Special Publication

Armed Forces Radiobiology Research Institute

Sensitivity of Guinea Pigs to Gamma Radiation

Sharon A. McBride
Tracy B. Kneisler
Steven M. Strocko
Roderick Dela Pena
Terry C. Pellmar

Special Publication 97-1

Special Publication

Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, Md. 20889-5603

Sensitivity of Guinea Pigs to Gamma Radiation

Sharon A. McBride
Tracy B. Kneisler
Steven M. Strocko
Roderick Dela Pena
Terry C. Pellmar

Special Publication 97-1 April 1997

This and other AFRRI publications are available to qualified users from the Defense Technical Information Center, Attention: OCP, 8725 John J. Kingman Road, Suite 0944, Fort Belvoir, VA 22060-6218; telephone (703) 767-8274. Others may contact the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161; telephone (703) 487-4650. AFRRI publications are also available from university libraries and other libraries associated with the U.S. Government's Depository Library System.

Contents

Introduction

Numerous studies investigated the effects of ionizing radiation on neuronal, gastrointestinal, and hematopoietic functioning in the guinea pig (Bond and Robertson, 1957; Batchelor et al., 1973; Lorenz et al., 1952; Lamarque and Gary-Bobo, 1956; Bond and Sugahara, 1968; Ellinger, 1945). Previous studies, the most recent of which was conducted nearly 25 years ago (Batchelor et al., 1973), reported $LD_{50/30}$ γ-radiation doses ranging from 2.5 to 6.5 Gy. The purpose of this work is to provide preliminary data for studies designed to assess the impact of sublethal γ radiation and nerve agent exposure on the seizure threshold. The efficacy of current therapy in humans before or after exposure to a nerve agent is more closely mimicked in the guinea pig than in any other animal model. The guinea pig model of nerve-agent-induced seizures has been extensively studied and currently is used in the development and testing of anticonvulsant drugs by the U.S. Army Institute of Chemical Defense. Given the wide variability in the $LD_{50/30}$ doses for γ radiation in guinea pigs presented in previous reports and the improvements in animal husbandry techniques over the past 25 years, this study was conducted to determine a physiologically relevant dose of γ radiation for use in our γ-radiation/nerve agent seizure threshold studies.

Methods

Animals

Male Hartley guinea pigs (Harlan), certified pathogen-free, were quarantined on arrival for 2 weeks and screened for disease before being released into the colony. Guinea pigs were housed six to a cage, and there was no evidence of fighting. Guinea pigs were fed with commercial guinea pig chow and acidified water (pH 2.5-3) *ad libitum*. Animal holding rooms were maintained at 21° ± 1°C with 50% ± 10% relative humidity using at least ten air changes per hour of 100% conditioned fresh air. Full spectrum lights were used to maintain a 12-hr light-dark cycle that commenced at 0600. Guinea pigs weighed 350-450 g at the beginning of the experiment.

Radiation Procedure

Guinea pigs were irradiated in a bilateral γ radiation field of the AFRRI ^{60}Co facility (Carter and Verrelli, 1973). For irradiation, guinea pigs were placed in well-ventilated acrylic plastic tubes. Animals were habituated to the plastic tubes for 1 hour daily for the 5 days before irradiation. The midline tissue doses to the animals were 1, 2, 3, 4, or 5 Gy and were delivered at a fixed dose rate of 1 Gy/min. An acrylic plastic stand holding the tubes in a stacked position allowed simultaneous irradiation of six animals. Before irradiation, the dose rate was established in an acrylic guinea pig phantom using a 0.5-cc tissue equivalent ionization chamber (calibration factor traceable to the National Institute of Standards and Technology). The tissue-to-air ratio was 0.95, and the field was uniform to within ± 2%. Dosimetric measurements were made in accordance with the American Association of Physicists in Medicine protocol for the determination of absorbed dose from high energy photon and electron beams (AAPM TG21). Sham irradiations consisted of animals being placed in restraining tubes and held in the cobalt facility exposure room for the same amount of time as the irradiated animals, with no radiation source present.

Data Collection and Analysis

Guinea pigs were weighed once daily (Monday through Friday) 5 days before irradiation and 29 days after irradiation. Statistical differences in weight gain during the observation period for each of the dose groups was determined using one-way ANOVA. Initial weight was used as a covariate in ANOVA of weight gain because the initial weights for the dose groups were significantly different. The number of animals surviving in each group was used to determine the $LD_{50/30}$ by probit analysis.

Results

LD50 and Lethality Timecourse

Guinea pigs were exposed to a range of doses of γ radiation (1–5 Gy) that was consistent with the range of previously reported $LD_{50/30}$ doses. The health of the animals was monitored for 30 days after radiation. Probit analysis of the number of survivors from each dose group at the 30-day endpoint revealed that the $LD_{50/30}$ for γ-irradiated test animals was 2.2 Gy (1.5 Gy–2.7 Gy, 95% confidence interval). Dose dependence is exhibited by the percentage of animals surviving 30 days postirradiation (table 1). All animals exposed to 1 Gy of γ radiation survived through the 30-day test period; however, none of the animals exposed to 5 Gy survived more than 9 days.

Survival is presented in figure 1. In each of the radiation dose groups, no deaths occurred until the sixth day after irradiation, and no deaths occurred after 11 days, suggesting that the window of days 6–11 after irradiation is the most critical. The days

Table 1. Deaths and percent survivors by dose after irradiation.

Dose (Gy)	Day of death postirradiation	Survivors (30 days postirradiation)
1	–	100%
2	9–11	55%
3	6–9	27%
4	9–11	8%
5	7–9	0%

of deaths due to irradiation in each dose group are presented in table 1.

Body Weights

Body weights before and after irradiation (mean ± standard error) for the exposure groups are presented

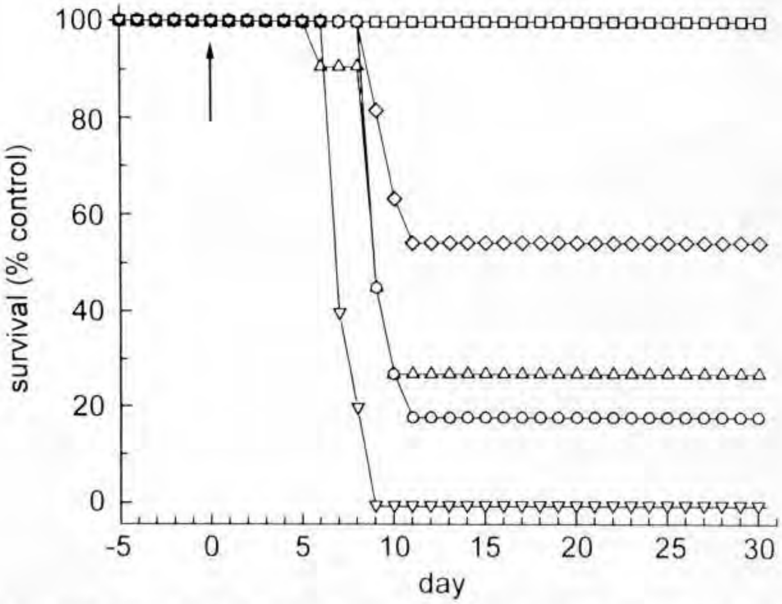

Fig. 1. Animal survival following γ irradiation. Guinea pigs were exposed on day 0 (at arrow) to 1 Gy (□, n=6), 2 Gy (◊, n=11), 3 Gy (Δ, n=11), 4 Gy (o, n=11), or 5 Gy (∇, n=5) or sham radiation (+, n=11 obscured from view by squares (1 Gy)). Animal survival is expressed as percent of surviving control animals for 30 days following γ irradiation. Arrow depicts day of irradiation.

Table 2. Mean body weights (g) ± standard error.

Dose (Gy)	Day 0	Day 4	Day 7	Day 15	Day 29
Sham	401 ± 16.4 *n*=11	444 ± 13.8 *n*=11	481 ± 15.2 *n*=11	504 ± 33.6 *n*=11	661 ± 16.0 *n*=11
1	440 ±10.1 *n*=6	448 ± 10.1 *n*=6	482 ± 10.6 *n*=6	564 ± 15.2 *n*=6	605 ± 19.9 *n*=6
2	362 ± 26.7 *n*=11	384 ± 25.1 *n*=11	408 ± 26.5 *n*=11	468 ± 21.7 *n*=6	613 ± 11.6 *n*=6
3	403 ± 16.8 *n*=11	418 ± 16.1 *n*=11	456 ± 17.9 *n*=10	418 ± 26.8 *n*=3	579 ± 43.8 *n*=34
4	399 ± 13.5 *n*=11	423 ± 12.1 *n*=11	443 ± 11.0 *n*=11	456 ± 5.2 *n*=2	574 ± 23.9 *n*=2
5	345 ± 2.8 *n*=5	385 ± 3.8 *n*=5	408 ± 4.5 *n*=2	ND *n*=0	ND *n*=0

ND, not determined

in table 2 and figure 2. Weights before irradiation were significantly different for the six groups (p = 0.053). The 1-Gy group had the largest average weight (440 ± 10.1 g), and the 5-Gy group had the smallest average weight (345 ± 2.8 g). Average weights for each group, therefore, were adjusted to the average starting weight of animals from all groups (399.5 g) for all additional analyses.

On days 4 and 7 after irradiation, weight gain was analyzed. Days 4 and 7 were chosen because they were several days after irradiation and before many

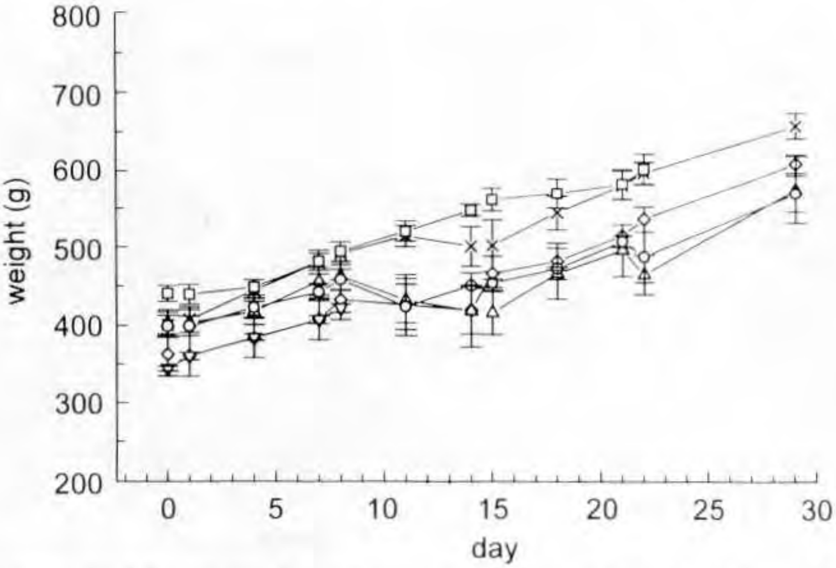

Fig. 2. Weight gain in guinea pigs following γ irradiation: 1 Gy (□, *n*=6), 2 Gy (◊, *n*=11), 3 Gy (Δ, *n*=11), 4 Gy (o, *n*=11), or 5 Gy (∇, *n*=5); sham-irradiated (x, *n*=11). Animals were weighed regularly for 29 days. Weight (average ± standard error) for each group is expressed as function of the day after irradiation.

radiation lethalities occurred. By day 4, significant differences in weight gain were found among the sham vs. radiation groups ($p < 0.001$). There were no significant weight gain differences, however, among the five radiation dose groups ($p = 0.1$). The average adjusted weight gain of irradiated animals was 20.0 $\pm$ 1.6 g, and that of the sham irradiated animals was 43.8 $\pm$ 3.1 g. On day 7, the effect of radiation also was significant ($p < 0.001$). The average adjusted weight gain of irradiated animals was 48.7 $\pm$ 1.8 g and that of the sham irradiated animals was 80.7 $\pm$ 3.7 g. Statistical comparisons among groups after day 7 were not feasible due to deaths of irradiated animals, but qualitative results suggest that the difference in weight gain between irradiated and sham-irradiated animals is consistent for all time points. There was no correlation between preirradiation body weight and lethality within the dose groups.

Pathological Observations

Lethality after γ irradiation occurred exclusively during the second week after exposure, with no animals dying until 6 days postirradiation. To establish the likely cause of death, several animals from each of the dose groups were necropsied. The most prominent lesions observed during the time period of maximum mortality were thin, watery blood (anemia) and mild pinpoint hemorrhages (petechiae) within both the mucosa of the gastrointestinal tract and the parenchyma of the lungs. During necropsy, mild hemorrhagic diarrhea was noted segmentally within the lumen of the small intestine of some animals, but no clinical signs of diarrhea were observed. Most major internal organ systems appeared pale, a consequence of anemia resulting from the hematopoietic syndrome.

Discussion

The $LD_{50/30}$ for guinea pigs exposed to γ radiation in this study (2.2 Gy) is less than expected from reports published through 1968. A review of the literature indicated that the $LD_{50/30}$ for guinea pigs exposed to γ radiation was between 2.5 and 6.5 Gy. We predicted that the $LD_{50/30}$ for guinea pigs exposed to γ radiation in this study would be nearer to 6.5 Gy than 2.5 Gy because of improvements in animal husbandry techniques and, consequently, laboratory animal health since the 1960s. Surprisingly however, in the present study, guinea pigs were as susceptible to the effects of radiation as they were before 1968. It is possible that the low $LD_{50/30}$ reported here may represent a decrease in immune competency of the guinea pigs due to closed colony breeding and/or a more sanitized housing environment.

Deaths after γ irradiation occurred between postirradiation days 6 and 11. A similar time pattern of death and the hematopoietic state has been described by Newton and Ter-Pogassian (1960) after γ irradiation of guinea pigs. Similarly, Rosenthal (1955) reported a coagulation defect in guinea pigs after doses of radiation in the $LD_{10/30}$ to $LD_{100/30}$ range. Hemorrhages were most prominent on days 9–13 after exposure and were closely related to thrombocytopenia. These factors tend to establish the importance of hematopoietic damage as the major factor leading to radiation deaths in guinea pigs. In this study, the observed difference between weight gain in irradiated versus sham-irradiated animals might be explained by the presence of gastrointestinal lesions in animals exposed to 2–5 Gy doses of γ radiation.

Future studies will be conducted to evaluate whether γ radiation decreases the threshold for nerve-agent-induced seizures in guinea pigs. Based on the present findings that identify 2.2 Gy as the $LD_{50/30}$ radiation dose and days 6-11 as the most critical time period following γ irradiation, our future studies on the combined effects of radiation and nerve agents on seizure threshold can be planned. For those studies, guinea pigs will be exposed to 2.2 Gy γ radiation, and seizure thresholds will be examined on post-irradiation day 7 or postirradiation day 15. Examining the effects of radiation on the seizure threshold immediately before the period when a predicted 50% of guinea pigs will not survive provides us the opportunity to evaluate the effects in eventual "survivors" and "nonsurvivors." Studies done on day 15 postirradiation will provide data for the effects of radiation on seizure threshold in an almost exclusive "survivor" population.

References

AAPM Task Group 21 (1983) A protocol for the determination of absorbed dose from high-energy photon and electron beams. Medical Physics 10:741-771

Bond VP, Sugahara T (1968) Comparative cellular and species radiosensitivity. Igaku Shoin, Ltd., Tokyo

Bond VP, Robertson JS (1957) Vertebrate radiobiology (lethal actions and associated effects). Annual Review of Nuclear Science 7:135-162

Batchelor AL, Corp MJ, Hulse EV (1973) The r.b.e. of fission neutrons, 250 kV X-rays, Cd (n,γ) and ^{60}Co gamma-rays for intestinal and haemopoietic deaths in guinea-pigs. International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine 24:15-24

Carter RE, Verelli DM (1973) AFRRI Cobalt Whole-Body Irradiation. Armed Forces Radiobiology Research Institute TN73-3

Ellinger F (1945) Lethal dose studies with x-rays. Radiology 44:125-142

Lamarque P, Gary-Bobo J (1956) A study of recovery following exposure of part or the whole of the body to ionizing radiation. In: Peaceful Uses of Atomic Energy, vol. 11, p 351, United Nations, NY

Lorenz E, Congdon CC, Uphoff D (1952) Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology 58:863-877

Newton WT, Ter-Pogassian M (1960) Median lethal dose for guinea pigs of Co^{60} gamma irradiation. Radiation Research 13:298-304

Rosenthal RL (1955) Relationship among hematopoiesis, blood coagulation, hemorrhage and mortality in the guinea pig after total body x-irradiation. Blood 10:510-527

DISTRIBUTION LIST

DEPARTMENT OF DEFENSE

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
ATTN: PUBLICATIONS BRANCH
ATTN: LIBRARY

ARMY/AIR FORCE JOINT MEDICAL LIBRARY
ATTN: DASG-AAFJML

ASSISTANT TO THE SECRETARY OF DEFENSE
ATTN: AE
ATTN: HA(IA)

DEFENSE SPECIAL WEAPONS AGENCY
ATTN: TITL
ATTN: DDIR
ATTN: RAEM
ATTN: MID

DEFENSE TECHNICAL INFORMATION CENTER
ATTN: ACQUISITION
ATTN: ADMINISTRATOR

FIELD COMMAND DEFENSE SPECIAL WEAPONS AGENCY
ATTN: DASIAC
ATTN: FCIEO

INTERSERVICE NUCLEAR WEAPONS SCHOOL
ATTN: DIRECTOR

LAWRENCE LIVERMORE NATIONAL LABORATORY
ATTN: LIBRARY

UNDER SECRETARY OF DEFENSE (ACQUISITION)
ATTN: OUSD(A)/R&E

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
ATTN: LIBRARY

DEPARTMENT OF THE ARMY

HARRY DIAMOND LABORATORIES
ATTN: SLCSM-SE

OFFICE OF THE SURGEON GENERAL
ATTN: MEDDH-N

U.S. ARMY AEROMEDICAL RESEARCH LABORATORY
ATTN: SCIENCE SUPPORT CENTER

U.S. ARMY CHEMICAL RESEARCH, DEVELOPMENT, & ENGINEERING CENTER
ATTN: SMCCR-RST

U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
ATTN: COMMANDER

U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
ATTN: MCCS-FCM

U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
ATTN: COMMANDER

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
ATTN: MCMR-UV-R

U.S. ARMY NUCLEAR AND CHEMICAL AGENCY
ATTN: MONA-NU

U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE
ATTN: DIRECTOR OF RESEARCH

U.S. ARMY RESEARCH LABORATORY
ATTN: DIRECTOR

WALTER REED ARMY INSTITUTE OF RESEARCH
ATTN: DIVISION OF EXPERIMENTAL THERAPEUTICS

DEPARTMENT OF THE NAVY

BUREAU OF MEDICINE & SURGERY
ATTN: CHIEF

NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY
ATTN: COMMANDING OFFICER

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
ATTN: CODE 42

NAVAL MEDICAL RESEARCH INSTITUTE
ATTN: LIBRARY

NAVAL RESEARCH LABORATORY
ATTN: LIBRARY

OFFICE OF NAVAL RESEARCH
ATTN: BIOLOGICAL & BIOMEDICAL S&T

DEPARTMENT OF THE AIR FORCE

BROOKS AIR FORCE BASE
ATTN: AL/OEBZ
ATTN: OEHL/RZ
ATTN: USAFSAM/RZB

OFFICE OF AEROSPACE STUDIES
ATTN: OAS/XRS

OFFICE OF THE SURGEON GENERAL
ATTN: HQ AFMOA/SGPT
ATTN: HQ USAF/SGES

U.S. AIR FORCE ACADEMY
ATTN: HQ USAFA/DFBL

U.S. AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
ATTN: DIRECTOR OF CHEMISTRY & LIFE SCIENCES

OTHER FEDERAL GOVERNMENT

ARGONNE NATIONAL LABORATORY
ATTN: ACQUISITIONS

BROOKHAVEN NATIONAL LABORATORY
ATTN: RESEARCH LIBRARY, REPORTS SECTION

CENTER FOR DEVICES AND RADIOLOGICAL HEALTH
ATTN: DIRECTOR

GOVERNMENT PRINTING OFFICE
ATTN: DEPOSITORY ADMINISTRATION BRANCH
ATTN: CONSIGNED BRANCH

LIBRARY OF CONGRESS
ATTN: UNIT X

LOS ALAMOS NATIONAL LABORATORY
ATTN: REPORT LIBRARY

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
ATTN: RADLAB

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
GODDARD SPACE FLIGHT CENTER
ATTN: LIBRARY

NATIONAL CANCER INSTITUTE
ATTN: RADIATION RESEARCH PROGRAM

NATIONAL DEFENSE UNIVERSITY
ATTN: LIBRARY TECHNICAL SERVICES

U.S. DEPARTMENT OF ENERGY
ATTN: LIBRARY

U.S. FOOD AND DRUG ADMINISTRATION
ATTN: WINCHESTER ENGINEERING AND ANALYTICAL CENTER

U.S. NUCLEAR REGULATORY COMMISSION
ATTN: LIBRARY

RESEARCH AND OTHER ORGANIZATIONS

AUSTRALIAN DEFENCE FORCE
ATTN: SURGEON GENERAL

AUTRE, INC.
ATTN: PRESIDENT

BRITISH LIBRARY
ATTN: ACQUISITIONS UNIT

CENTRE DE RECHERCHES DU SERVICE DE SANTE DES ARMEES
ATTN: DIRECTOR

FEDERAL ARMED FORCES DEFENSE SCIENCE AGENCY FOR NBC PROTECTION
ATTN: LIBRARY

FOA NBC DEFENCE
ATTN: LIBRARY

INHALATION TOXICOLOGY RESEARCH INSTITUTE
ATTN: LIBRARY

INSTITUTE OF NUCLEAR MEDICINE AND ALLIED SCIENCES
ATTN: DIRECTOR

INSTITUTE OF RADIOBIOLOGY, ARMED FORCES MEDICAL ACADEMY
ATTN: DIRECTOR

OAK RIDGE ASSOCIATED UNIVERSITIES
ATTN: MEDICAL LIBRARY

RESEARCH CENTER OF SPACECRAFT RADIATION SAFETY
ATTN: DIRECTOR

RUTGERS UNIVERSITY
ATTN: LIBRARY OF SCIENCE AND MEDICINE

UNIVERSITY OF CALIFORNIA
ATTN: DIRECTOR, INSTITUTE OF TOXICOLOGY & ENVIRONMENTAL HEALTH
ATTN: LIBRARY, LAWRENCE BERKELEY LABORATORY

UNIVERSITY OF CINCINNATI
ATTN: UNIVERSITY HOSPITAL, RADIOISOTOPE LABORATORY

XAVIER UNIVERSITY OF LOUISIANA
ATTN: COLLEGE OF PHARMACY

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1997	Special Report

4. TITLE AND SUBTITLE

Sensitivity of Guinea Pigs to Gamma Radiation

5. FUNDING NUMBERS

NWED QAXM

6. AUTHOR(S)

McBride SA, Kneisler TB, Strocko SM, Dela Pena R, Pellmar TC

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, MD 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER

SP97-1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Given the wide variability in the $LD_{50/30}$ doses for γ radiation in guinea pigs presented in older reports and the improvements in animal husbandry techniques over the past 25 years, this study was conducted to determine a physiologically relevant dose of γ radiation for use in our planned γ-radiation/nerve agent seizure threshold studies. Guinea pigs received bilateral, whole-body, midline tissue doses of 1-5 Gy of γ rays from a ^{60}Cobalt source. Probit analysis of the number of survivors from each dose group at the 30-day endpoint revealed the $LD_{50/30}$ for γ irradiation in our test animals was 2.2 Gy (1.5 to 2.7 Gy, 95% CI). In each of the radiation dose groups, no animal deaths were seen prior to 6 days after irradiation, and no animal died after 11 days postirradiation, suggesting that the window of 6-11 days after irradiation is the most critical. By day 4, significant differences in weight gain were found among sham and radiation groups ($p<0.001$), but there were no significant weight gain differences among the five radiation dose groups ($p=0.1$). The observed difference in weight gain in irradiated versus sham-irradiated animals may be explained by the presence of gastrointestinal lesions in animals exposed to 2-5 Gy of γ radiation. Based on the present findings that identify 2.2 Gy as the $LD_{50/30}$ radiation dose and days 6-11 as the most critical time period following γ irradiation, our future studies on the combined effects of radiation and nerve agents on seizure threshold can be planned.

14. SUBJECT TERMS	15. NUMBER OF PAGES
	19
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

SECURITY CLASSIFICATION OF THIS PAGE

CLASSIFIED BY:
DECLASSIFY ON:

SECURITY CLASSIFICATION OF THIS PAGE