

AD-A156 772

BIOASSAY OF MILITARY RELEVANT COMPOUNDS FOR
CARCINOGENIC ACTIVITY BY THE... (U) MEDICAL COLL OF OHIO
AT TOLEDO DEPT OF PATHOLOGY G D STONER JAN 85

1/1

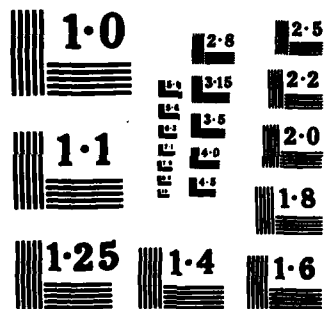
UNCLASSIFIED

DAMD17-R1-C-1178

F/G 8/5

NL

END
DATE
FILMED
8-85



AD-A156 772

BIOASSAY OF MILITARY RELEVANT COMPOUNDS FOR CARCINOGENIC ACTIVITY
BY THE STRAIN A MOUSE LUNG TUMOR BIOASSAY

FINAL PHASE REPORT

Gary Stoner, Ph.D.

January 1985

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-81-C-1178

Medical College of Ohio
Department of Pathology
3000 Arlington Avenue
Toledo, Ohio 43699

DTIC
ELECTE
JUL 19 1985
S D

Contract Officer Technical Representative:
Robert Finch, Ph.D.

Health Effects Research Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

Approved for public release;
distribution unlimited

The findings of this report are not to be construed as an official Department
of the Army position unless so designated by other authorized documents.

DTIC FILE COPY

85 7 08 093

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Bioassay of Military Relevant Compounds for Carcinogenic Activity by the Strain A Mouse Lung Tumor Bioassay		5. TYPE OF REPORT & PERIOD COVERED Final Phase Report March 1983 - May 1984
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Gary D. Stoner, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) Contract No. DAMD17-81-C-1178
9. PERFORMING ORGANIZATION NAME AND ADDRESS Medical College of Ohio, Department of Pathology 3000 Arlington Avenue Toledo, Ohio 43699		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A-3M161102BS10-CC-344
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command; Attn: SGRD-RMS Fort Detrick, Frederick, MD 21701-5012		12. REPORT DATE January 1985
		13. NUMBER OF PAGES 17
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) U.S. Army Medical Bioengineering Research and Development Laboratory Fort Detrick, Frederick, MD 21701-5010		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) C.I. Solvent Yellow 33 Carcinogenicity C.I. Solvent Green 3 Dyes Strain A mouse lung adenoma bioassay		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Two dye compounds, C.I. Solvent Yellow 33 and a mixture of C.I. Solvent Green 3-C.I. Solvent Yellow 33, utilized in colored smoke grenades, were tested for carcinogenic potential in the strain A mouse lung tumor bioassay. The maximum tolerated dose for each dye was determined to be 25 mg/kg. At total doses of 600, 300 and 120 mg/kg, C.I. Solvent Yellow 33 and a mixture of C.I. Solvent Green 3-C.I. Solvent Yellow 33 did not produce an increase in the lung tumor response (i.e., the percentage of mice with lung tumors or the mean number of lung tumors per mouse) when compared to vehicle (tricaprylin).		

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 68 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Cont 11

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

controls. Therefore, at the dose levels administered both dyes were non-carcinogenic in the strain A mouse lung tumor bioassay under the conditions of the test. At necropsy, mice tested with either compound had significant quantities of accumulated dye in their tissues. This observation suggests that the dyes were either not metabolized or only partially metabolized by the animals.

As a basis for	
DTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	



Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	iv
INTRODUCTION	1
MATERIALS AND METHODS	3
Animals	3
Chemicals	3
Preliminary toxicology	7
Bioassays	7
RESULTS	7
Toxic Effects	7
Lung Tumor Response	8
Lesions in Other Organs	8
CONCLUSION.....	8
REFERENCES	11
DISTRIBUTION LIST	12
TABLES	
1. Impurities in C.I. Solvent Yellow 33 Dye	2
2. Impurities in C.I. Solvent Green 3/C.I. Solvent Yellow 33 Dye Mixture.....	4
3. Histopathological Evaluation of Strain A/J Mice	5
4. Quantitation of C.I. Solvent Yellow 33 and C.I. Solvent Green 3 in Dye Samples.....	6
5. Lung Tumor Response in Untreated, Vehicle-Treated and Urethan-Treated A/J Mice	9
6. Lung Tumor Response in A/J Mice to C.I. Solvent Yellow 33 and C.I. Solvent Green 3/C.I. Solvent Yellow 33 Mixture	10

FOREWORD

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

INTRODUCTION

The objectives of this study were to examine two dye compounds; i.e., C.I. Solvent Yellow 33 and a C.I. Solvent Green 3/C.I. Solvent Yellow 33 dye mixture, for carcinogenic activity in the strain A mouse lung tumor bioassay. Both dyes are incorporated into colored smoke grenades and, during the manufacture of these munitions, workers are exposed to the compounds via the dermal and inhalation routes. Both dyes are insoluble in water and there is no information on the metabolism of the compounds. The strain A mouse lung tumor bioassay was chosen to evaluate the potential carcinogenic activity of the dyes since the standard route of administration for this bioassay, intraperitoneal, may allow a greater amount of the dyes to reach the target organ (1).

The green and yellow smoke munitions both contain 42 percent by weight of the dyes. The green smoke munition contains 29.5 percent C.I. Solvent Green 3 and 12.5 percent C.I. Solvent Yellow 33. The yellow smoke munition contains 42 percent C.I. Solvent Yellow 33 (2). Worker exposure would be to the C.I. Solvent Yellow 33 or the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture.

The yellow dye is 2-(2-quinoly)-1,3-indandione and synonyms are:

C.I. Solvent Yellow 33
C.I. No. 47000
D&C Yellow No. 11

The FDA allows the use of the insoluble dye in externally applied drugs. The batch must be not less than 96 percent pure dye and contain not more than 0.3 percent phthalic acid and 0.2 percent quinaldine. The C.I. Solvent Yellow 33 dye has been tested for purity by high-pressure liquid chromatography (2) (see Table 1). The dye tested for purity was used in the animal studies.

Experimental animal studies with C.I. Solvent Yellow 33 have used the oral and dermal routes of administration. The acute oral LD50 for adult male albino rats was reported to be greater than 10 g/kg and no toxic signs were observed. In a six-week range finding study in rats, the dye was fed at dietary levels of 0.1, 0.23, 0.55, 1.29 and 3.0 percent. A definite toxic effect was noted at the highest (3%) level and consisted of growth suppression, increased liver/body weight ratios and histologic alterations in the spleen, liver, kidney, thyroid, and bone marrow. At the four lower dietary levels body weight gains for the test animals were lower than those for the controls and, at the 0.55 and 1.29 percent levels, liver/body weight ratios were higher than the controls. Histologic alterations similar to, but less severe than those seen in the organs of animals receiving 3.0 percent in the diet, were also observed in some of the animals at each of the four lower levels.

In a one year study, C.I. Solvent Yellow 33 was fed to male and female albino rats at dietary levels of 0.03, 0.1, 0.3 and 1.0 percent. Growth suppression was noted in the males at all levels, but only in the 1.0 percent female group. A yellow or yellow-green coloration of the kidneys and livers was noted in animals at all levels. Morphologic alterations were present in the liver, spleen and kidneys of animals receiving 1.0 percent dye. Pigment deposition was also present in the livers and kidneys of rats given the lower dietary levels, but there was no associated cellular damage. Applications of

Table 1. Impurities in C.I. Solvent Yellow 33 Dye

	Weight % (mean + SD) ^a
Phthalic anhydride and/or Phthalic acid	<1.8 ± 0.1
Quinaldine	<0.4
Impurity A	2.2 ± 0.1 ^b

^a n = 5

^b area of A ÷ area of C.I. Solvent Yellow 33

the dye in both hydrophilic ointment and white petrolatum bases at 0.1 and 1.0 percent to rabbit abdominal skin produced no adverse effects following 15 (abraded skin) or 65 (intact skin) repeated exposures.

The green dye used in the green/yellow dye mixture is 1,4-di-p-toluidino-anthraquinone (CAS 128-80-3; 1,4-bis-[4-(methylphenyl)-amino]-9,10-anthracenedione. Synonyms are:

C.I. Solvent Green 3
C.I. 61565
D&C Green No. 6

The FDA limits the use of this color for dyeing surgical sutures. The toxicity, sensitization or carcinogenic properties of this dye are not known. The FDA requires that lifetime feeding studies be conducted with the dye and the closing date for submission of the results was January, 1981. In an April 1982 Federal Register announcement, the dye was listed for external use in surgical sutures. The C.I. Solvent Green 3/C.I. Solvent Yellow 33 dye mixture has been tested for purity by high-pressure liquid chromatography (2) (See Table 2). The dye mixture tested for purity was used in the animal studies.

MATERIALS AND METHODS

Animals. Six-to-eight week-old male and female mice were used in the bioassays. They were obtained from our breeding colony, derived from A/J mice purchased from the Jackson Laboratories, Bar Harbor, Maine. The mice were kept on corn cob bedding in temperature (22°C) and humidity (50%) controlled rooms with a 12-hr light/dark cycle. Certified Purina laboratory chow and water were provided ad libitum.

The health status of the breeding colony and the mice used in the bioassays was examined on a yearly basis by: (a) complete gross necropsy, tabulating gross lesions, and general condition and body weight of each animal; (b) histopathological evaluation of formalin fixed tissues involving all organs listed in Table 1; and (c) serological tests for the presence of murine virus infections. The serological tests were conducted by Microbiological Associates in Bethesda, Maryland.

As indicated in Table 3, histopathological examination of the major organs of ten animals indicated that all organs were normal in appearance except the livers of two animals that contained occasional lesions typical of those seen in viral hepatitis.

Chemicals. Chemical analysis of these lots of dyes was performed, using high-pressure liquid chromatography, by the Lovelace Inhalation Toxicology Research Institute, Albuquerque, New Mexico, which also was performing the work under a project order. C.I. Solvent Yellow 33 was tested as a 93.1% pure compound whereas the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture contained these two dyes in a ratio of 71%:24%, respectively (Table 4) (2). The results demonstrated that the C.I. Solvent Yellow 33 dye has one major impurity, designated impurity A (Table 1). The dye is very homogeneous in composition. High-pressure liquid chromatography of the C.I. Solvent Green 3/C.I. Solvent Yellow 33 dye mixture indicated that impurity A was also the major impurity in the mixture (Table 2). The dye mixture appeared to be homogeneous in composition.

Table 2. Impurities in C.I. Solvent Green 3/C.I. Solvent Yellow 33 Dye Mixture

	<u>Weight % (mean + SD)^a</u>
p-Toluidine	0.10 ± 0.03
Impurity A	0.70 ± 0.04 ^b
Impurity B	0.09 ± 0.01 ^b
Impurity C	0.49 ± 0.02 ^b
Quinaldine	< 0.21
Quinazarine	< 0.05

^a (weight of impurity + weight of injected dye) x 100

^b (area of impurity peak + sum of area of C.I. Solvent Yellow 33 and C.I. Solvent Green 3) x 100

Table 3. Histopathological Evaluation of Strain A/J Mice

<u>Organ</u>	<u>No. of Animals</u>	<u>Morphology</u>
Lung	10	Normal
Liver	10	8 animals had normal-appearing livers. 2 animals had a few lesions similar to those observed in mice with viral hepatitis.
Kidneys	10	Normal
Intestines, Stomach	10	Normal
Spleen	10	Normal
Heart	10	Normal
Brain	10	Normal
Salivary and Endocrine Glands	10	Normal
Urinary Bladder	10	Normal

Table 4. Quantitation of C.I. Solvent Yellow 33 and C.I. Solvent Green 3 in Dye Samples

Dye	Weight % (mean + SD)		
	C.I. Solvent Yellow 33	C.I. Solvent Green 3	Total
C.I. Solvent Yellow 33 ^a	93.1 $\pm$ 1.3	-	93.1 $\pm$ 1.3
C.I. Solvent Green 3/ C.I. Solvent Yellow 33 mixture ^b	24.1 $\pm$ 0.5	70.9 $\pm$ 1.1	95.0 $\pm$ 1.0

^a n = 5

^b n = 4

Both compounds were supplied by the U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland. The chemicals were stored at 4°C in the dark.

With sterile technique, the chemicals were administered intraperitoneally (i.p.) by injection as 0.1 ml/dose in tricapylin (glycerol trioctanoate; Eastman Kodak, Rochester, N.Y.). Solutions were freshly prepared immediately before administration. Amber colored bottles were used to protect the chemicals from fluorescent light.

Preliminary toxicology. The maximum solubility of both C.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture in tricapylin is 5 mg/ml. Six repeated i.p. injections of both dyes at a concentration of 0.5 mg/mouse/injection (25 mg/kg) during a two week period did not result in mortality or weight loss of any of the treated animals. Therefore, the MTD for the bioassay was 25 mg/kg.

Bioassays. C.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture were tested at three dose levels: the MTD, 0.5 MTD and 0.2 MTD, with 50 mice (25 males and 25 females) per dose. The groups were injected thrice weekly for eight weeks for a total of 24 administrations. Control groups consisted of untreated, vehicle (tricapylin)-treated, and urethan-treated mice. Urethan is the reference carcinogen in the lung tumor bioassay and, historically, the response to urethan has been approximately 1 tumor/mg dose (3,4,5,6).

Mice given the two test compounds were killed by cervical dislocation 30 weeks after initiation of the bioassay. The lungs were removed and fixed for 48 hr in 70% ethanol containing 5% glacial acetic acid and 5% formaldehyde. Lung tumors, which appeared as pearly-white nodules on the surface of the lung (3,4) were counted and randomly sampled for histological evaluation and confirmation of adenoma. Duplicate tumor counts were obtained by two technicians working independently. In addition, the liver, kidneys, spleen, intestines, thymus, stomach, and the salivary and endocrine glands were examined grossly. If gross lesions were observed, they were examined histologically for the presence of neoplasms.

The tumor incidence (i.e., the percentage of mice that developed lung tumors) was compared between dye-treated and control animals by Chi square analysis. The tumor multiplicity (i.e., the number of lung tumors per mouse) was compared between dye-treated and control animals by analysis of variance (ANOVA).

RESULTS

Toxic effects. The MTD for both C.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture was 25 mg/kg. During the chronic bioassay, 10% of the mice that were treated with the MTD of the C.I. Solvent Yellow 33, and 26% of the animals that received the MTD of the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture, died.

The probable reason for the mortality among mice treated with either compound was the development of peritonitis in the peritoneal cavity of the treated mice. The peritonitis may have been caused by the accumulation of dye(s) in the peritoneal cavity, and the ensuing generalized inflammatory response.

Lung tumor response. Table 5 presents results on the occurrence of lung adenomas in untreated, vehicle-treated and urethan-treated A/J mice. In all groups, the percentage of animals with lung tumors and the average number of lung tumors per mouse in male mice were not significantly different ($p > 0.05$) from those in females. Data from untreated mice represent the "spontaneous" occurrence of lung tumors and are in agreement with earlier results on strain A mice of the same age (3,4,5,6). The tumor responses in mice administered i.p. tricaprylin were lower than in untreated mice of the same sex, but the differences were not significant ($p > 0.05$). Therefore, the occurrence of lung tumors was not affected by the injections of vehicle. The tumor response to urethan was dose-related with approximately 1 tumor per mg dose. This response is in close agreement with previous results on the lung tumor response of strain A mice to this reference carcinogen (3,4,5,6).

Data obtained from bioassays of the two compounds, C.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture, are summarized in Table 6. Chi square analysis of the data on tumor incidence (i.e., the percentage of mice that had lung tumors) showed that none of the dye-treated groups had a significantly higher incidence of tumors than the tricaprylin or untreated controls. Therefore, administration of the dyes had no effect on the likelihood of the mice to develop tumors. In addition, analysis of variance (ANOVA) indicated that the number of lung tumors per mouse in dye-treated mice was not significantly higher than in controls.

Lesions in other organs. The only neoplasm other than the lung adenomas observed at necropsy was a salivary gland tumor in an untreated animal. Salivary gland tumors are common in strain A mice (3); therefore, the presence of this tumor was not an unusual finding.

CONCLUSION

Based on the results of the assays, C.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture are non-carcinogenic by the criterion of lung tumor induction in strain A/J mice. However, higher doses of either compound may have produced tumors. In addition, a negative response for lung tumor induction in strain A mice does not preclude the possibility of a carcinogenic response in another bioassay system.

Table 5. Lung Tumor Response in Untreated, Vehicle-Treated and Urethan-Treated A/J Mice

Treatment	Total Dose (mg/kg)	Sex	Number of Animals Survivors/ Initial	Mice With Lung Tumors (%)	Average Number Lung Tumors/ Mouse	No. of Mice According to the No. of Lung Tumors per Mouse										
						0	1	2	3	4	5	6	7	8	10	>10
Untreated	-	M	24/25	42	0.79 ± 0.29 ^a	14	7	1	0	1	0	1	0	0	0	0
		F	23/25	26	0.39 ± 0.15	17	3	3	0	0	0	0	0	0	0	0
		M+F	47/50	34	0.60 ± 0.17	31	10	4	0	1	0	1	0	0	0	0
Tricaprylin ^b	-	M	22/25	32	0.45 ± 0.17	15	5	1	1	0	0	0	0	0	0	0
		F	25/25	20	0.24 ± 0.10	20	4	1	0	0	0	0	0	0	0	0
		M+F	47/50	26	0.34 ± 0.10	35	9	2	1	0	0	0	0	0	0	0
Urethan ^c	1000	M	4/4	100	23.25 ± 1.25	0	0	0	0	0	0	0	0	0	0	4
		F	4/4	100	26.00 ± 4.30	0	0	0	0	0	0	0	0	0	0	4
		M+F	8/8	100	24.62 ± 2.14	0	0	0	0	0	0	0	0	0	0	8
500	500	M	4/4	100	6.25 ± 2.36	0	0	0	1	1	0	1	0	0	1	0
		F	4/4	100	8.50 ± 1.55	0	0	0	3	0	0	0	0	1	0	0
		M+F	8/8	100	7.37 ± 1.37	0	0	0	4	1	0	1	0	1	1	0
200	200	M	4/4	100	5.75 ± 1.55	0	0	0	2	0	0	1	0	0	0	1
		F	4/4	100	4.25 ± 1.25	0	0	0	0	0	1	0	1	0	1	1
		M+F	8/8	100	5.00 ± 0.96	0	0	0	2	0	1	1	1	0	1	2

Values are mean ± standard error.
^bTricaprylin was administered i.p. thrice-weekly during a period of 8 weeks at 0.1 ml/dose.
^cUrethan was administered in a single i.p. injection at dose levels of 1000, 500 and 200 mg/kg.

Table 6. Lung Tumor Response in A/J Mice to C.I. Solvent Yellow 33 and C.I. Solvent Green 3/C.I. Solvent Yellow 33 Mixture

Compound	Amount Per Injection ^b (mg/kg)	Total Dose ^a (mg/kg)	Sex	Number of Animals Survivors/ Initial	Mice With Lung Tumors (%)	Average Number Lung Tumors/ Mouse	No. of Mice According to the No. of Lung Tumors per Mouse			
							0	1	2	3
C.I. Solvent Yellow 33	25	600	M	21/25	43	0.52 + 0.15 ^c	12	7	2	0
			F	24/25	33	0.37 + 0.12	16	7	1	0
			M+F	45/50	38	0.44 + 0.09	28	14	3	0
	12.5	300	M	24/25	17	0.21 + 0.10	20	3	1	0
			F	25/25	24	0.32 + 0.13	19	4	2	0
			M+F	49/50	20	0.27 + 0.08	39	7	3	0
	5	120	M	23/25	35	0.39 + 0.12	15	7	1	0
			F	23/25	22	0.30 + 0.13	18	3	2	0
			M+F	46/50	28	0.35 + 0.09	33	10	3	0
C.I. Solvent Green 3/C.I. Solvent Yellow 33 Mixture	25	600	M	20/25	25	0.25 + 0.10	15	5	0	0
			F	17/25	12	0.12 + 0.08	15	2	0	0
			M+F	37/50	19	0.19 + 0.07	30	7	0	0
	12.5	300	M	22/25	36	0.50 + 0.16	14	5	3	0
			F	23/25	26	0.26 + 0.09	17	6	0	0
			M+F	45/50	31	0.39 + 0.09	31	11	3	0
	5	120	M	25/25	28	0.48 + 0.17	18	3	3	1
			F	25/25	28	0.28 + 0.09	18	7	0	0
			M+F	50/50	28	0.38 + 0.10	36	10	3	1

^aTotal cumulative dose per animal.

^bC.I. Solvent Yellow 33 and the C.I. Solvent Green 3/C.I. Solvent Yellow 33 mixture were given i.p. 3 times per week for 8 weeks for a total of 24 administrations.

^cValues are mean ± standard error.

REFERENCES

1. Stoner, G.D., Greisiger, E.A., Schut, H.A.J., Pereira, M.A., Loeb, T.R., Klaunig, J.E. and Branstetter, D.G. A comparison of the lung adenoma response in strain A/J mice after intraperitoneal and oral administration of carcinogens. *Toxicol. Appl. Pharmacol.* 72:313-323, 1984.
2. Henderson, R.F., Bimonthly Technical Progress Report No. 2 entitled, "Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions," Army Project Order No. 3807, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, July 15, 1983, to U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, Maryland.
3. Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K. and Gori, G.B. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. *Cancer Res.* 33:3069-3085, 1973.
4. Shimkin, M.B. and Stoner, G.D. Lung tumors in strain A mice: Application to carcinogenesis bioassay. *Adv. Cancer Res.* 21:1-58, 1975.
5. Stoner, G.D. and Shimkin, M.B. Strain A mouse lung tumor bioassay, *J. Amer. Coll. Toxicol.* 1:145-169, 1982.
6. Stoner, G.D., Shimkin, M.B., Troxell, M.C., Thompson, T.L., and Terry, L.S. Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. *Cancer Res.* 36:1744-1747, 1976.

DISTRIBUTION LIST

25 copies

Commander
US Army Medical Bioengineering
Research and Development Laboratory
ATTN: SGRD-UBG
Fort Detrick
Frederick, MD 21701

4 copies

USAMRDC (SGRD-RMS)
Fort Detrick
Frederick, MD 21701

12 copies

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDA
Cameron Station
Alexandria, VA 22314

1 copy

Dean
School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

1 copy

Commandant
Academy of Health Sciences, US ARMY
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

1 copy

Commander
US Army Medical Bioengineering
Research and Development Laboratory
ATTN: SGRD-UBD-A/Librarian
Fort Detrick
Frederick, MD 21701

DATE
FILMED
-8