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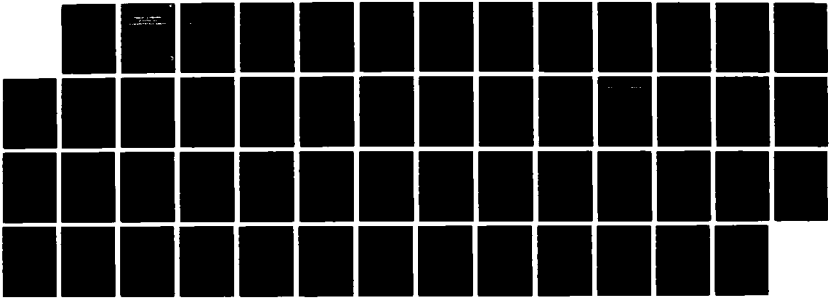

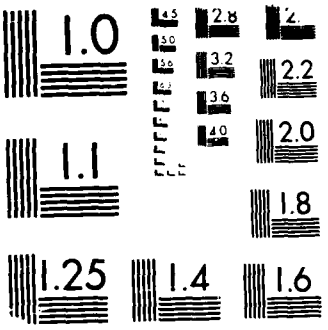

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PRIMARY SCREENING OF POTENTIAL RADIOPROTECTIVE AGENTS

ANNUAL REPORT

CURTIS P. SIGDESTAD, PH.D.

1 October 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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Louisville, Kentucky 40292

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This report deals with primary screening of potential radiation protective agents. The drugs to be tested were provided by the U.S. Army Medical Research and Development Command, Fort Detrick, Maryland. The compounds were tested in toxicity screens to determine the maximum tolerated dose (MTD) effects. Limited available drug amounts precluded more refined testing. The second screen involved Cobalt-60 gamma radiation. The agents to be tested were injected intraperitoneally into CDI female Swiss mice, thirty minutes prior to irradiation with either 9.0 or 9.5 Gy. The latter value was found to be the radiation LD100(30) for this mouse strain. Survival was measured and the degree of protection was determined.

Dose modification factors were determined on a limited number of agents as directed by the COR.

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SUMMARY

This report deals with primary screening of potential radiation protective agents. The drugs to be tested were provided by the U. S. Army Medical Research and Development Command, Fort Detrick, Maryland. The compounds were tested in toxicity screens to determine the maximum tolerated dose (MTD) which was defined as the highest dose that produces no lethal effects. Limited available drug amounts precluded more refined testing. The second screen involved Cobalt-60 gamma radiation. The agents to be tested were injected intraperitoneally into CD1 female Swiss mice, thirty minutes prior to irradiation with either 9.0 or 9.5 Gy. The latter value was found to be the radiation LD100(30) for this mouse strain. Survival was measured and the degree of protection was determined.

Dose modification factors were determine on a limited number of agents as directed by the COR.

FOREWORD

In conducting research using animals, the investigator(s) 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 Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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INTRODUCTION

The Armed Forces of the United States have a mandate to provide health services to its members. This includes prophylactic care for numerous conditions of which the protection from ionizing radiation is only one. The U. S. Army has spearheaded the search for effective anti-radiation drugs since the first description that an agent can protect animals from the adverse effects of x-rays. It has been through their efforts that the development of WR-2721 has been shown to be the most effective protector. This benchmark protector is, however, not entirely optimal, inasmuch as it shows some toxicity, is effective only for a few hours, does not pass the blood-brain barrier, and it is not well absorbed when taken orally. for these reasons the search goes on for better protectors which will provide the needed protection for military personnel in the event of having to perform their duties in an environment that will likly expose them to levels of ionizing radiation which will be detrimental to their well being.

This report describes the initial testing of potential radiation protective agents. It reports results on toxicity determinations, radiation effectiveness screens and studies in some depth the better protectors identified.

MATERIALS and METHODS

1. Animals:

The animals used in the toxicity and radioprotection screens were viral antibody free (VAF) CD1 Swiss female mice. They are obtained from Charles Rivers Laboratories and shipped from their Portage, Michigan facilities. Animals are delivered in filtered crates to the University's Animal Care Center. Upon receipt the animals are examined and any sign of ill health is reported immediately before any of the animals are caged. Mice are housed 5 to a cage and are kept for 14 days before being used in experimental trials. The cages are placed on racks in a laminar

flow unit. The animals are kept on a 12 hr light cycle, they are fed Purina Lab Chow 5010 ad libitum and are maintained on hyperacidified water (pH 2.7) to inhibit the growth of Pseudomonas species.

Serological monitoring for Sendai, PVC, MHV and Mycoplasm is routinely performed by the vendor and repeated by the Veterinary staff upon receipt and at weeks one and two after arrival. Standard bacteriological sampling is part of the quality control program. Animal care personnel are outfitted with shoe covers, disposable gowns, caps, masks and gloves when handling the animals. The animal housing facility, cages, water bottles, bedding material and feed are subjected to a strict regimen of sanitation and sterilization procedures.

Animals surviving the thirty day test period are disposed of by means of Carbon Dioxide euthanasia under conditions described in the "Guide for Laboratory Animal Facilities and Care".

2. Test Drugs:

The compounds to be tested in the toxicological and radioprotection screening are supplied by the U. S. Army Medical Research and Development Command. Technical support is provided by the Contracting Officers Representative (COR) at the Walter Reed Institute for Research. Table one lists the drugs submitted for testing along with the submitters.

In order to avoid possible degradation of the test agents extreme care is taken to provide optimal storage contiditons. Upon receipt the drugs are immediately stored according to the instructions provided on the accompanying data sheets. They are kept under desiccation with Drierite either in a refrigerator or freezer. Possible photodegradation is minimized by storage in amber bottles and avoiding direct exposure to light. Before testing the compounds are allowed to equilibrate to room temperature. The drugs are weighed and dissolved or suspended in a suitable vehicle immediately before injection. Drugs soluble in water are dissolved in sterile, nonpyrogenic water for injection.

TABLE ONE

SUBMITTER	WR	COMPOUNDS
Lamar Field Vanderbilt Univ.	255541	Sodium 3(p-tolyldithio) propanesulfinate $C_{10}H_{13}O_2S_3 \cdot Na$
Lamar Field Vanderbilt Univ.	255542	Disodium(1,4-butylene bis dithio)bis(3-propanesulfinate) $C_{10}H_{20}O_4S_6 \cdot 2Na \cdot H_2O$
Lamar Field Vanderbilt Univ.	255544	Disodium 3,3'trithio bis (propanesulfinate) $2x\ (C_6H_{12}O_4S_3) \cdot 4Na\ 3 \cdot H_2O$
Ludwig Bauer U.of Illinois	254353	S-[N[2-[1-(4-Fluorophenyl)-2-adamantyl] ethyl]carbamidinium] methyl phosphorothioate Monohydrate $C_{20}H_{28}FN_2O_3PS \cdot H_2O$
Ludwig Bauer U.of Illinois	254593	S-(N-[2(2-Phenyl-1-adamantyl)ethyl]carbamidinium]methyl) phosphorothioate $C_{20}H_{29}N_2O_3PS$
A.L.Ternay U.of Texas	254407	L- cysteine cysteamine disulfide Hydrochloride $C5H_{12}N_2O_2S_2 \cdot HCl$
A. L. Ternay U. of Texas	256107	Cysteamyl 2-(3amino-propylamino)ethyldisulfide Trihydrochloride $C_7H_{18}N_2S_2 \cdot 3HCl$
A. L. Ternay U. cf Texas	256234	2-(3-aminopropylamino) ethyl 2-hydroxyethyl disulfide Dihydrochloride $C_7H_{18}N_2OS_2 \cdot 2HCl$
Ash Stevens, Inc.	2721	S-2-Aminopropylamino) ethyl phosphorothioic acid Trihydride $C_5H_{15}N_2O_3PS \cdot 3H_2O$

TABLE ONE Cont.

SUBMITTER	WR	COMPOUNDS
Ash Stevens, Inc.	1065	2-(3-Aminopropylamino) ethyl Mercaptan Dihydrochloride $C_5H_{14}N_2S \cdot 2\ HCl$
Ash Stevens, Inc.	151327	S-3-(3-Methylaminopropylamino) propylphosphorothioic Acid Trihydrate $C_7H_{19}N_2O_3PS \cdot 3H_2O$
Ash Stevens, Inc.	254677	S-[2-(3-Aminopropylamino) ethylthio]-L-cysteine Dihydrochloride $C_8H_{19}N_3O_2S_2 \cdot 2HCl$
Ash Stevens, Inc.	255549	2-(3-Aminopropylamino) ethylsulfinic acid Hydrochloride $C_5H_{14}N_2O_2S \cdot 2HCl$
Ash Stevens, Inc.	255591	2-[(3-Methylaminopropyl) amino]ethanethiol Dihydrochloride $C_6H_{16}N_2S \cdot 2HCl$
Ash Stevens, Inc.	151326	3-(3-Methylaminopropyl amino)propyl Mercaptan Dihydrochloride $C_7H_{18}N_2S \cdot 2HCl$
F. I. Carroll	254638	S-2-(2'-Thiocarbamido ethylamino)ethyl Lithium Hydrogen Phosphorothioate Trihydrate $C_5H_{12}N_3O_3PS_2 \cdot Li \cdot 3H_2O$
F. I. Carroll	254676	S-2-(2'Amidinoethyl-amino)ethylphosphoro-thioic Acid Hemihydrate $2x\ C_5H_{14}N_3O_3PS \cdot H_2O$

TABLE ONE Cont.

SUBMITTER	WR	COMPOUNDS
F. I. Carroll	254721	S-2(2'-N-Methyl-amidinoethylamino)ethyl-phosphorothioic Acid Trihydrate $C_6H_{16}N_3O_3PS \cdot 3H_2O$
F. I. Carroll	255830	S-2[2'-(4,5-Dihydroimidazoyl)ethyl-amino]ethyl Lithium Hydrogen Phosphorothioate Hydrate $C_7H_{15}N_2O_3PS \cdot Li \cdot H_2O$
F. I. Carroll	256281	S-2-(2'-tert-butylcarbamoylethylamino) ethyl Dilithium Phosphorothioate Hemihydrate $2x\ C_9H_{19}N_2O_4PS \cdot 4Li \cdot H_2O$
F. I. Carroll Research Triangle Institute	257614	4-(3-Methylaminopropyl)-5,6-Dihydro-1,2,4-3(4H) Dithiazinethione Hydrochloride $C_7H_{14}N_2S_3 \cdot HCl$
James C. Piper Southern Research Institute	255538	S,S'-2-(3-Methylaminopropylamino)-trimethylenebis(phos-phorothioic Acid) Monohydrate $C_7H_{20}N_2O_6P_2S_2 \cdot H_2O$
James C. Piper Southern Research Institute	255709	1-{[3-(3-aminopropyl)] thiazolidin-2-yl}-D-gluco-1,2,3,4,5-pentane-pentol Dihydrochloride $C_{11}H_{24}N_2O_5S \cdot HCl$

TABLE ONE Cont.

SUBMITTER	WR	COMPOUNDS
James C. Piper Southern Research Institute	257623	S-3-(3-Methylamino- propylamino)propyl Thioacetate Dihydrobromide $C_9H_{20}N_2OS \cdot 2HBr$
Klayman/Scoville	3689	S-[2-(Methylaminopropyl) aminoethyl]phosphoro- thioic Acid Monohydrate $C_6H_{17}N_2O_3PS \cdot H_2O$
Southwest Research Institute	255796	2-(3-Aminopropylamino) ethane sulfonic Acid Hydrochloride $C_5H_{14}N_2O_3S \cdot HCl$
Sigma Company	015443	a-Ketoglutaric Acid Crystalline Monosodium Salt $C_5H_5O_5Na$
W. O. Foye	254115	$C_{20}H_{28}N_2S$ I

Drugs which are found to be insoluble in water are suspended 0.3% methylcellulose, 15% ethanol and water or as indicated on the data sheet. The drug amount is formulated so that injections are administered at 1% of individual body weight. The acidity of the highest injected dose is measured and recorded. All drug doses mentioned represent the free base weight and are corrected for salt and water content of the individual compounds. The drugs are administered by intraperitoneal injection thirty minutes prior to irradiation.

3. Drug Toxicity Studies:

Groups of 5 to 10 mice are injected i.p. with the test agent. At least three doses are used to determine the highest dose that resultes in 100% survival which is considered the maximum tolerated dose (MTD).

4. Irradiation Procedures:

An Atomic Energy of Canada (AECL) Therac 780 Cobalt Teletherapy unit is used as a radiation source for all radiation protection testing. The dose rate is 1.1 Gy/minute at a Source to Surface Distance (SSD) of 78.5 cm. The surface field size is 35 x 35 cm and the backscatter factor is determined to be 1.084. Dosimetry is performed by the Departmental radiological physics staff using a Victoreen Condensor R Meter with additional Thermoluminescence dosimetry (TLD).

The animal holder is placed on an electric device which rotates animals at about 4 rpm in the irradiation field. This procedure assures a uniform dose delivered to each mouse and correctes for any field flatness problems.

Originally, the mice were allowed to freely move in a well ventilated leucite cylindrical container 30 cm in diameter and 4 cm high. Ultimately a animal holding device with the same dimensions but divided into twelve individual compartments is utilized. This provides greater precision in individual mouse dosimetry.

A. Control Mice: Radiation Sensitivity

Unprotected mice were extensively studied to determine baseline radiation sensitivity. This included Probit Analysis for six and thirty day mortality which reflects gastrointestinal and hematopoietic related deaths respectively.

B. Radiation Protection Screens:

Assays of radiation protection utilize drug doses at the maximum tolerated dose (MTD), one-half the MTD and one-fourth the MTD. Ten mice are each injected i.p. with the appropriate dose and irradiated with a dose which assures 100% lethality of control, unprotected mice. Survival is followed for thirty days.

C. Dose Modification Factors:

Probit Analysis is applied in the determination of the dose modification factor (DMF). Six radiation doses, which are expected to bracket the LD50, are selected at an equal log interval. Mice are either injected i.p. with the test agent or its solvent (control, unprotected) and irradiated whole-body thirty minutes later. Survival is determined for thirty days post irradiation. DMFs are determined by multiple probit analysis which results in a potency ratio with 95% confidence limits.

RESULTS and DISCUSSION

1. Animals:

Cultures from mouth, eye and sipper tubes were taken, periodically, to determine whether pathogenic bacteria were modifying the response to irradiation. In addition, sterile blood cultures were obtained before and after drug or radiation treatment. The results indicated that there was no contamination of pathogenic organisms, specifically Peusdomonas. Blood cultures were sterile and blood counts did not indicate an infection.

2. Irradiated, Unprotected Test Animals:

A. Comparison of Irradiation Procedures:

This experiment was performed because the original

irradiation procedure allowed mice to roam free in a leucite chamber while being rotated in the gamma beam. The mice were observed to crawl over one another or 'pile up' at the edge of the container. This presented dosimetry problems which could add scatter to the data. A comparative study was designed to test if irradiation in a container with individual compartments improved the precision from the original procedure. The 30-day lethality of unrestricted and restricted animals at either 9.0, 9.5 or 10 Gy was compared. Figures 1-3 compare the three doses individually, while figures 4-5 compare restricted vs. unrestricted for all doses tested.

Mice irradiated with a dose of 9 Gy showed 20% survival when animals were allowed to roam free in the irradiation chamber. As the dose increased to 9.5 Gy this difference was abolished. A second important finding is seen in figure 3, where 10 Gy was administered. Here it can be noticed that gastrointestinal death is definitely included at this dose level. Early deaths between days 5 and 7 should be considered gut related.

Figures 4-5 compare survival time of irradiated restricted or unrestricted test animals at all doses. When mice were irradiated in the restricted container 100% lethality was noted at all three radiation doses, while unrestriced mice showed 20% survival at 9.0 Gy. As in the previous figures the inclusion of gastrointestinal syndrome was noted with 10.0 Gy.

B. Gastrointestinal Death:

Initial studies to determine the sensitivity of the gastrointestinal epithelium of the CD1 female mouse were performed. Table 2, shows the results of these studies. The lethal dose to 50% of the mice was found to be 12.77 $\pm$ 0.3 Gy. The resultant probit curve was linear with a probability of 99.8%.

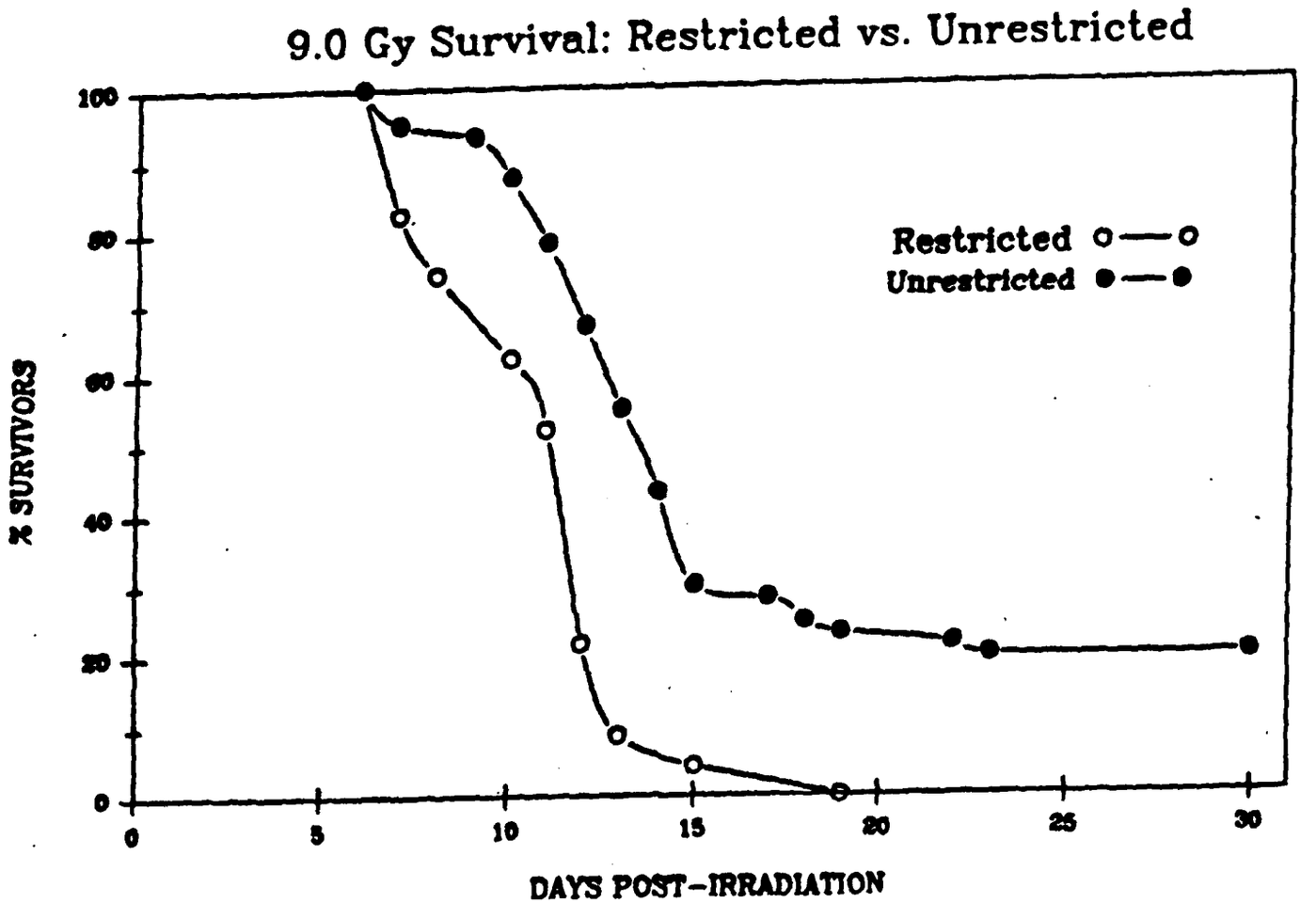
9.0 Gy Survival: Restricted vs. Unrestricted
% SURVIVORS
100
80
60
40
20
0
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
Restricted
Unrestricted

Fig. 1

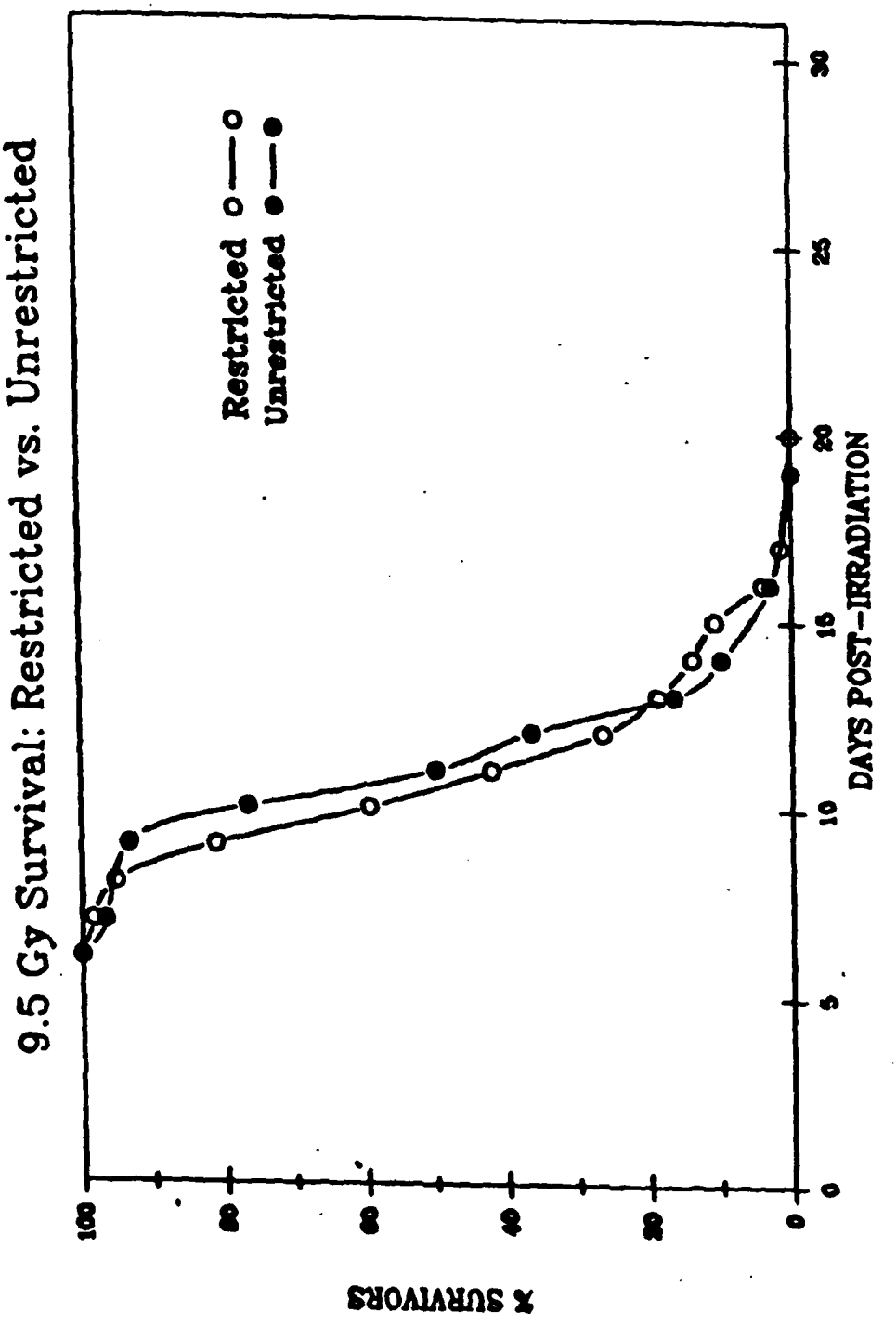
9.5 Gy Survival: Restricted vs. Unrestricted
Restricted o—o
Unrestricted ●—●
% SURVIVORS
100
80
60
40
20
0
0
5
10
15
20
25
30
DAYS POST-IRRADIATION

Fig. 2

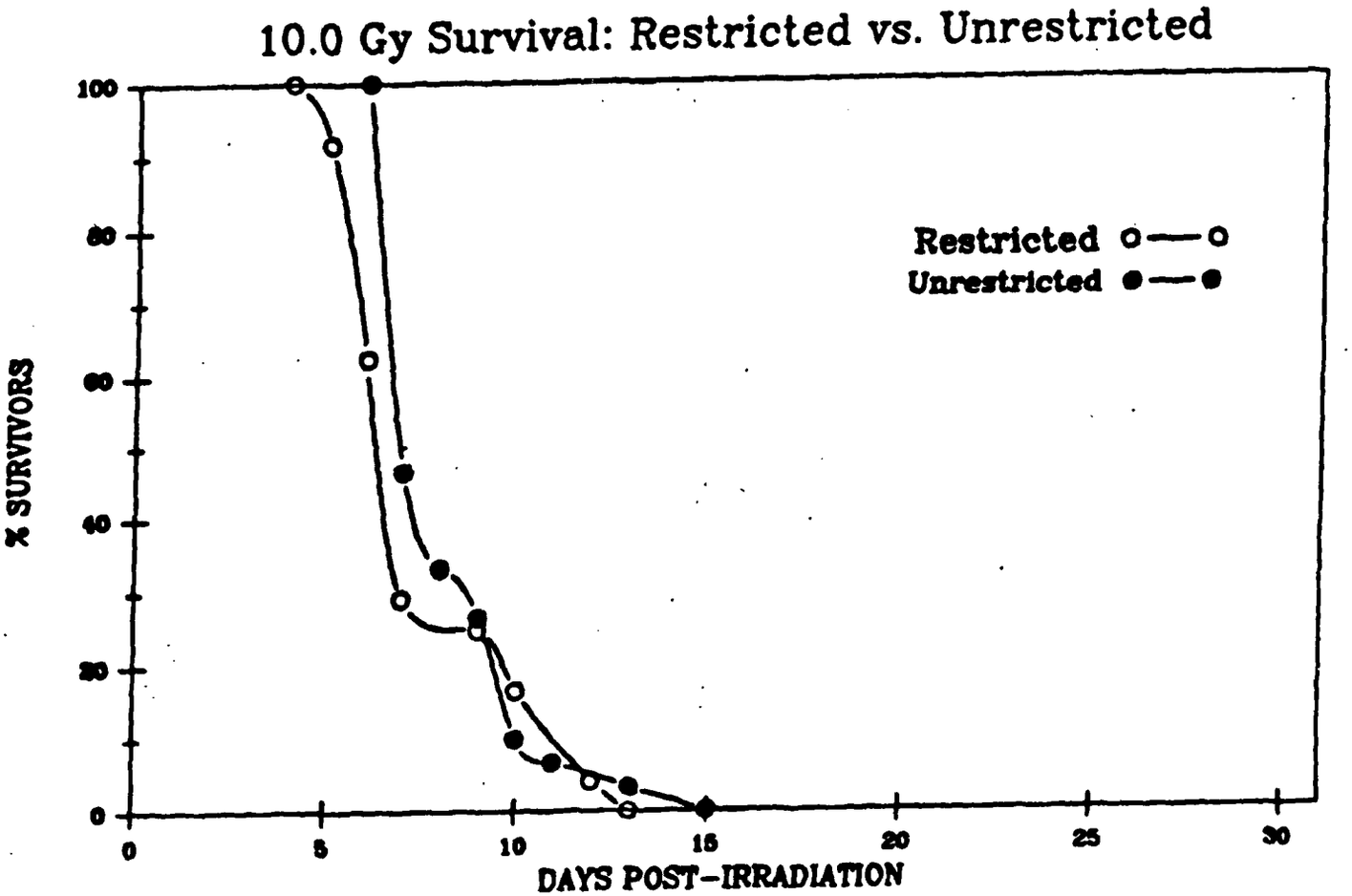
10.0 Gy Survival: Restricted vs. Unrestricted
% SURVIVORS
100
80
60
40
20
0
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
Restricted
Unrestricted

Fig. 3

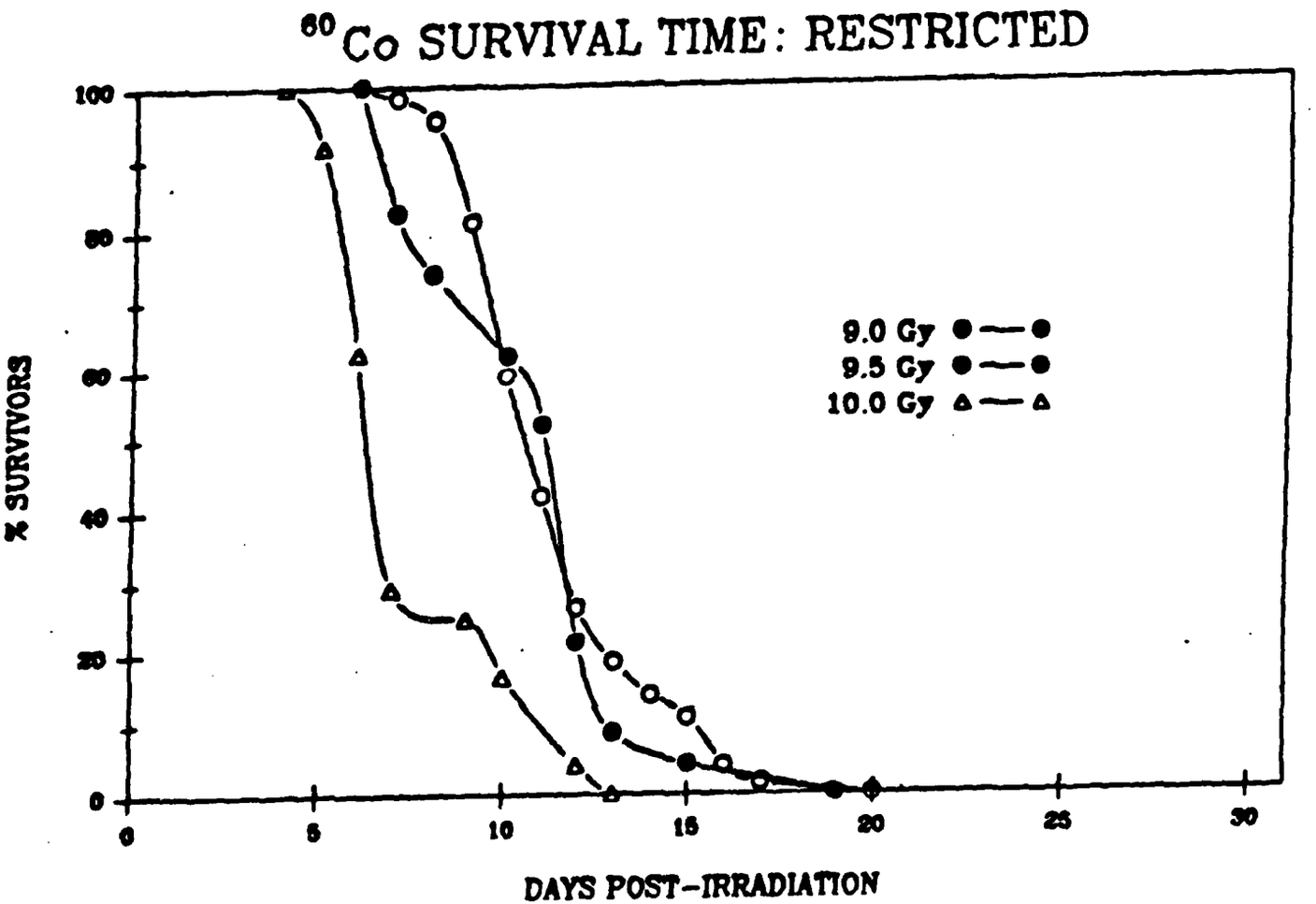
^{60}Co SURVIVAL TIME: RESTRICTED
% SURVIVORS
100
80
60
40
20
0
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
9.0 Gy
9.5 Gy
10.0 Gy

Fig. 4

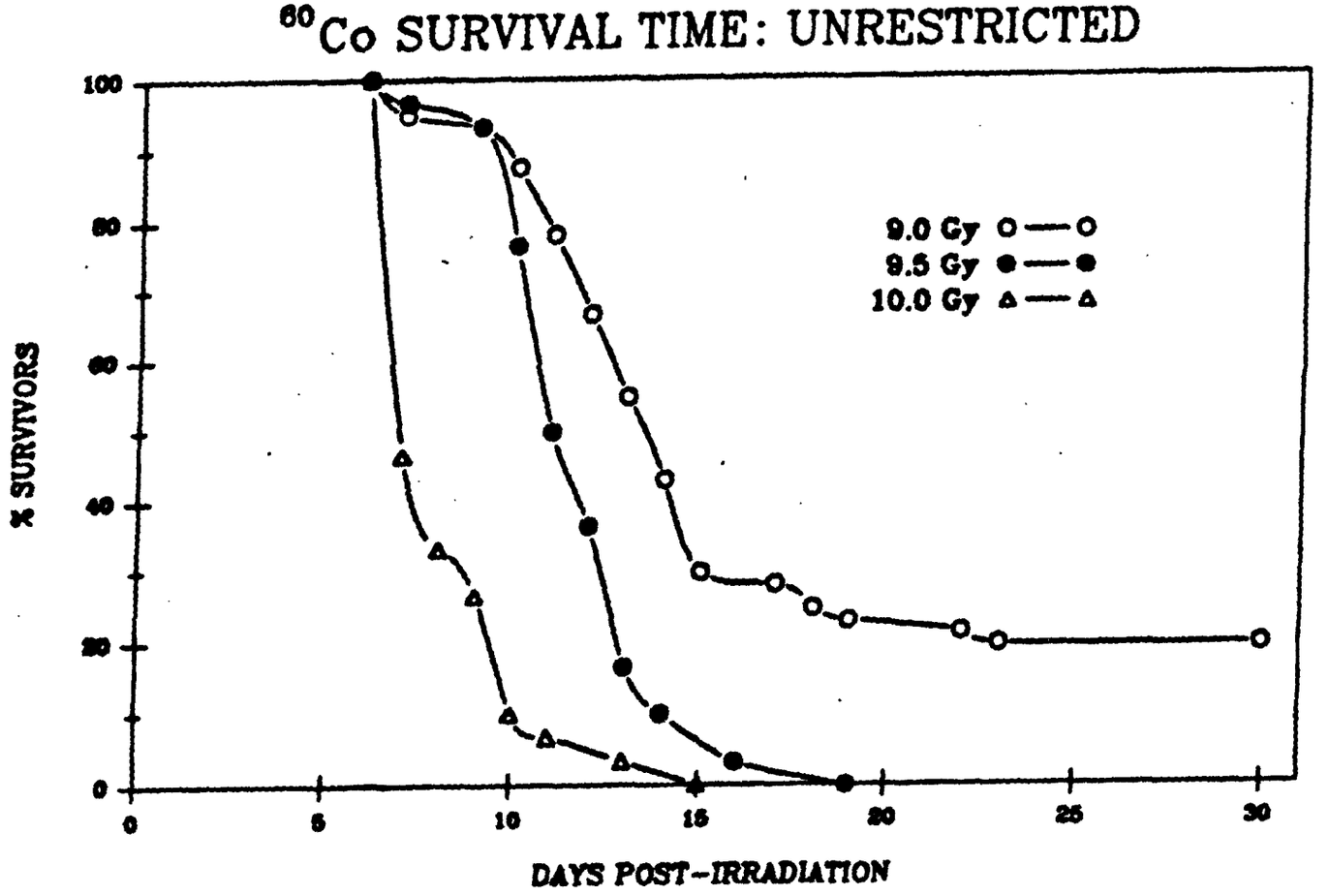
^{60}Co SURVIVAL TIME: UNRESTRICTED
% SURVIVORS
100
80
60
40
20
0
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
9.0 Gy
9.5 Gy
10.0 Gy

Fig. 5

TABLE 2

Seven Day Mortality after Cobalt-60 Irradiation

Dose (Gy)	n	Lethality	Percent
Exp 87-8			
11.17	15	3	20
12.29	15	4	27
13.52	15	8	53
14.88	15	8	53
16.36	15	15	100
$LD_{50(7)}$ = 12.77 ± 0.33 Gy		Linearity = 99.8%	

C. Hematopoietic Death:

Three determinations of the LD50(30) were performed during the contract year. The initial study which tested only 10 mice per dose resulted in a LD50 of 7.19 Gy which was apparently a low estimate of this value. Table 3, shows the results of this experiment, and figure 6 depicts the survival times for the six highest radiation doses used in this study.

The second study in this series utilized 22 mice per point and gave results which appear more probable. The LD50 was found to be 7.92 ± 0.05 Gy (Table 4). Figure 7 shows the survival time of mice after various radiation doses. This correlated well with the third experiment the results of which are shown in Table 5 and Figure 8. The LD50 was found to be 7.73 ± 0.07 Gy which is not statistically significantly different from the second study.

TABLE 3

Thirty Day Lethality after Cobalt-60 Irradiation

Dose (Gy)	n	Lethality	Percent
Exp 86-2			
5.75	10	0	0
6.61	10	0	0
7.60	10	8	80
8.74	10	10	100
10.05	10	10	100
11.56	10	10	100
13.30	10	10	100
$LD_{50(30)}$ = 7.19 Gy $\pm$ 0.37 Gy		Linearity - 99.59%	

TABLE 4

Thirty Day Mortality after Cobalt-60 Irradiation

Dose (Gy)	n	Lethality	Percent
Exp 87-14			
6.00	22	0	0
6.60	22	0	0
7.26	22	2	9
7.98	22	14	64
8.78	22	20	91
9.66	22	22	100
$LD_{50(30)}$ = 7.92 $\pm$ 0.08 Gy		Linearity = 84.5%	

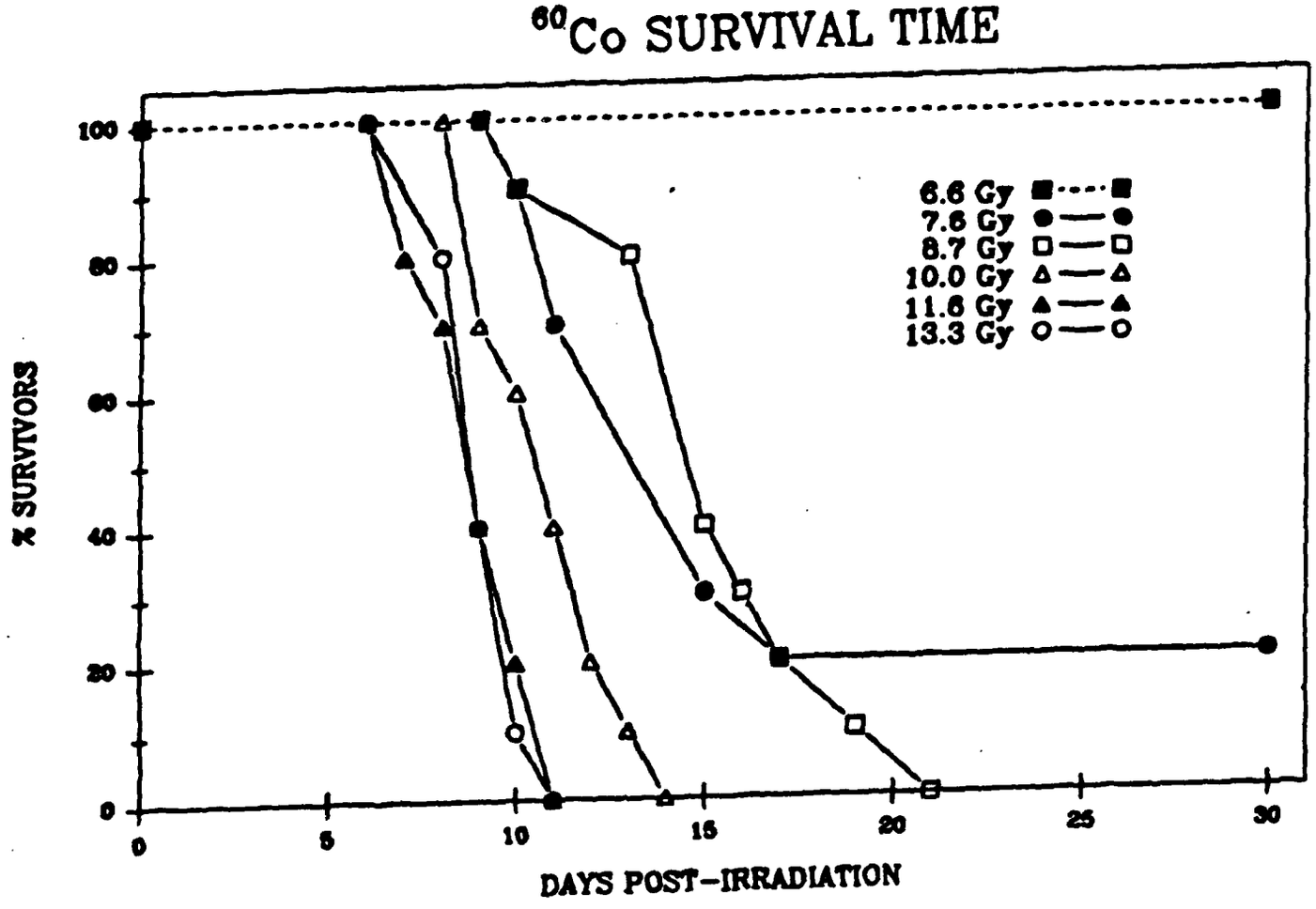
^{60}Co SURVIVAL TIME
% SURVIVORS
DAYS POST-IRRADIATION
6.6 Gy
7.6 Gy
8.7 Gy
10.0 Gy
11.6 Gy
13.3 Gy
0
20
40
60
80
100
0
5
10
15
20
25
30

Fig. 6

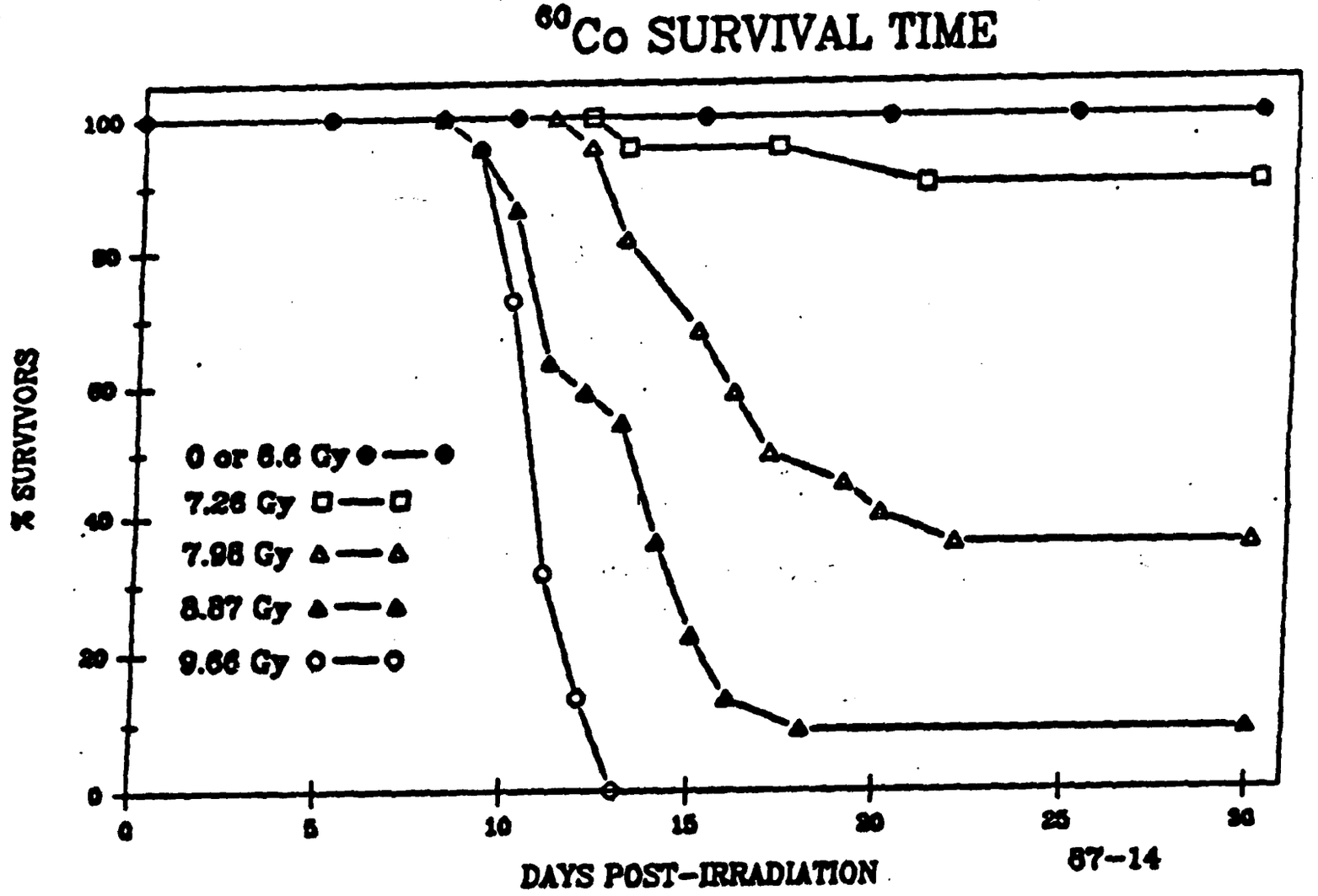
^{60}Co SURVIVAL TIME
% SURVIVORS
100
80
60
40
20
0
0 or 6.6 Gy
7.26 Gy
7.96 Gy
8.87 Gy
9.66 Gy
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
87-14

TABLE 5

Thirty Day Lethality after Cobalt-60 Irradiation

Dose (Gy)	n	Lethality	Percent
Exp 87-16			
6.21	24	0	0
7.02	24	5	21
7.93	36	22	61
8.96	24	21	88
10.13	12	12	100
$LD_{50(30)}$ = 7.73 Gy ± 0.07 Gy		Linearity - %	

3. WR-2721 Studies

A. Toxicity

Mice were injected i.p. with WR-2721 in doses which ranged from 737 to 1107 mg/kg (base). Probit analysis indicated a LD50 of 972 mg/kg. Subsequent experiments used 600 mg/kg base as WR-2721 benchmark studies.

B. Radiation Protection with WR-2721:

Dose modification factors were determined for four drug doses: 150, 300, 476 and 600 mg/kg base. The results are shown in Figure 9 and in Table 6-7.

C. Time of Injection:

Mice were injected with WR-2721 (600 mg/kg, base) at 5, 15, 30, 45, 60 and 90 minutes and 3, 6, 12, 24 and 48 h prior to irradiation with Cobalt-60 gamma rays. A dose of 12 Gy was selected to assure lethality when protection was minimal. This dose of WR-2721 afforded 100% survival as early as 5 minutes prior to irradiation. This level of protection continued for injection times up to and including 90 minutes. At three hours, however, protection was reduced to 80% and at 6 hours, no protection was noted (Table 8). If a lower radiation dose would have been used, perhaps, protection would have been extended beyond the three hour time interval noted in these experiments.

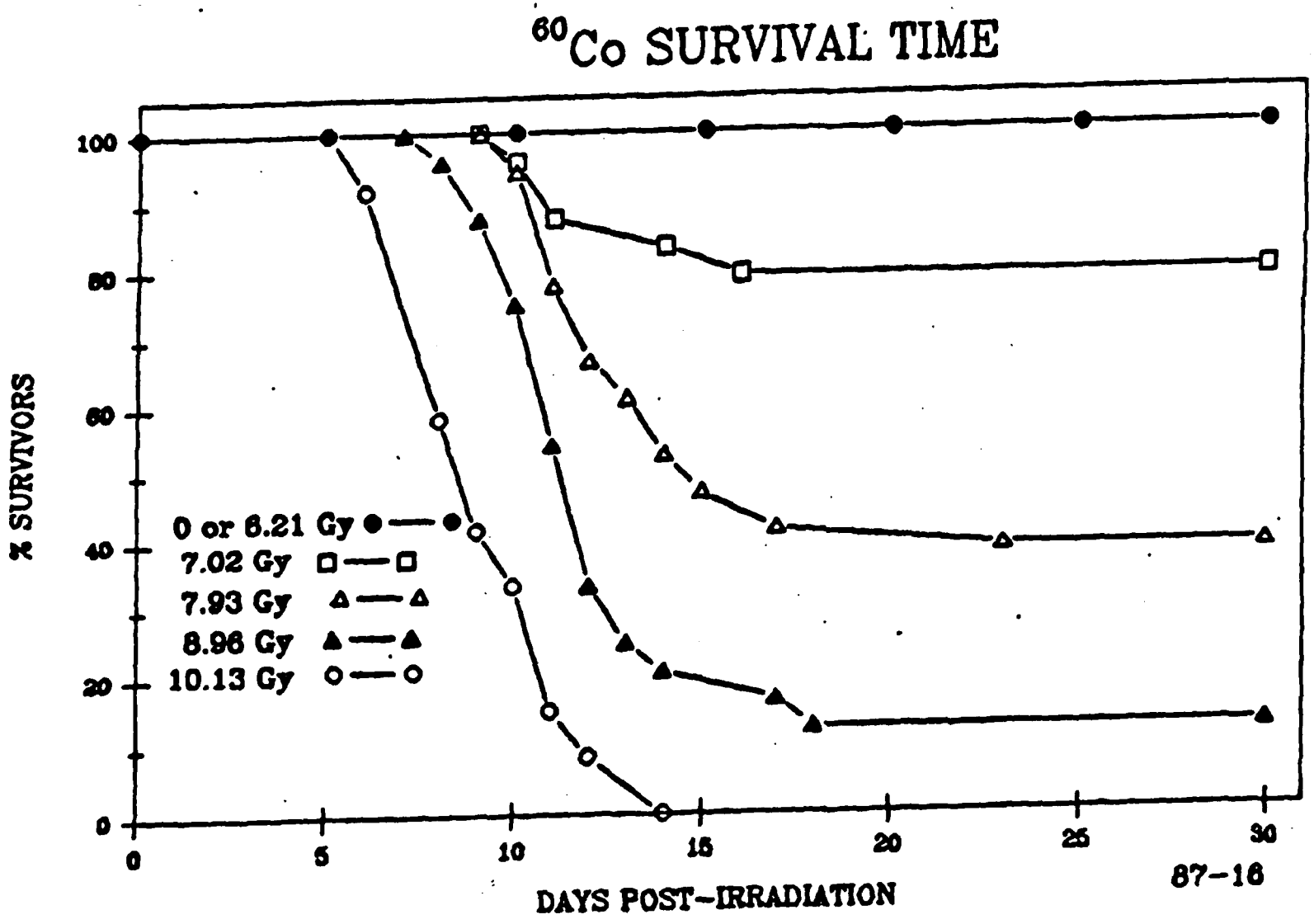
^{60}Co SURVIVAL TIME
% SURVIVORS
100
80
60
40
20
0
0 or 6.21 Gy
7.02 Gy
7.93 Gy
8.96 Gy
10.13 Gy
0
5
10
15
20
25
30
DAYS POST-IRRADIATION
87-16

Fig. 8

TABLE 6

DOSE MODIFICATION BY WR-2721

DOSE (mg/kg)	RADIATION Dose (Gy)	SURVIVORS	PERCENT	$LD_{50(30)}$	95% CL
0				7.83	7.79-7.88
150	9.76	9/10	90	11.67	11.41-11.94
	10.74	5/9	56		
	11.82	6/10	60		
	13.00	2/10	20		
	14.30	0/10	0		
	15.73	0/10	0		
	17.30	0/10	0		
300	13.22	10/10	100	19.06	18.00-23.00
	14.55	9/10	90		
	16.00	10/10	100		
	17.60	9/10	90		
	19.36	4/10	40		
	21.29	0/10	0		
476	13.63	14/15	93	20.21	19.67-20.74
	15.00	15/15	100		
	16.50	15/15	100		
	18.15	0/15	0	Spurious Deaths	
	19.97	15/15	100		
	21.96	5/15	33		
	24.16	2/15	13		
	26.57	1/15	7		
	29.23	0/15	0		

TABLE 6 (Cont.)

DOSE MODIFICATION BY WR-2721 (Cont.)

DOSE (mg/kg)	RADIATION Dose (Gy)	SURVIVORS	PERCENT	$LD_{50(30)}$	95% CL
600	20.00	13/15	87	23.80	23.65-23.96
	22.00	13/15	87		
	24.20	7/15	47		
	26.62	2/15	13		
	29.28	0/15	0		
	32.21	0/15	0		

TABLE 7

DMF of WR2721

Dose		DMF	
mg/kg [Base]	LD50(30)	773 RAD*	792 RAD*
150	1167	1.51	1.47
300	1906	2.47	2.41
476	2020	2.61	2.55
600	2380	3.08	3.01

* Values used as the denominator of the DMF calculation as determined in Experiments 87-14 and 87-16.

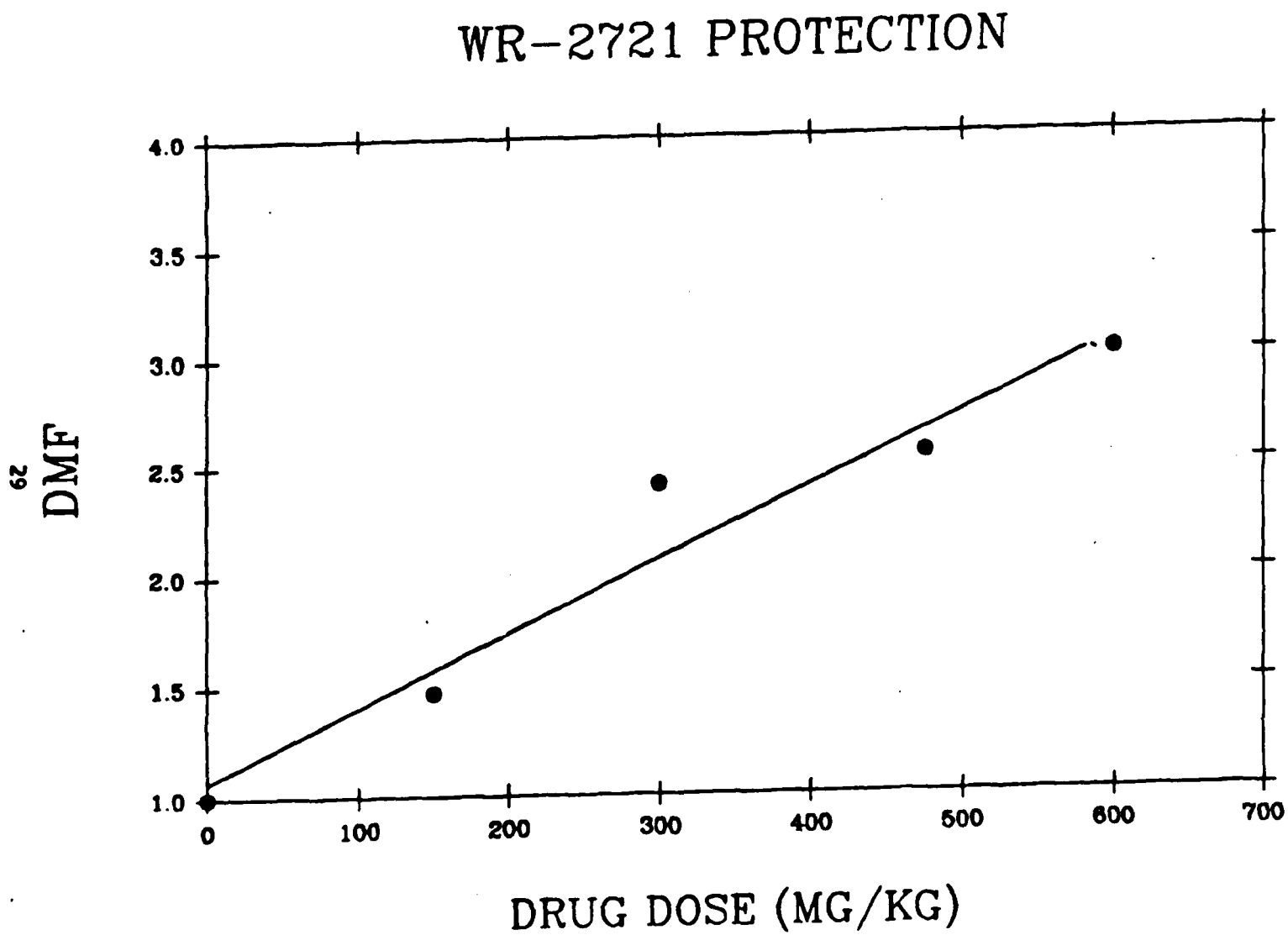
WR-2721 PROTECTION
^{62}DMF
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
100
200
300
400
500
600
700
DRUG DOSE (MG/KG)

Fig. 9

TABLE 8

TIME OF INJECTION

WR-2721 (600 mg/kg) and 12.0 Gy

TIME PRIOR TO IRRADIATION	30-DAY SURVIVORS	SURVIVAL PERCENT
5 Min	10/10	100
15 Min	10/10	100
30 Min	10/10	100
45 Min	10/10	100
60 Min	9/10	90[1]
90 Min	10/10	100
3 Hr	8/10	80
6 Hr	0/10	0
12 Hr	0/10	0
24 Hr	0/10	0
48 Hr	0/10	0

[1] Spurious Death

4. Toxicity Screening:

Twenty eight compounds were received from the USAMRDC for toxicity and radioprotection screening. Table 1 gives a detailed listing of these drugs and their submitters, respectively. The toxicity screenings for these compounds have been completed and the data are presented in table 9.

Of these drugs four (WR-254115; WR-254353; WR-254593; WR-257614) were found to be rather toxic with a maximum tolerated dose(MTD) of 37.5 mg/kg or less. Six of the tested agents were relative non-toxic with no lethalities observed at the 600 mg/kg dose level (see table 10). The majority of the radioprotective agents had MTDs were in the range between 150 and 300 mg/kg [Base].

With three drugs: WR-254676 and the adamantyl-amidinium compounds WR-254353 and WR-254593 difficulties in dissolving or suspending them were encountered. Several vehicles containing varying ratios of Methylcellulose, Ethanol and Tween-80 were tried to improve the solubility of the above mentioned agents. However, none of the tested vehicles resulted in a homogeneous suspension. The results for these agents should, therefore, be judged with care.

Another problem was noted, concerning the increase in toxicity in three drugs between the initial toxicity screening and the radioprotection testing, although all compounds were handled and stored with utmost care. For the drug WR-254593 the MTD decreased from 37.5 to 9.4 mg/kg; the MTD for WR-255830 decreased from 150mg/kg to 100mg/kg and for WR-3689 the MTD changed from 1200 to 1000mg/kg.

5. Radioprotection Screening:

Out of the twenty seven compounds (excluding WR-2721) which were received for testing of their radioprotective potential nearly one third (28%) afforded 100% protection against radiation induced death (see Table 11). Three of these drugs were submitted by Ash Stevens Inc., two were synthesized by F.I. Carroll and one came each from J.C. Piper and A.L. Ternay,

TABLE 9

TOXICITY SCREENS

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
1065	BK 71365	1200	Water	i.p.	5/5	0/5	0
		600			5/5	0/5	0
		300			5/5	0/5	0
		150			0/5	5/5	100
		75			0/5	5/5	100
3689	BN 62848	1200	Water	i.p.	0/5	5/5	100
		600			0/5	5/5	100
		300			0/5	5/5	100
15443	BL 09435	1200	Water	i.p.	6/10	4/10	40
		600			0/10	10/10	100
		300			0/10	10/10	100
151326	BL 00101	300	Water	i.p.	3/5	2/5	40
		250			1/5	4/5	80
		200			1/5	4/5	80
		150			0/5	5/5	100
151327	BK 98991	1200	Water	i.p.	5/5	0/5	0
		600			0/5	5/5	100
		300			0/5	5/5	100

[1] 3% Methyl Cellulose, 0.4% Tween-80, 15% Ethanol. [2] 0.3% Methyl Cellulose, 15% Ethanol. [3] 5% Sodium Bicarbonate.

TABLE 9 (Cont.)

TOXICITY SCREENS[1]

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
254115	ZP 55243	75	see [1]	i.p.	10/10	0/10	0
		37.5			10/10	0/10	0
		18.75			9/10	1/10	10
		9.38			0/10	10/10	100
254353	ZP 55289	300	see [2]	i.p.	5/5	0/5	0
		75			5/5	0/5	0
		37.5			5/5	0/5	0
		18.75			0/5	5/5	100
254407	ZP 54399	1200	Water	i.p.	5/5	0/5	0
		600			0/5	5/5	100
		300			0/5	5/5	100
254593	ZP 55305	300	see [2]	i.p.	5/5	0/5	0
		75			5/5	0/5	0
		37.5			0/5	5/5	100
		18.75			0/5	5/5	100
254638	ZP 55467	750	Water	i.p	5/5	0/5	0
		600			3/5	2/5	40
		300			0/5	5/5	100

TABLE 9 (Cont.)

TOXICITY SCREENS

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
254676	BL 08830	300	see [2]	i.p.	2/10	8/10	80
		150			1/10	9/10	90
		75			0/10	10/10	100
254677	BL 08778	1200	Water	i.p.	10/10	0/10	0
		600			10/10	0/10	0
		300			5/10	5/10	50
		150			0/10	10/10	100
254721	BL 09346	300	see [2]	i.p.	5/10	5/10	50
		150			0/10	10/10	100
		75			0/10	10/10	100
255538	BK 40404	1200	see [3]	i.p.	5/5	0/5	0
		600			5/5	0/5	0
		300			5/5	0/5	0
		150			0/5	5/5	100
		75			0/5	5/5	100
		37.5			0/5	5/5	100

TABLE 9 (Cont.)

TOXICITY SCREENS

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
255541	BK 40468	300	Water	i.p.	5/5	0/5	0
		150			4/5	1/5	20
		75			0/5	5/5	100
255542	BK 40477	600	Water	i.p.	5/5	0/5	0
		450			3/5	2/5	40
		300			0/5	5/5	100
255544	BK 40486	750	Water	i.p.	4/5	1/5	20
		600			2/5	3/5	60
		300			0/5	5/5	100
255549	BK 40780	1200	Water	i.p.	0/5	5/5	100
		600			0/10	10/10	100
		300			0/10	10/10	100
255591	BL 24405	1200	Water	i.p.	5/5	0/5	0
		600			5/5	0/5	0
		300			0/5	5/5	100
		150			0/5	5/5	100
		75			0/5	5/5	100

TABLE 9 (COnt.)

TOXICITY SCREENS

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
255709	BK 75176	1200	Water	i.p.	5/5	0/5	0
		600			5/5	0/5	0
		300			0/5	5/5	100
		150			0/5	5/5	100
		75			0/5	5/5	100
255796	BL 21977	1200	Water	i.p.	0/5	5/5	100
		600			0/5	5/5	100
		300			0/5	5/5	100
255830	BL 22358	1200	Water	i.p.	10/10	0/10	0
		600			10/10	0/10	0
		300			9/10	1/10	10
		150			0/10	10/10	100
256107	BL 26892	300	Water	i.p.	10/10	0/10	0
		150			10/10	0/10	0
		75			0/10	10/10	100

TABLE 9 (Cont.)

TOXICITY SCREENS

WR	BN	DOSE(mg/kg)	VEHICLE	ROUTE	LETHALITY	SURVIVAL	%
256234	BL	300	Water	i.p.	5/5	0/5	0
	27915	150			0/5	5/5	100
		75			0/10	10/10	100
		37.5			0/10	10/10	100
256281	BL	1200	Water	i.p.	10/10	0/10	0
	28529	600			10/10	0/10	0
		300			0/10	10/10	100
		150			0/10	10/10	100
257614	BL	150	Water	i.p.	5/5	0/5	0
	49073	75			5/5	0/5	0
		37.5			1/5	4/5	80
		18.75			0/5	5/5	100
257623	BL	200	Water	i.p.	5/5	0/5	0
	49242	150			1/5	4/5	80
		100			0/5	5/5	100

TABLE 10

DISTRIBUTION OF MAXIMUM TOLERATED DOSE (mg/kg)

FOR TEST AGENTS

9.38	18.75	37.5	75	100	150	300	600	1200
254115	254353	254593[1]	254676	257623	1065	254638	15443	3689[1]
	257614		255107	255541	151326	255542	151327	255549
					254677	255544	254407	255796
					254721	255591		
					255538	255709		
					255830[1]	256281		
					256234			

[1]Toxicity increase since initial screening.

respectively. Two agents (8.3%), one of which was a sulfinate containing compound prepared by L. Field and the well documented compound WR-3689 submitted by Klayman/Scoville protected ninety percent of the test animals. Three drugs accounting for 12.5% all from the laboratory of F. I. Carroll lead to 80% survival in animals injected with these drugs. A survival rate of 70% was obtained with two drugs; both were submissions from Ash Stevens, Inc. A sulfinate compound from L. Field and one amidinium containing drug systhesized by L. Bauer yielded 60% protection. The remaining eight drugs (33.3%) from several different synthesis groups produced radioprotection of 50% or less. The detailed of the radiation protection screens for all drugs are presented in Table 12.

A. Ash Stevens, Inc.

From the compounds submitted by this synthesizer, WR-255591 (the free thiol of WR-3689) a new drug which has never been tested before, proved to be an excellent radioprotector, yielding 100% protection from a lethal radiation dose at all three drug dose levels (300, 150 and 75 mg/kg) tested. A dose modification study is in progress using this protector.

The methylated analog of WR-2721 compound WR-151327 exhibited 100% protection at the MTD of 600mg/kg and at one half MTD. Seventy percent survival was achieved with the dose of 150 mg/kg at 9.0 Gy. This compound is currently being retested at 9.5 Gy. The well examined protector WR-1065 afforded 90; 100 and 10% protection when tested at 150; 75 and 37.5mg/kg at a radiation dose of 9.0 Gy. However, only 70; 60 and 0% survival was noted at the higher dose of 9.5 Gy.

Of the two other drugs from the same submitter WR-254677, which yields WR-1065 and cysteine, and WR-255549, WR-1065 oxidized to the sulfinate, only WR-254677 provided 70% protection at the MTD of 150mg/kg. No effect was seen at the lower drug doses. The agent WR-255549 revealed no protective potential at all.

B. F.I.Carroll

Two compounds synthezised by F.I.Carroll exhibited good protection at the highest administered doses. Drug WR-254638 a congener of WR-2721 led to 100; 40 and 10% survival, while with WR-254676, an amidine analog of WR-3689 animal survival was 100; 70 and 10%. The other analogs of WR-3689; WR-254721, WR-255830 and WR-256281 all afforded 80% protection at the highest tested drug doses but only 50%, 20% and 60% respectively at one-half MTD.

C. Lamar Field

Two disulfide and one trisulfide-containing compounds from this submitter were screened. Only one of these, WR-255542, led to 90% survival at the highest administered dose. Moderate protection of 60% of the treated animals was observed with the drug WR-255541 while the third compound WR-255544 showed only a minimal protection effect.

D. A.L.Ternay

Three drugs came from the laboratory of this synthesizer. The L-cysteine cysteamine disulfide WR-254407 led to 100; 80 and 10% survival for the three tested drug doses. The two other compounds, WR-256107 (which hydrolyzes to cysteamine and WR-1065) and WR-256234 (which yields WR-1065 and β-mercaptoethanol) proved to have only moderate protective capabilities. With both drugs only 50% of the irradiated test animals survived.

E. J.C. Piper

Two protective agents were submitted by J.C.Piper. With the Phosphorothioate WR-255538 100% protection at the highest dose was achieved, while WR-255709, a thiazolidin containing agent provided very marginal (30%) protection at the highest tested dose.

F. Ludwig Bauer

The drugs prepared by this submitter were WR-254593 and WR-254353. These compounds are Adamantyl-amidinium containing agents with a covered thiol function. With both

drugs, which are rather toxic, a moderate survival rate of 60 and 50% was achieved at the MTDs of 18.75mg/kg.

G. Others

The remaining compounds were submissions from different synthezisers. The known protector WR-3689, the methyl analog of WR-2721 was prepared by Klayman/Scoville and was tested at irradiation doses of 9.0 and 9.5 Gy. In both screens this compound afforded 90% animal survival at all three dose levels. W.O.Foye submitted WR-254115 a compound that revealed only minimal protection (30%) as did the Sodium -Ketoglutarate from Sigma Company, which had shown activity against cyanide challenge.

TABLE 11

WR DRUGS AND THEIR RESULTANT PERCENT SURVIVAL

(9.0[1] Gy and 9.5 Gy)

Submitter	100	90	80	70	60	50	40-30	20-10	0
A.Stevens	1065[2] 151327[1] 255591[1]			1065[2] 254677					255549
F.I.Carroll	254638 254676		254921 255830 256281						
Lamar Field		255542			255541			255544	
James C. Piper	255538[1]						255709[1]		
A. Ternay	254407[1]					256107 256234			
L. Bauer					254593	254353			
W. O. Foye Klayman Scoville Sigma Corp.			3689[2]				254115	15443	

[1]Screen performed with 9.0 Gy

[2]Screen performed with both 9.0 and 9.5 Gy.

TABLE 12

RADIOPROTECTION SCREEN

WR	BN	DOSE[1] (mg/kg)	VEHICLE	ROUTE	RADIATION DOSE (Gy)	THIRTY DAY SURVIVORS	PERCENT SURVIVAL
NONE					9.5	0/30	0
						0/12	0
						0/15	0
1065	BK 71365	150	Water	i.p.	9.0	9/10	90
		75				10/10	100
		37.5				1/10	10
1065	BK 71365	150	Water	i.p.	9.5	7/10	70
		75				6/10	60
		37.5				0/10	0
3689	BN 62848	1200	Water	i.p.	9.0	9/10	90
		600				9/10	90
		300				10/10	100
3689	BN	1200	Water	i.p.	9.5	2/10[4]	20
		600				9/10	90
		300				9/10	90
15443	BL 09435	600	Water	i.p.	9.5	2/10	20
		300				2/10	20
		150				3/10	30

TABLE 12 (Cont.)

RADIOPROTECTION SCREEN

WR	BN	DOSE[1] (mg/kg)	VEHICLE	ROUTE	RADIATION DOSE (Gy)	THIRTY DAY SURVIVORS	PERCENT SURVIVAL
151327	BK 98991	600 300 150	Water	i.p.	9.0	10/10 10/10 7/10	100 100 70
254115	ZP 55243	9.38 4.69 2.35	see [2]	i.p.	9.5	3/10 3/10 0/10	30 30 0
254353	ZP 55289	18.75 9.38 4.69	see [2]	i.p.	9.5	5/10 6/10 2/10	50 60 20
254407	ZP 54399	600 300 150	Water	i.p.	9.0	10/10 8/10 1/10	100 80 10
254593	ZP 55305	37.5 18.75 9.38 4.69	see [2]	i.p.	9.5	0/10 6/10 2/10 0/10	0[4] 60 20 0

TABLE 12 (Cont.)

RADIOPROTECTION SCREEN

WR	BN	DOSE[1] (mg/kg)	VEHICLE	ROUTE	RADIATION DOSE (Gy)	THIRTY DAY SURVIVORS	PERCENT SURVIVAL
254638	ZP 55467	300 150 75	Water	i.p.	9.5	10/10 4/10 1/10	100 40 10
254676	BL 08830	150 75 37.5	see [2]	i.p.	9.5	10/10 7/10 1/10	100 70 10
254677	BL 08778	150 75 37.5	Water	i.p.	9.5	7/10 0/10 0/10	70 0 0
254721	BL 09346	150 75 37.5	see [2]	i.p.	9.5	8/10 5/10 3/10	80 50 30
255538	BK 40404	150 75 37.5	see [2]	i.p.	9.0	10/10 2/10 4/10	100 20 40

TABLE 12 (Cont.)

RADIOPROTECTION SCREEN

WR	BN	DOSE[1] (mg/kg)	VEHICLE	ROUTE	RADIATION DOSE (Gy)	THIRTY DAY SURVIVORS	PERCENT SURVIVAL
255541	BK 40468	100	Water	i.p.	9.5	6/10	60
		50				2/10	20
		25				0/10	0
255542	BK 40477	300	Water	i.p.	9.5	9/10	90
		150				4/10	40
		75				2/10	20
255544	BK 40486	300	Water	i.p.	9.5	2/10	20
		150				1/10	10
		75				0/10	0
255549	BK 40780	1200	Water	i.p.	9.5	0/10	0
		600				0/10	0
		300				0/10	0
255591	BL 24405	300	Water	i.p.	9.0	10/10	100
		150				10/10	100
		75				10/10	100

TABLE 12 (Cont.)

RADIOPROTECTION SCREEN

WR	BN	DOSE[1] (mg/kg)	VEHICLE	ROUTE	RADIATION DOSE (Gy)	THIRTY DAY SURVIVORS	PERCENT SURVIVAL
255709	BK	300	Water	i.p.	9.0	3/10	30
	75176	150				1/10	10
		75				2/10	20
255830	BL	150	Water	i.p.	9.5	3/10	30[4]
	22358	100				8/10	80
		75				2/10	20
		37.5				0/10	0
256107	BL	75	Water	i.p.	9.5	5/10	50
	26892	37.5				1/10	10
		18.88				0/10	0
256234	BL	150	Water	i.p.	9.5	5/10	50
	27915	75				2/10	20
		37.5				0/10	0
256281	BL	300	Water	i.p.	9.5	8/10	80
	28529	150				6/10	60
		75				4/10	40

[1] 3% Methyl Cellulose, 0.4% Tween-80, 15% Ethanol. [2] 0.3% Methyl Cellulose, 15% Ethanol. [3] 5% Sodium Bicarbonate. [4] Toxicity related death.

CONCLUSIONS

1. The screening procedures were developed and tested with new investigators and a new mouse strain. The results obtained, with previously tested compounds appeared to be in agreement with data reported in the past.

2. The lethal dose to 50% of CD1 female mice was found to be 7.83 Gy. The gastrointestinal LD50 was found to be 12.77 Gy.

3. The optimal time of injection for WR-2721 was found to be between 5 to 90 minutes prior to irradiation.

4. In addition to WR-2721 the following drugs protected mice from the $LD_{50(30)}$ when administered at the maximum tolerated dose: WR-1065, WR-151327, WR-254638, WR-254676, WR-254407, WR-255538 and WR-255591.

5. WR-255591 showed 100% protection from the LD100 dose at the MTD, 0.5 MTD and 0.25 MTD.

PUBLICATIONS

1. C. P. Sigdestad, D.J. Grdina, A.M. Connor and W.R. Hanson, A Comparison of Radioprotection from Three Neutron Sources and Cobalt-60 by WR-2721 and WR-151327. Radiat. Res. 106:224-233 (1986).

2. J. S. Zhang, C.P. Sigdestad, M.A. Gemmell and D. J. Grdina, Modification of Radiation Response in Mice by Fractionated Extracts of Panex ginseng. Radiat. Res. 112:156-163 (1987).

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