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Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

Tumorigenicity Studies of Diesel Fuel-2, Red Smoke Dye and Violet Smoke Dyes in the SENCAR Mouse Skin Tumorigenesis Bioassay System

Final Report

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R. J. M. Fry

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5010

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The tumorigenicities of Diesel Fuel-2, Red Smoke Dye and Violet Smoke Dye were tested in the SENCAR Mouse Skin Bioassay System. The Diesel Fuel-2 gave a significant tumor response when tested as a tumor promoter but negative results when tested as a complete carcinogen. There were no tumor responses to either the Red or Violet Smoke Dyes when tested as complete carc few tumors occurred in the Red and Violet Smoke Dye tumor the response was not significantly different from that of

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Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council [DHEW Publication No. (NIH) 78-23, Revised 1978].

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Introduction

This report summarizes the findings of tumorigenicity studies of reference grade Diesel Fuel-2 obtained from Phillips Petroleum Company. This study was carried out as part of a program to determine the possible long range health hazard to personnel exposed to diesel fuel disseminated as an aerosol to provide an obscuring battlefield smoke.

A number of the U.S. Army's pyrotechnic devices contain compounds which produce colored smokes for signaling and marking. Because of the potential for exposure of workers to the dyes and of instructors and trainees to the disseminated smokes, the U.S. Army Medical Research and Development Command has supported research to study the immediate and delayed health effects of such exposure. This report also describes the results of the tumorigenicity assays of the red and violet colored smoke dyes. The red dye is composed of 1-methylaminoanthraquinone (MAA). The violet dye is made up of 80 percent by weight 1,4-diamino-2,3-dihydroanthraquinone (DDA) and 20 percent by weight MAA.

The assessment of tumorigenicity has been carried out in the SENCAR mouse skin tumorigenesis bioassay system (Slaga et al., 1978; Boutwell, 1964; Slaga et al., 1982). Skin tumors are induced by the sequential application of a subthreshold dose of a carcinogen followed by repetitive treatment with a chemical promoter (Slaga, 1983). In testing a compound, the compound is substituted for either the carcinogen and/or the promoter and the endpoints for this assay are: (1) the incidence of papillomas, i.e., the percentage of papilloma-bearing mice, (2) the multiplicity of papillomas, i.e., the number of papillomas per mouse, (3) the incidence of carcinomas, i.e. the percent of carcinoma-bearing mice, and (4) the multiplicity of carcinomas, i.e. the number of carcinomas per mouse.

This assay system has a well documented response to polycyclic aromatic hydrocarbons (PAH) (Slaga et al., 1978). The system has also been used to identify many chemicals other than PAHs as potential carcinogens (Table 1). These chemicals represent a wide variety of classes of chemicals, including aldehydes, carbamates, epoxides, haloalkylethers, haloaromatics, haloalkylcarbonyls, hydroxylamines, lactones, nitrosamides, sulfonates, sultones, and ureas. The 32 chemicals listed in Table 1 include such well known chemical carcinogens as aflatoxin B1, bis(chloromethyl)ether, chloromethyl methyl ether, urethane, N-acetoxy-2-acetamidofluorene, β-propiolactone, N-methyl-N'-nitro-N-nitrosoguanidine, 1,3-propanesultone, N-nitroso-methyl urea, triethylenemelamine, and 4-nitroquinoline-N-oxide. The mouse skin tumorigenesis bioassay can also detect chemicals that cause tumors in the respiratory tract of animals (Table 2). Of 11 known animal respiratory carcinogens, the mouse skin tumorigenesis system has to date detected carcinogens from the PAH, quinoline, and carbamate groups. Of the chemicals tested from the list of highly suspect occupational respiratory carcinogens, chloromethyl ethers and coke oven emissions have been found to be tumorigenic in the mouse skin

Table 1. Chemicals Other Than PAH Detected by Mouse Skin Bioassay[a]

Class	Chemical	Reference
Aldehyde	Malonaldehyde	Shamberger et al., 1974
Carbamate	Urethane	Salaman and Roe, 1953 Slaga et al., 1973
	Vinyl carbamate	Dahl et al., 1978
	Ethyl N-phenylcarbamate	Roe and Salaman, 1955
Epoxide, diepoxide	Glycidaldehyde	Shamberger et al., 1974
	1,2,3,4-Diepoxybutane	Van Duuren et al., 1965
	1,2,4,5-Diepoxypentane	Van Duuren et al., 1965
	1,2,6,7-Diepoxyheptane	Van Duuren et al., 1965
	Chloroethylene oxide	Zajdela et al., 1980
Haloalkylether	Bis(chloromethyl)ether	Van Duuren et al., 1969 Zajdela et al., 1980 Slaga et al., 1973
	Chloromethyl methyl ether	Slaga et al., 1973 Van Duuren et al., 1969
Haloaromatic	2,3,4,5-Tetrachloronitrobenzene	Searle, 1966
	2,3,4,6-Tetrachloronitrobenzene	Searle, 1966
	2,3,5,6-Tetrachloronitrobenzene	Searle, 1966
	Pentachloronitrobenzene	Searle, 1966
Haloalkyl-carbonyl	Chloroacetone	Searle, 1966
	3-Bromopropionic acid	Searle, 1966

Table 1. (Cont'd)

Class	Chemical	Reference
Hydroxylamine	N-Acetoxy-4-acetamidobiphenyl	Scribner and Slaga, 1975
	N-Acetoxy-2-acetamidofluorene	Scribner and Slaga, 1975 Slaga et al., 1978
	N-Hydroxy-2-aminonaphthalene	Clayson and Garner, 1976
	N-Acetoxy-2-acetoamidophenanthrene	Scribner and Slaga, 1975
	N-(4-Methoxy)benzoyloxypiperidine	Scribner and Slaga, 1975
	N-(4-Nitro)benzoyloxypiperidine	Scribner and Slaga, 1975
	N-Acetoxy-4-acetamidostilbene	Scribner and Slaga, 1975
Lactone	β-Propiolactone	Roe and Salaman, 1955 Slaga et al., 1973 Hennings and Boutwell, 1969
Multifunctional	Triethylenemelamine	Roe and Salaman, 1955
	4-Nitroquinoline-N-oxide	Hennings and Boutwell, 1969
Natural products	Aflatoxin B1	Lindenfelser et al., 1974
Nitrosamide	N-Methyl-N'-nitro-N-nitrosoguanidine	Hennings et al., 1978 Fujii, 1976
Sulfonate	Allyl methysulfonate	Roe, 1957
Sultone	1,3-Propanesultone	Slaga et al., 1973
Ureas	N-Nitrosomethylurea	Graffi and Hoffman, 1966

[a]Nesnow et al., 1981.

Table 2. Response of Carcinogens in Humans, Animals and Mouse Skin[a]

Sample	Occupational Respiratory Carcinogen[b]	Animal Respiratory Carcinogen[b]	Mouse Skin Tumorigen[c]
Arsenic	+		
Asbestos	+	+	
Beryllium	+	+	
Carbamates		+	+
Chloromethyl ethers	+	+	+
Chromium	+		
Coke oven	+		+
Isopropyl oil	+		
MOCA[d]	+	+	
Mustard gas	+	+	
Nickel	+	+	
Nitrosamines		+	
PAH		+	+
Quinolines		+	+
Vinyl chloride	+	+	

[a]From Nesnow et al., 1981.
[b]Frank, 1978.
[c]Slaga et al., 1978; Van Duuren, 1976.
[d]Methylene bis(ortho-chloroaniline).

tumorigenesis system (Nesnow et al., 1981). These results indicate that the mouse skin tumorigenesis bioassay seems particularly well suited for evaluation of the tumorigenic potential of compounds and mixtures known to be dermal carcinogens and also some compounds and mixtures that are thought to cause cancers at other sites (Nesnow et al., 1982).

Materials and Methods

A. Materials

1. The carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), was obtained from Sigma Chemical Co.

2. The promoter, 12-0-tetradecanoylphorbol 13-acetate (TPA), was obtained from L. C. Systems of Woburn, Mass.

3. Diesel Fuel Type 2-D (Diesel Fuel-2, DF-2) as specified in the code of Federal Regulations, Title 45, Subtitle A, Part 1210, Sub Part J, paragraph 120, 121, Commercial fuel was obtained from Phillips Petroleum Co. Our test sample was a portion from the bulk quantity supplied to ORNL for this project. A detailed chemical analysis of this fuel was performed by the Analytical Chemistry Division, ORNL, and reported by Jenkins (1983).

4. Red (RSD) and Violet (VSD) smoke dyes were supplied to ORNL for this project courtesy of Pine Bluff Arsenal. The samples used in the tumorigenicity assays were characterized by the Analytical Chemistry Division, ORNL, and reported by Rubin (1983). "The VSD is formulated to contain 80 percent 1,4-diaminoanthraquinone and 20 percent 1-methylaminoanthraquinone. The RSD is formulated to contain only one dye component, 1-methylaminoanthraquinone."

5. Spectral grade acetone from Baker Chemical Co. was used to dilute or dissolve all compounds to the desired concentration.

6. SENCAR mice, male and female, ages 5 to 9 weeks, either bred at the Oak Ridge National Laboratory, Oak Ridge, TN, or from Harlan Sprague Dawley, Indianapolis, IN. In one experiment, namely, complete carcinogenesis with smoke dyes, SENCAR mice obtained from Harlan Sprague-Dawley (HSD) were used. The breeding stock used by HSD was supplied by ORNL.

B. Methods

These studies used forty 5 to 9 week-old SENCAR mice per treatment group (20 of each sex). The animals were housed in plastic (Maryland Plastics #E0670 cage, 8 5/8" x 13 7/8" x 5 1/8" with bar type stainless steel tops) cages (10/cage) with hardwood chip bedding (Sanichip, P. J. Murphy Forest Products Corp., Rochelle Park, NJ), fed Purina Laboratory

Chow 5001 and water ad libitum, and maintained at 20°-23°C with 10 changes of air per hour. Animals were treated and housed in gold-lighted rooms (General Electric F40GO: 12 hrs of light/day) reducing the possibility of UVB-induced reactions with the treatment chemicals or cocarcinogenic effects of the UVB on the treated animals. The hair was shaved from the backs of the mice with surgical clippers 2 days before the initial treatment. All sample doses were applied in topical treatment volumes of 0.2 mL. Spectral quality acetone was used as a solvent for the samples, the positive control, DMBA (2.52 µg in 0.2 mL), and the promoter, TPA (2.0 µg in 0.2 mL).

After the topically administered initiation dose, the animals were promoted 1 or 2 times per week as outlined in Tables 3 and 4. Skin tumor formation was recorded weekly and papillomas greater than 2 mm in diameter were included in the cumulative total if they persisted for one week or longer. Both the number of mice with tumors and the number of tumors per mouse were determined and recorded weekly. Tumors were randomly removed for histological verification. Group animal weights were determined every three to four weeks throughout the study.

The maximum dose level for the smoke dyes was determined by their solubility in acetone. The maximum dose of the DF-2 was the undiluted liquid.

The Fisher Exact Test (Fisher, 1935) was used to determine if the tumor responses were significantly above background controls.

1. Diesel Fuel-2

A. Results

When the mice were treated with diesel fuel one time a week at 1X, 1/10X, 1/100X concentration, for 38 weeks, no treatment group gave a tumor yield above historic background controls (Table 5).

In the tumor promotion study where the mice were initiated with DMBA and then given the 1X, 1/10X or 1/100X concentration dose of diesel fuel two times a week, some positive results were obtained (Table 6). Visibly, all three test concentrations gave a positive tumor response but only the 1X dose gave a statistically positive tumor response significantly above background controls. In addition, 5.5% of the 1X test animals developed carcinomas. Even though the data for the 1/10X and 1/100X concentrations are not statistically different from the controls, the results indicate a trend that may suggest that the Diesel Fuel-2 is a promoting agent.

An additional observation on the tumor promotion data is that there is a tumor response time of 8 to 11 weeks difference between the males and females (Table 7). A difference in tumor response time of 14 to 21 days between sexes is often observed but 8 to 11 weeks is unusual. The males are more sensitive to the diesel fuel than the females.

Table 3. Diesel Fuel-2 Protocols

	Initiation	7 days later	Promotion
A. Complete Carcinogenesis[a]			
Group 1[b]	DF-2 1x conc. 0.2 mL		DF-2 1x conc. 0.2 mL 1x/wk
Group 2	DF-2 1/10x conc. 0.2 mL		DF-2 1/10x conc. 0.2 mL 1x/wk
Group 3	DF-2 1/100x conc. 0.2 mL		DF-2 1/100x conc. 0.2 mL 1x/wk
Group 4	DMBA 2.52 g		DMBA 2.52 µg 1x/wk
B. Tumor Promotion[a]			
Group 1[b]	DMBA 2.52 µg		DF-2 1x, 0.2 mL 2x/wk
Group 2	DMBA 2.52 µg		DF-2 1/10x, 0.2 mL 2x/wk
Group 3	DMBA 2.52 µg		DF-2 1/100x, 0.2 mL 2x/wk
Group 4	DMBA 2.52 µg		TPA 2 µg 2x/wk

[a]38 weeks duration of test program.
[b]Each group consisted of 20 male and 20 female SENCAR mice, 5-9 weeks old.

Table 4. Colored Smoke Dye Protocols

	Initiation	7 days later	Promotion
A. Complete Carcinogenesis[a]			
Group 1 Smoke Dye[b]	2 mg/0.4 mL[c]		Smoke Dye 1 mg/0.2 mL 2x/wk
Group 2 Smoke Dye	1 mg/0.2 mL		Smoke Dye 1 mg/0.2 mL 1x/wk
Group 3 Smoke Dye	0.1 mg/0.2 mL		Smoke Dye 0.1 mg/0.2 mL 1x/wk
Group 4 DMBA	2.52 µg		DMBA 2.52 µg 1x/wk
B. Tumor Initiation[a]			
Group 1 Smoke Dye[b]	2 mg/0.4 mL		TPA 2 µg 2x/wk
Group 2 Smoke Dye	1 mg/0.2 mL		TPA 2 µg 2x/wk
Group 3 Smoke Dye	0.1 mg/0.2 mL		TPA 2 µg 2x/wk
Group 4 DMBA	2.52 µg		TPA 2 µg 2x/wk

[a]Thirty weeks duration of test program.
[b]Each group consisted of 20 male and 20 female SENCAR mice, 5-9 weeks old.
[c]Maximum solubility of Smoke Kyes was 5 mg/mL of acetone.
In Group 1, 1 mg/0.2 mL was applied and as soon as the acetone dried, this was repeated to give a 2 mg dose.

Table 5. Complete Skin Carcinogenesis of Diesel Fuel-2 When Applied Topically Once a Week.

Summary at 38 Weeks

Dose of DF-2	No. of Mice 0 Wks	No. of Mice 38 Wks	Avg. Wt. Mice 0 Wks	Avg. Wt. Mice 38 Wks	Mice/ Papillomas	Mice/ Carcinomas
1X Conc.	40	38	33.5	40.9	0/0	0/0
1/10 Conc.	40	39	33.6	41.0	1/1	0/0
1/100 Conc.	40	38	32.9	41.6	0/0	0/0
DMBA 2.52 µg (1x/wk)	40	40	31.3	41.0	10/41	0/0
*Acetone 0.2 mL (1x/wk)	40	40	34.3	42.7	0/0	0/0

Dose	% Mice with Paps	No. of Paps/ Mouse	No. of Ca/ Mouse	% Mice with Ca	Fishers Exact Test on Mice with Paps
1X	0	0	0	0	-
1/10X	2.9	0.025	0	0	Not significant
1/100X	0	0	0	0	-
DMBA	25	1.025	0	0	
Acetone	0	0	0	0	

*Control data terminated at 30 weeks of test. 20 male and 20 female SENCAR mice/test group.
In historic controls, 1 or 2 papillomas are expected in a test group of 40.

Table 6. Skin Tumor Promoting Activity of Diesel Fuel-2 When Given Twice a Week after DMBA Initiation.

Dose of DF-2	Number of Mice 0 wk	30 wk	Avg. Wt. Mice 0 wk	30 wk	Mice/ Papillomas	Mice/ Carcinomas	% Mice with Paps
1X Conc.	40	37	32.6	39.9	24/118	0/0	60
1/10X Conc.	40	38	32.5	40.1	4/17	0/0	10
1/100X Conc.	40	38	32.95	42.7	2/5	0/0	5
DMBA 2.52 µg	40	35	33.5	39.1	35/432	3/3	100
Acetone 0.2 mL + TPA 2 µg	40	39	30.5	41.5	1/1	0/0	2.5

Dose	No. of Paps/Mouse	No. of Ca/Mouse	% Mice With Ca	Fishers Exact Test on Mice with Paps
1X Conc.	2.95	2/2 mice	5.5	P<.001 Significant
1/10X Conc.	0.43	0	0	Not significant
1/100X Conc.	0.13	0	0	Not significant
DMBA	10.3	0.075	7.5	
Acetone + TPA	0.025	0	0	

20 male and 20 female SENCAR mice/test group.

Table 7. Diesel Fuel-2 Promotion, Male and Female Tumor Responses

Weeks of Testing	1X Conc. Paps/Mouse Male/Female (40 mice, 32.6 gm/mouse)		1/10X Conc. Paps/Mouse Male/Female (40 mice, 32.5 gm/mouse)		1/100X Conc. Paps/Mouse Male/Female (40 mice, 32.95 gm/mouse)		DMBA 2.52 µg Paps/Mouse Male/Female (40 mice, 33.5 gm/mouse)	
1	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	2.0	5.0
9	0	0	0	0	0	0	2.0	7.0
10	.15	0	0	0	0	0	4.7	8.6
11	.15	0	0	0	0	0	6.7	11.7
12	.4	0	0	0	0	0	7.1	13.4
13	.45	0	0	0	0	0	–	–
14	.55	0	0	0	0	0	8.2	14.9
15	.95	0	0	0	0	0	8.8	14.5
16	1.05	0	0	0	0	0	11.2	14.45
17	1.1	0	0	0	0	0	10.3	13.45
18	1.35	0	0.21	0	0	0	11.2	15.15
19	1.45	0	0.22	0	0	0	–	–
20	1.9	0	0.27	0	0	0	11.45	14.05
21	2.35	0	0.27	0	0	0	11.5	13.6[b]
22	2.45	0.10	0.33	0	0	0	11.4	13.7
23	2.40	0.20	0.33	0	0	0	11.8	14.15
24	2.9	0.45	0.33	0	0	0	12.1	13.9
25	3.0	0.50	0.38	0	0	0	12.4	14.5
26	3.05	0.60	0.5	0	0	0	–	–
27	3.15	0.60	0.66	0.05	0	0	10.8	12.13
28	3.45	0.90	0.66	0.05	0	0	–	–
29	3.45	1.2	0.77	0.05	0	0	10.95	11.5
30	3.75	1.3	0.83	0.1	0.1	0	11.11	12.3

Table 7. (Cont'd)

Weeks of Testing	1X Conc. Paps/Mouse Male/Female (40 mice, 32.6 gm/mouse)		1/10X Conc. Paps/Mouse Male/Female (40 mice, 32.5 gm/mouse)		1/100X Conc. Paps/Mouse Male/Female (40 mice, 32.95 gm/mouse)		DMBA 2.52 µg Paps/Mouse Male/Female (40 mice, 33.5 gm/mouse)	
31	3.75	1.5	0.77	0.1	0.1	0	11.9	12.9
32	3.37	1.55	0.77	0.1	0.15	0	-	-
33	3.17	1.8	0.77	0.1	0.2	0	11.6	12.4
34	3.22	2.0	0.83	0.1	0.25	0	12.2	12.5
35	3.44	2.0	0.83	0.1	0.25	0	-	-
36	3.61	2.0	0.83	0.1	0.25	0	11.7	9.8
37	3.66	2.11	0.83	0.1	0.25	0	-	-
38	3.83	2.47	0.83	0.1	0.26	0[a]	12.6	9.8
39	Terminated (37 mice, 39.8 gm)		(38 mice, 40.1 gm)		(38 mice, 42.7 gm)		(23 mice, 40.0 gm)	

[a]Treatment of this group had to be terminated shortly before a response in females would have been expected, assuming that the female response was later than that in the males, as had been observed in the two higher dose groups.

[b]Carcinomas began to appear after 22 weeks. 12 mice died over last 8 weeks of test due to massive papillomas and carcinomas.

B. Discussion

In the two-stage carcinogenesis system using mouse skin, the initiation phase requires only a single application of either a direct or indirect carcinogen at a subthreshold dose and is essentially irreversible. The promotion phase requires repetitive treatments after initiation and is initially reversible, later becoming irreversible. A promoting agent is one which, when applied repeatedly after a single dose of a tumor-initiating agent, results in tumors. Tumor promoters can be either weak carcinogens or noncarcinogens (Slaga et al., 1982).

The chemical characterization of DF-2 is presented in Table 8. A number of the chemical fractions contain compounds or groups of compounds that give a positive tumor response in the animal system (NIH Pub. #80-453). However, the quantity of these compounds per gram or mL of DF-2 is very low. For example, the benzo(a)pyrene [B(a)P] analysis gave an average concentration of 72 nanograms/mL. This is 14.4 nanograms of B(a)P per 0.2 mL of DF-2 which was the maximum initiation dose. With such minute amounts of carcinogens given to the mouse in the tumor initiation studies, at maximum dose, it is not surprising that there were no tumors due to this component obtained in the tumor initiation study. The concentration of other carcinogens in the fuels is also low. Unless there were marked interactions including, perhaps, synergisms, a very low degree of potency would be expected.

The positive tumor response in the DF-2 promotion study reflects the amplified sensitivity of the SENCAR two-stage mouse skin system. After multiple doses of the DF-2, tumors were detected. If it is assumed that the multiple exposures to the very low doses of the carcinogenic constituents of the fuel did not act as initiators they must have acted as promoters. In support of this contention, the cumulative dose of B(a)P was 6% of a dose that is known to be sufficient for initiation (2.52 μg, Slaga et al., 1982) and far less than that required for complete carcinogenesis. It should be noted that with the multiplicity of compounds in the DF-2, some of which are carcinogens, complex interactions that might include cocarcinogenesis cannot be eliminated as the cause of our findings.

2. Colored Smoke Dyes

A. Results

Table 9 shows that there was no tumor response to either the red or violet smoke dyes when tested as complete carcinogens. Although a few tumors occurred in the tumor initiation studies (Table 10) the results are not significantly different from the TPA controls when compared by the Fisher Exact Test.

B. Discussion

The colored smoke dyes are anthraquinones. As a group, the anthraquinones have a molecular configuration that is not carcinogenic. Literature references of specific anthraquinones give tumor data of background levels only (NIH Publication No. 80-453). Our tumor initiation and promotion studies agree with these findings.

Table 8. Major Diesel Fuel-2 Components Characterized by Jenkins et al. (1983)

Components		
Fraction A	Aliphatic Fraction Straight & branched chain hydrocarbons	Approx. 700 mg/gm of fuel
Fraction B	Alkyl-substituted benzene compounds	Approx. 160 mg/gm of fuel
Fraction C	Two-ring aromatic Naphthalene, alkylated naphthalenes, alkylated biphenyls and small amounts of triopenes	Approx. 120 mg/gm of fuel
Fraction D	Three-ring aromatic Fluorene, alkylated fluorene, phenanthrene and alkylated phenanthrenes	Approx. 20 mg/gm of fuel
Semi-Polar Fraction	Alkyl-substituted indols & Carbazols, Carbozole	Approx. 2 mg/gm fuel
B(a)P Analysis	B(a)P	Average level was 72 nanograms/mL of fuel
Trace Elements	21 Trace Elements 2 are Chromium (<0.01 g/mL) and Cadmium (0.01 µg/mL)	Approx. total <.48 g/mL

Table 9. Complete Carcinogenic Activity of Red & Violet Smoke Dyes at 30 Weeks.

		Mice		Avg Wt/Mouse		Mice/	Mice/
		0 Wks	0 Wks	0 Wks	30 Wks	Papillomas	Carcinomas
RSD	1 mg (2x/wk)	42	40	33.3 gm	43.9 gm	0	0
RSD	1 mg (1x/wk)	39	38	33.3 gm	44.9 gm	0	0
RSD	1/10 mg (1x/wk)	39	38	32.6 gm	39.1 gm	0	0
VSD	1 mg (2x/wk)	40	40	32.1 gm	43.0 gm	0	0
VSD	1 mg (1x/wk)	39	39	32.2 gm	40.2 gm	0	0
VSD	1/10 mg (1x/wk)	41	41	32.3 gm	42.0 gm	0	0
DMBA	2.52 µg (1x/wk)	40	39	31.3 gm	41.0 gm	10/41	0/0
Acetone	0.2 mL of stock (2x/wk)	40	40	34.3 gm	42.8 gm	0	0

20 male and 20 female SENCAR mice/test group.

Table 10. Tumor Initiating Activity of Red & Violet Smoke Dyes of 30 Weeks

		Mice		Avg Wt/Mouse		Mice/	Mice	% Mice
		0 Wks	30 Wks	0 Wks	30 Wks	Papillomas	Carcinomas	w/Paps
RSD	2 mg	40	40	33.6 gm	41.9 gm	5/6	0/0	12.5
RSD	1 mg	40	40	32.8 gm	40.8 gm	0/0	0/0	0
RSD	1/10 mg	40	40	34.3 gm	40.9 gm	3/4	0/0	7.5
VSD	2 mg	40	38	32.6 gm	41.2 gm	2/2	0/0	5.2
VSD	1 mg	40	38	32.5 gm	41.2 gm	3/3	0/0	7.5
VSD	1/10 mg	40	39	33.0 gm	41.4 gm	1/1	0/0	2.6
DMBA	2.52 µg	40	35	33.5 gm	39.1 gm	35/432	3/3	100
Acetone + TPA	2 µg	40	40	34.6 gm	42.1 gm	1/1	0/0	2.6

Dose	Mean Paps/Mouse	Fishers Exact Test Mice with Paps
RSD 2 mg	0.15	Not significant
RSD 1 mg	0.0	Not significant
RSD 1/10 mg	0.1	Not significant
VSD 2 mg	0.05	Not significant
VSD 1 mg	0.075	Not significant
VSD 1/10 mg	0.025	Not significant
DMBA 2.52 µg	10.8	
Acetone + TPA	0.025	

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