatic erythrocytes after intraperitoneal (i.p.) injections of solvent red 1 at up to 0.1 g/kg of body weight (Manthei et al. 1983). In contrast, in vitro studies using a mouse lymphoma cell line showed increases in thymidine kinase mutants and increases in micronuclei (Harrington-Brock et al. 1991). Experiments conducted in Chinese hamster ovary cells showed that solvent red 1 was cytotoxic and induced an increase in sister-chromatid-exchange frequency but was not mutagenic or clastogenic (Brooks et al. 1989). Studies on the mutagenicity of solvent red 1 in the bacterial system *Salmonella typhimurium* (Ames test) have also yielded conflicting results. Some investigators determined that solvent red 1 was not mutagenic in this system (Manthei et al. 1983; Brooks et al. 1989). Another investigator determined that it was positive in one strain (TA100) when a mammalian bioactivating system was used (Moore et al. 1989). Taken together, these mutagenicity studies suggest that solvent red 1 is a weak mutagen. The possibility that a contaminant in the dye is responsible for the mutagenic effect cannot be dismissed.

TABLE E-2 Summary of Mutagenicity Studies Conducted with Solvent Red 1

Experimental system	Exposure Conditions	End Points and Comments	Reference
Swiss albino male mice	i.p. injections, 0.001, 0.01, 0.1 g/kg, at 30 hr and 6 hr before euthanasia	No increase in micronucleated-femoral polychromatic erythrocytes	Manthei et al. 1983
Mouse lymphoma cells	0-9 g/L, 4 hr	+S9, 100 thymidine kinase mutants per 10^6 survivors; 16 micronuclei per 1,000 cells at 22% survival	Harrington-Brock et al. 1991
Salmonella typhimurium (strains TA1535, TA1537, TA 1538, TA98, TA100) ± S9	0.0001-1 g/plate	Not mutagenic	Manthei et al. 1983
Salmonella typhimurium (strains TA1537, TA1538, TA98, TA100, TA102, TA104) ± S9	0-0.3 g/plate	Positive in TA100 +S9 with mammalian bioactivating system	Moore et al. 1990
Salmonella typhimurium (strains TA1535, TA1538, TA98, TA100) ± S9	0-0.8 g/plate,	Not mutagenic	Brooks, et al. 1989
Chinese hamster ovary cells	0-40 g/L, 3 hr	Cytotoxic, increased sister-chromatid-exchange frequency, not mutagenic or clastogenic	Brooks et al. 1989

Abbreviation: i.p., intraperitoneal.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

Experimental data are insufficient to assess the toxic effects of solvent red 1. The studies on contact hypersensitivity suggest that additional studies in the area might be advisable given the demonstrated dermal-sensitization potential of other dyes.

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Appendix F

Disperse Red 9

BACKGROUND

DISPERSE RED 9 is also called 1-(methylamino)-9,10-anthracenedione (MAA) (after 1972) and 1-*N*-methylamino-9,10-anthraquinone (before 1972). This compound is a component of the old red-and violet-dye mixtures.

TOXICOKINETICS

Oral administration of disperse red 9 to sheep at a concentration of 0.05 grams per kilogram (g/kg) of body weight showed that 40% of the total dose could be accounted for as unmetabolized disperse red 9 or its colored metabolites (Martin et al. 1983). Those colored metabolites were approximately 90% glucuronide conjugates. Greater than 50% of the remaining dose was represented as noncolored metabolites. Sendelbach (1989) concluded that disperse red 9 was essentially nontoxic and rapidly metabolized in mammals.

TOXICITY SUMMARY

Effects in Humans

DERMAL EXPOSURE

Disperse red 9 is reported to be a skin irritant and sensitizer in humans (Dacre et al. 1979; Owens and Ward 1974). Exposure concentrations were not reported.

Effects in Animals

INHALATION EXPOSURE

Disperse red 9 has been assigned a toxicity rating of 1 when inhaled or swallowed (Parent 1964). A rating of 1 is defined as a slightly toxic material whose effects are temporary and disappear upon termination of exposure (Parent 1964).

DERMAL EXPOSURE

Application of disperse red 9 at a concentration of 2 g/kg of body weight on the shaved and abraded backs of rabbits resulted in negligible effects (Martin et al. 1983).

OCULAR EXPOSURE

Martin et al. (1983) exposed rabbits to disperse red 9 at a concentration of 0.05 g per eye. The exposure caused no toxic effects.

ORAL EXPOSURE

Griswold et al. (1968) administered disperse red 9 at a dose of 0.5 g per rat by gastric tube for 10 doses over a 30-day period (total dose, 5 g per

rat) to female Sprague-Dawley rats. The rats were observed for 9 months, and little toxicity was observed. Disperse red 9 administered orally to dogs at up to 8 grams per kilogram (g/kg) of body weight resulted in "minimal toxicity" (Martin et al. 1983). Disperse red 9 has a toxicity rating of 1 which means that it is slightly toxic and its effects are temporary.

MUTAGENICITY AND CARCINOGENICITY STUDIES

Studies have used the Ames assay to test disperse red 9 for mutagenicity. Lundy and Eaton (1994) reported a positive response. Dacre et al. (1979) cited a study that concluded that there was no evidence of mutagenicity. A review of anthraquinone dyes as candidates for nomination to the National Cancer Institute's Chemical Selection Working Group for carcinogenesis bioassay is described by Sigman et al. (1985). Disperse red 9 was considered for study because it gave positive results for mutagenicity in mouse lymphoma cells with and without activation and positive effects in the unscheduled DNA synthesis assay with mouse liver S9. Other mutagenicity tests (i.e., Ames, dominant lethal, mitotic gene conversion) gave negative results.

In a carcinogenicity study by Griswold et al. (1968), one kidney tumor was identified in a female rat 9 months after disperse red 9 was administered at 5 g per rat (total dose) by gavage. The results produced inadequate evidence of carcinogencity.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

The experimental data are insufficient to assess the toxic effects of disperse red 9.

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Appendix G

Disperse Red 11

BACKGROUND

DISPERSE RED 11 is also called 1,4-diamino-2-methoxy-anthraquinone (DMA). Disperse red 11 is a component of the new red-dye mixture.

TOXICOKINETICS

No information is available on the toxicokinetics of disperse red 11 in humans. One study instilled a suspension containing disperse red 11 to determine its retention in the lungs of rats. The study determined that disperse red 11 is rapidly absorbed into the blood with only 66% of the instilled dose remaining at 5 min and 3.5% remaining at 24 hr (Henderson et al. 1988).

TOXICITY SUMMARY

Effects in Humans

One study was conducted with six females with histories of allergic contact dermatitis (Lisboa et al. 1994). The patients were patch tested with a series of 15 disperse dyes used in the textile industry. All six

patients had a reaction to two or more of the disperse dyes; however, none of the patients reacted to disperse red 11.

Effects in Animals

A minimal battery of toxicity studies have been conducted with disperse red 11. Those studies are summarized in Table G-1. Acute-toxicity studies using two dye lots of disperse red 11 exposed female rats by either the oral or dermal routes and established the lethal dose for 50% of the animals (LD_{50}) to be greater than 5 grams per kilogram (g/kg) of body weight (Smith et al. 1986). In contrast, for male rats, the LD_{50} after oral exposure was determined to be between 0.7 and 1.0 g/kg of body weight. Acute lethal doses after dermal exposure in rabbits are greater than 2 g/kg of body weight. Disperse red 11 was found to be negative in an eye irritation study conducted in rabbits (Smith et al. 1986). However, depending upon the dye lot used, disperse red 11 was found to be moderately to mildly irritating in a dermal irritation study (Smith et al. 1986). Mice studies using an ear-swelling test or a local lymphoid assay determined disperse red 11 to be negative for production of contact hypersensitivity (Sailstad et al. 1994).

Mutagenicity

Mutagenicity studies with disperse red 11 are summarized in Table G-2. The mutagenic effects of disperse red 11 have been investigated in three experimental systems: mouse lymphoma cells, *Salmonella typhimurium* bacterial assay (Ames test), and Chinese hamster ovary cells. The results of those studies are conflicting. Increased mutants and micronuclei were noted in the mouse lymphoma-cell system (Harrington-Brock et al. 1991), and an increased frequency of sister-chromatid-exchange and hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutant frequency were seen in Chinese hamster ovary cells (Brooks et al. 1989). However, studies using bacterial assays have shown mixed results. The studies of Brown and Brown (1976) showed that disperse red 11 was not mutagenic, but the studies of Moore et al. (1989) showed that disperse red 11 was positive in one test strain. Brooks et al. (1989) concluded that mutagenicity of disperse red 11 was dependent upon the dye lot

TABLE G-1 Summary of Toxicity Studies Conducted with Disperse Red 11

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Acute toxicity	Rat, Fischer 344, M	Oral, acute, 5 g/kg	Lot 1: 5/5 died, 4-6 d; Lot 2: 5/5 died, 4-5 d	Smith et al. 1986
Acute toxicity	Rat, Fischer 344, M	Oral, acute, Lot 1: 0.6, 0.7, 0.9, 1.4 g/kg; Lot 2: 0.5, 0.9, 1.4 g/kg	Lot 1: 0/5, 0/5, 4/5, 5/5 died; Lot 2: 0/5, 3/5, 3/5 died; LD_{50} 1.0 g/kg	Smith et al. 1986
Acute toxicity	Rat, Fischer 344, F	Oral, acute, 5 g/kg	Lot 1: 0/10 died at 14 d; LD_{50} > 5 g/kg Lot 2: 0/10 died at 14 d; LD_{50} > 5 g/kg	Smith et al. 1986
Acute toxicity	Rabbit, New Zealand White, M, F	Dermal, acute, 2 g/kg for 24 hr	Lot 1: 0/10 died at 14 d; LD_{50} > 2 g/kg; Lot 2: 0/10 died at 14 d; LD_{50} > 2 g/kg	Smith et al. 1986
Eye irritation	Rabbit, New Zealand White, M or F	0.1 g/eye	Lot 1: negative; Lot 2: negative	Smith et al. 1986
Dermal irritation	Rabbit, New Zealand White, M, F	Clipped skin, 0.5 g/kg for 24 hr	Lot 1: primary irritation score 2.7; moderately irritating; Lot 2: primary irritation score 0.73; mildly irritating	Smith et al. 1986
Contact hypersensitivity	Mice, Balb/c, F	Mouse ear-swelling test: 0.0006 g/d shaved back, 2 d; challenge on d 5	Negative	Sailstead et al. 1994
Contact hypersensitivity	Mice, Balb/c, F	Local lymph node assay: 0.00005 g/d both ears, 3 d	Negative	Sailstead et al. 1994
Contact dermatitis	Humans, 6 F with allergic contact dermatitis	1% solution	Negative	Lisboa et al. 1994

Abbreviations: M, male; F, female.

TABLE G-2 Summary of Mutagenicity Studies Conducted with Disperse Red 11

Experimental System	Exposure Conditions	End Points and Comments	Reference
Mouse lymphoma cells	0-40 µg/mL, 4 hr	264 thymidine kinase mutants per 10^6 survivors; 109 micronuclei per 1,000 cells at 15% survival	Harrington-Brock et al. 1991
Salmonella typhimurium (strains TA1535, TA1537, TA1538, TA98, TA100, TA1978) ± S9	Not specified	Not mutagenic	Brown and Brown 1976
Salmonella typhimurium (strains TA1537, TA1538, TA98, TA100, TA102, TA104) ± S9	0-7, 500 µg/plate	Positive in TA102 +S9	Moore et al. 1989
Salmonella typhimurium (strains TA1535, TA1538, TA98, TA100) ± S9	0-1,000 µg/plate,	Lot 1: not mutagenic; Lot 2: mutagenic activity in TA98 and TA1538 +S9	Brooks et al. 1989
Chinese hamster ovary cells	0-40 µg/mL, 3 hr	Lot 1 and 2: increased frequency of sister chromatid exchange; delay in cell growth; no increase in chromosomal aberrations; Lot 2: increase in HGPRT mutant frequency	Brooks et al. 1989

Abbreviations: HGPRT, hypoxanthine guanine phosphoribosyl transferase.

type, suggesting that the observed mutagenicity might be due to a contaminant in the dye lot.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

The experimental data are insufficient to assess the toxic effects of disperse red 11.

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Appendix H

1,4-Diamino-2,3-Dihydroanthraquinone

BACKGROUND

ALTERNATIVES NAMES for 1,4-diamino-2,3-dihydroanthraquinone (DDA) (after 1972) include 1,4-diamino-2,3-dihydro-9,10-anthracenedione (before 1972) and leuco-1,4-diaminoanthraquinone. DDA is a component of the old violet-dye mixture.

TOXICOKINETICS

No data are available on the toxicokinetics of DDA.

TOXICITY SUMMARY

Effects in Humans

No data are available on the effects of DDA in humans.

Effects in Animals

No data are available on the systemic toxicity of DDA in animals.

Mutagenicity Studies

In a report by Lundy and Eaton (1994), DDA was found to produce positive results in the Ames assay. In a study by Brown and Brown (1976), as cited by Dacre (1979), DDA was reported to produce "marginally adequate" evidence of mutagenicity in the Ames assay.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

Experimental data are insufficient to assess the toxic effects of DDA.

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Appendix I

1,4-Diaminoanthraquinone

BACKGROUND

1,4-DIAMINOANTHRAQUINONE (DAA) is a combustion product of 1,4-diamino-2,3-dihydroanthraquinone, a component of the old violet-dye mixture.

TOXICOKINETICS

No data are available on the toxicokinetics of DAA.

TOXICITY SUMMARY

Effects in Humans

No data are available on the effect of DAA in humans.

Effects in Animals

DAA was found to produce moderate eye irritation in rabbits at a dose of 0.5 grams (g) for 24 hr (Lundy and Eaton 1994). DAA has a reported

lethal dose for 50% of the test animals (LD_{50}) of 4.9 g per kilogram (kg) of body weight (route of exposure not reported) (RTECS 1981-82, as cited in Lundy and Eaton 1994).

Mutagenicity Studies

DAA was reported to produce positive effects in the Ames assay in a report by Lundy and Eaton (1994). Rubin (1982) also reported positive effects with DAA in the Ames assay and made the point that the results showed DAA to be more active in the Ames assay than DDA.

SUBCOMMITTEE EVALUATION OF DYE TOXICITY

Experimental data are insufficient to assess the toxic effects of DAA.

REFERENCES

Lundy, D., and J. Eaton. 1994. Occupational Health Hazards Posed by Inventory U.S. Army Smoke/Obscurant Munitions (Review Update). WRAIR/RT-94-0001. AD-A276-774. U.S. Army Medical Research Detachment, Wright-Patterson Air Force Base, OH.

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