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# A Novel Technique for Performing Space Based Radiation Dosimetry Using DNA : Results from GRaDEx -I and the Design of GRaDEx -II

Joe Ritter[1,2], R. Branly[2,3], C. Theodorakis[4], J. Bickham[4], C. Swartz[4], R. Friedfeld[5], E. Ackerman[3], C. Carruthers[3], A. DiGirolamo[3], J. Faranda[6], E. Howard[7], C. Bruno[3]

1) Space Optics Manufacturing Technology Center SD 71, NASA George C. Marshall Space Flight Center AL. 35812 Email: Joe.Ritter@msfc.NASA.gov
2) Florida Space Institute, University of Central Florida, Orlando FL
3) Broward Community College, Department of Natural Science, Davie FL
4) Texas A&M University, Department of Fisheries and Wildlife, College Station TX
5) Steven F. Austin State University, Nacogdoches TX
6) Georgia State Department of Physics, Atlanta GA
7) Florida International University, Miami FL

## Abstract

Because of the large amounts of cosmic radiation in the space environment relative to that on earth, the effects of radiation on the physiology of astronauts is of major concern. Doses of radiation which can cause acute or chronic biological effects are to be avoided, therefore determination of the amount of radiation exposure encountered during space flight and assessment of its impact on biological systems is critical.

Quantifying the radiation dosage and damage to biological systems, especially to humans during repetitive high altitude flight and during long duration space flight is important for several reasons. Radiation can cause altered biosynthesis and long term genotoxicity resulting in cancer and birth defects etc. Radiation damage to biological systems depends in a complex way on incident radiation species and their energy spectra. Typically non-biological, i.e. film or electronic monitoring systems with narrow energy band sensitivity are used to perform dosimetry and then results are extrapolated to biological models. For this reason it may be desirable to perform radiation dosimetry by using biological molecules e.g. DNA or RNA strands as passive sensors.

A lightweight genotoxicology experiment was constructed to determine the degree to which in-vitro naked DNA extracted from tissues of a variety of vertebrate organisms is damaged by exposure to radiation in a space environment. The DNA is assayed by means of agarose gel electrophoresis to determine damage such as strand breakage caused by high momentum particles and photons, and base oxidation caused by free radicals. The length distribution of DNA fragments is directly correlated with the radiation dose. It is hoped that a low mass, low cost, passive biological system to determine dose-response relationship (increase in strand breaks with increase in exposure) can be developed to perform radiation dosimetry in support of long duration space flight, and to predict negative effects on biological systems (e.g. astronauts and greenhouses) in space. The payload was flown in a 2.5 cubic foot Get Away Special (GAS) container through NASA's GAS program. It was subjected to the environment of the space shuttle cargo bay for the duration of the STS-91 mission (9 days). Results of the genotoxicology and radiation dosimetry experiment (GRaDEx-I) as well as the design of an improved follow on payload are presented.

## Introduction

Traditionally, radiation dosimetry has been accomplished using narrow energy bandwidth devices such as electronic detectors or radiation-sensitive film badges. Extrapolation of the measured doses to biological effects can be problematic, especially when people are exposed to radiation with a complex energy spectra (Pissarenko 1993). It would be advantageous to develop dosimeters, which could provide more biologically relevant data.

Because many of the more serious effects of radiation toxicity are due to damage of DNA molecules, any dosimeter which could incorporate DNA in its design would be a likely candidate for such a biological dosimeter. These types of dosimeters are currently being developed for measuring terrestrial exposure to solar ultra-violet radiation (unpublished data), but their use for monitoring cosmic ionizing radiation has yet to be realized.

The purpose of this study is to develop a low cost, high sensitivity, DNA dosimeter with a wide dynamic range, which can be used in space e.g., aboard space shuttle flights, on the International Space Station, and eventually on long duration flights e.g., to Mars, for monitoring cosmic radiation exposure. These simple dosimeters consist of a naked DNA solution sealed into quartz cuvettes. After exposure, the DNA is analyzed for radiation-induced damage via agarose gel electrophoresis.

Biosynthesis is essential to life. Altered biosynthesis can occur in several ways. Radiation can damage DNA, RNA, and proteins. Proteins can be replaced, new RNA can be pattered from DNA, but radiation damage to a cells DNA can be catastrophic. If a cell undergoes extreme radiation damage it usually is no longer viable, can not reproduce, and simply dies. Replicating cells are particularly sensitive to such damage. Of concern e.g. to astronauts are smaller radiation doses, which may not cause immediate cell death but can show up later when a cell tries to duplicate. For example of serious concern are genetic mutations which do not impair cell reproduction, but impair the function of future generations of cells. Such cells may become cancerous, or in the case of mutation to e.g. a sperm cell, offspring of radiated subjects can be affected.

Radiation can cause nucleotide substitutions. The process involves the initial production of single or double strand breaks, either as a result of the direct interaction of the DNA with the particle or photon, or by interaction with radicals. Radiation-induced DNA damage occurs primarily by the production of unstable free radicals by the ionizing radiation. If a radioactive particle interacts with a DNA molecule, it can produce a DNA radical (Drouin et al. 1996). Such a DNA radical is unstable, and quickly leads to a break in the DNA strand (Kraft et al.1989). DNA is a long, double-stranded molecule, so strand breaks can occur in adjacent sites in both of the adjacent strands or in only one of the strands. Alternatively, the high momentum particle or photon may interact with a molecule in solution, most commonly an oxygen or water molecule, leading to the production of oxy- or hydroxy- free radicals. If these free radicals encounter a DNA molecule, they may strip away an electron, which can form a strand break. Free Radicals may also form covalent bonds with the DNA itself, thus leading to oxidation of a portion of the DNA molecule (Shulte-Frohlinde and Von Sonntag 1985). After the dosimeters returned from the shuttle flight, the DNA within them was analyzed for DNA strand breakage and oxidation.

Only strand breaks and base oxidation are measured with this experimental design; however, there is a direct correlation between the number of strand breaks and the mutation rate. In order to detect another type of mutation in in-vivo DNA, seeds of the mustard plant Arabodopsis were flown, and will be grown on earth to look for the effects of chromosome damage in somatic cells by means of flow cytometry. This plant s frequently used by plant geneticists as its genetic sequence is known. Results of the Arabodopsis assay are not presented here.

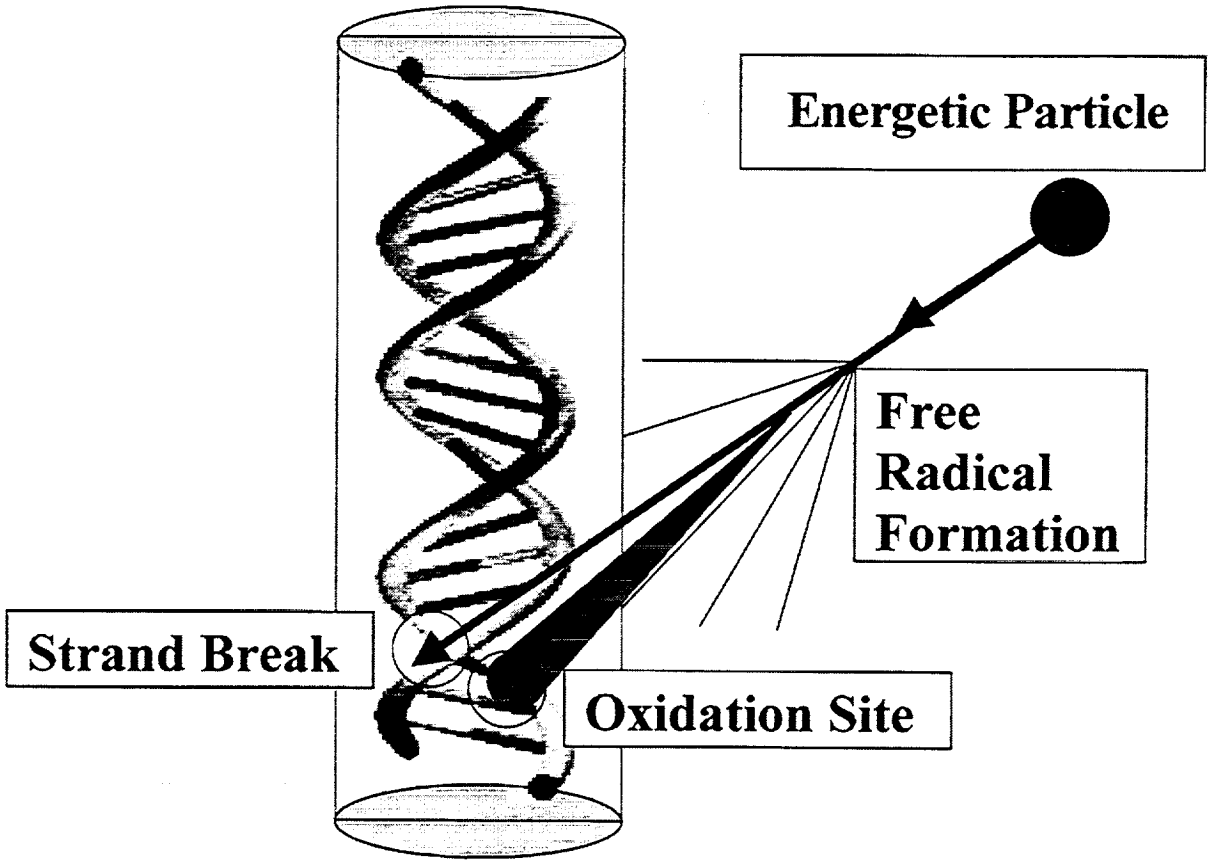
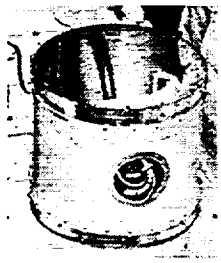



Figure 1 DNA damage mechanisms.



**The Payload**

ASPR-GRaDEx-I (Genotoxicology and Radiation Dosimetry Experiment I) was a variant of another experiment ASPR and Texas A&M are constructing (MET4-GPSE) which will fly as part of the TAMSE GAS payload (Ritter et al., 1998). Because of TAMSE's complexity (9 instruments) an opportunity for an early flight of the simpler GRaDEx payload arose.

Naked DNA was sealed in quartz cuvettes, and then placed in an airtight monolithic 6061-T-6 aluminum container having 1/4 inch thick walls. This was then installed in and flown in a standard, 2.5 cubic foot Get Away Special (GAS) container through NASA's GAS payload program. The payload was subjected to the environment of the space shuttle Discovery's cargo bay for the duration of the STS-91 mission (9 days).

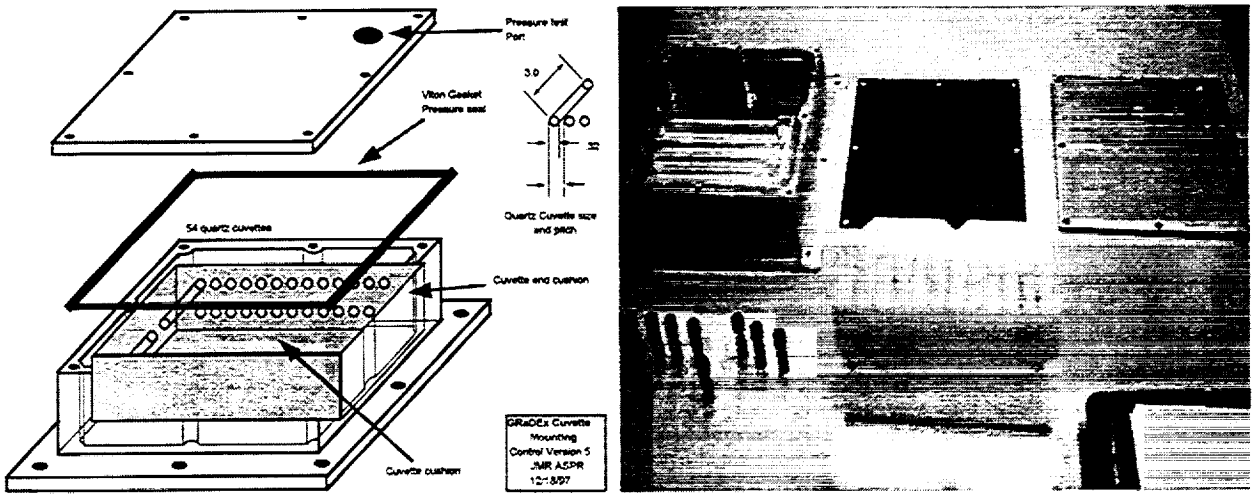



Figure 2 Genotoxicology and Radiation Dosimetry Experiment (ASPR-GRaDEx-I)

**Methods**

DNA single-stand breaks were determined by alkaline gel electrophoresis. The alkaline treatment separates the double-stranded DNA into single-stands, allowing determination of single-strand breaks. Double-strand breaks can be caused not only by radiation but also by shearing of the molecule due to physical disruption by shake, thermal cycling, and even the formation and shearing of ice crystals in the buffer solution. Thus, the number of double-strand breaks is not determined because it is difficult to distinguish between double-strand breaks caused by shearing and those caused by radiation using our current techniques.

The number of oxidized bases was determined by digesting the DNA with enzymes which produce single-stranded DNA breaks at each site of oxidation damage (Boiteux et al. 1992, Dizdaroglu et al. 1993). The number of single-strand breaks produced by this treatment, and thus the number of oxidized bases, can be determined by comparing the number of single-strand breaks with and without such treatment (Freeman and Thompson 1990).

DNA was extracted from mosquitofish (Gambusia affinis), chicken (Gallus gallus), and tiger salamander (Ambystoma tigrinum) by homogenizing approximately 200 mg tissue in 500:1 TEN buffer (10 mM trizma base, 1mM EDTA, 100 mM NaCl) plus 1% sarcosyl. The homogenate was then extracted with 500:1 phenol/chloroform/isoamyl alcohol (25:24:1, v:v:v) and then 500:1 chloroform. The DNA was precipitated with 2 volumes of ice-cold ethanol. The precipitate was then resuspended in 50 mM phosphate buffer (sterile) at a concentration of 20 mg/ml. Human placental DNA was purchased from Sigma Chemical Co. (St. Louis, MO) and resuspended in phosphate as above. Five hundred µl of DNA solution was then loaded into quartz cuvettes and heat-sealed. Some of the cuvettes were flown on the space shuttle Discovery on STS-91 (flight samples), while some were kept in the laboratory (control samples).

After exposure to cosmic radiation, the flight DNA was precipitated by adding 50:1 3 M sodium acetate, 100 g mussel glycogen and 1 ml ice-cold ethanol, and cooling this mixture for 15 min at -80° C. The DNA was then pelleted by centrifugation at 13,000 rpm for 10 minutes in a microcentrifuge. The pellet dried for 10 min under vacuum and was then resuspended in 50:1 TE (10 mM trizma base, 1 mM

EDTA, pH 7.0). The DNA concentration was then quantified spectrophotometrically at 260 nm (1 AU = 50 μg DNA).

For enzymatic digestion, 1.1μg DNA was diluted in digestion buffer (5 mM HEPES buffer, 100 mM KCl final concentration), and 2μg each of endonuclease III and formamidopyrimidine (FaPy) glycosylase (Fpg protein), in a final volume of 30:l. This mixture was incubated at 37° C for 1 hour. Then proteinase K and NaCl were added to a final concentration of 20 mg/ml and 50 mM, respectively, and the mixture was incubated for an additional 1 hour. Samples were incubated both with and without endonuclease. The digested DNA was then analyzed via alkaline or neutral gel electrophoresis.

For alkaline gel electrophoresis, 10.5 ml digestion solution, 3 ml tracking dye (15% Ficoll, 0.025% bromophenol blue), and 1.5 ml of 1 N NaOH and incubated for 15 min. at room temp. All of this mixture was then loaded into a 1% agarose gel and subjected to electrophoresis in alkaline solution (30 mM NaOH, 2 mM EDTA, pH 12.5) at 7 V/cm for 5 hours. The gels were then stained with ethidium bromide (a fluorescent DNA-binding stain) and photographed under ultra-violet light.

For neutral gel electrophoresis, 4 ml digestion reaction was loaded into a 0.3% agarose gel and subjected to electrophoresis in TBE (90 mM trizma base, 90 mM boric acid, 1 mM EDTA, pH 8.0) at 1.5 V/cm for 15 hrs. The gel was then stained and photographed as above.

The average molecular length (Ln) of each sample was calculated with molecular length standards (coliphage T4 DNA, lambda phage DNA Hind III digestion, and 100 base pair ladder) using the methods of Freeman and Thompson (1990) . The amount of DNA damage was calculated according to the formula: $D=(1/Ln1 - 1/Ln2) \times 100$ .

The number of oxidized bases, Ln1, is the molecular length of the sample with enzyme and Ln2 is the molecular length of the sample without enzyme (both determined using alkaline gel electrophoresis). For number of single-strand breaks, Ln1 is determined using alkaline gel electrophoresis, and Ln2 is determined using neutral gel electrophoresis (both are without enzyme). The amount of damage is reported as number of oxidized bases or number of single strand breaks per $10^5$ base pairs.

**Results**

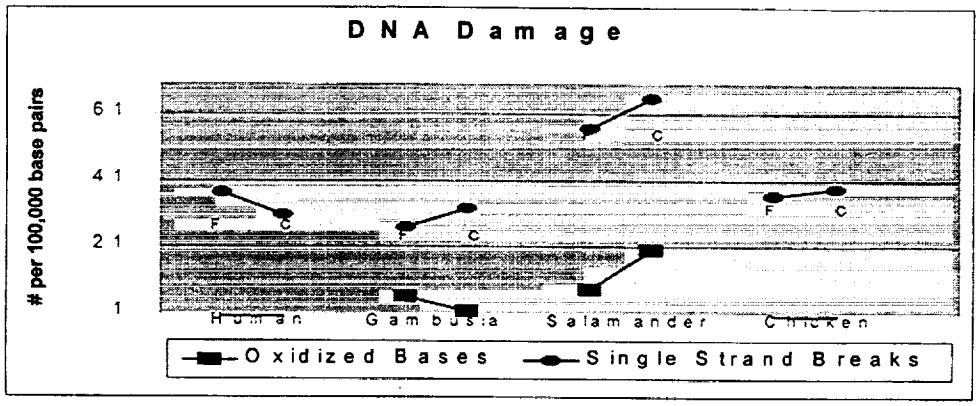



Figure 4 Electrophoresis strand break analysis results.

The number of oxidized bases in the Gambusia flight (F) DNA was greater than that in the control (C) DNA. The opposite was true for the salamander DNA. No other samples showed evidence of

oxidized base damage. Unlike the positive (irradiated) controls in prior ground experiments, there were no obvious trends in the number of single-strand break data. For the human DNA, the flight samples had roughly 7 more strand breaks/$10^5$ base pairs, but for the other species this trend seemed to be reversed.

| Species | # Oxidized Bases | # Single Strand Breaks |
|---|---|---|
| Human Flight | 0.00 | 37.56 |
| Human Control | 0.00 | 30.78 |
| Gambusia Flight | 5.98 | 26.92 |
| Gambusia Control | 1.49 | 32.82 |
| Salamander Flight | 8.19 | 56.76 |
| Salamander Control | 19.97 | 65.76 |
| Chicken Flight | 0.00 | 36.57 |
| Chicken Control | 0.00 | 38.71 |

Table 1 - Number of DNA single-strand breaks and oxidized nucleotide bases (per 105 bases) in DNA samples from different species exposed to cosmic and solar radiation. "Flight" samples were exposed to cosmic and solar radiation aboard the space shuttle. "Control" samples remained in the laboratory during the same period of time.

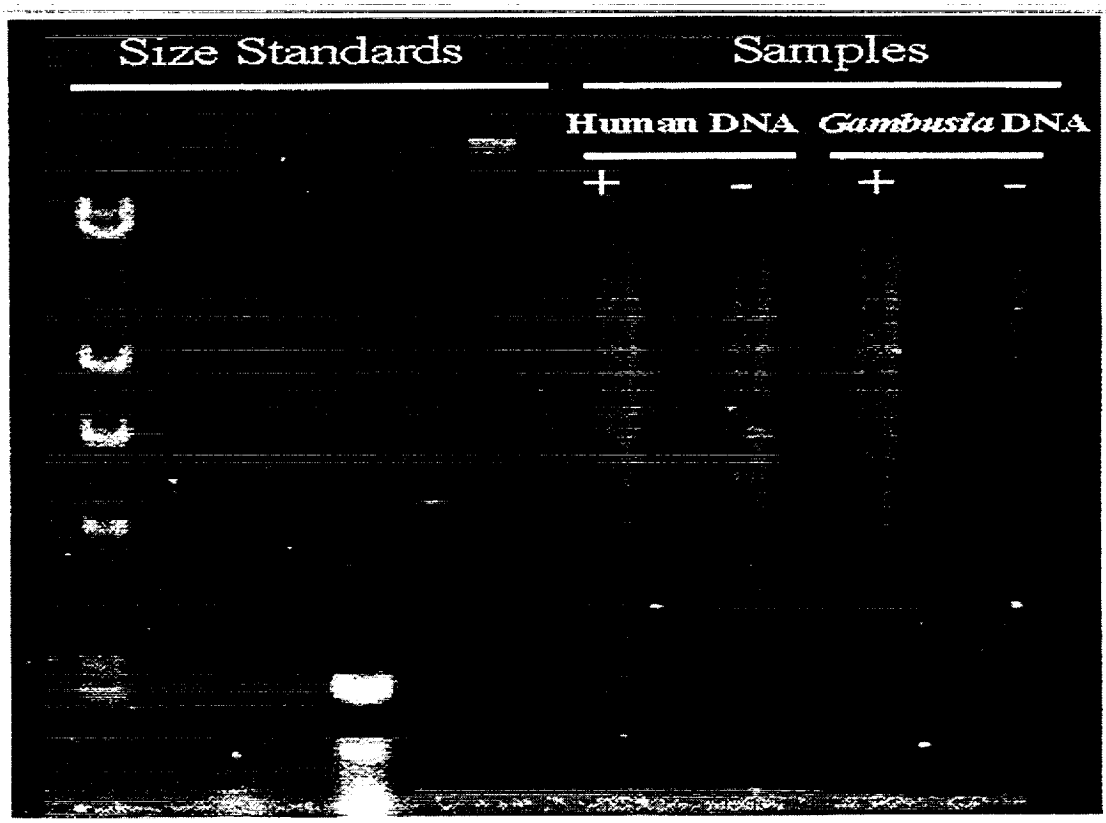



Figure 5 An agarose gel stained with ethidium bromide and photographed under UV light. "Size standards" are DNA fragments of known molecular length. The 1st 3 lanes on the left of the gel are standards: Lambda phage DNA cut with Hind III (high ladder) ; 100 base pair ladder (low ladder); and coliphage T4 DNA (single band). The next 4 lanes are samples from cuvettes. The samples are human flight (with then without enzyme digestion) and gambusia flight DNA (with then without enzyme digestion).

## Conclusions

There was no clear evidence of cosmic radiation-induced DNA damage. It could be that the amount of damage from the cosmic radiation was so small that it is beyond the *current* detection limit of this assay. However, the DNA samples also had small amounts of trizma and EDTA mixed in with them. It is known that trizma (tris(hydroxymethyl) amino-methane) is a radioprotectant free-radical scavenger (Stanton et al. 1993), and EDTA may also be a free-radical scavenger, as are many other organic acids. Thus these compounds may have had a protective effect on the DNA in the samples. Also, the amount of DNA used in this experiment was low, so that the fluorescence of the DNA was small compared to the background fluorescence of the ethidium bromide stain in the gel. Thus the background fluorescence may have contributed extraneous variation when reading data from the gels.

In GraDEx-II, an effort will be made to ensure that no radioprotective compounds are included in with the DNA samples. Also, more DNA will be used in the cuvettes, so that a higher concentration of DNA can be loaded into a well, thus minimizing the effect of background fluorescence in the determination of Ln. Additionally strands of known length e.g., a Coliphage enzymatically cut to a known length may be used as a substrate. Future work will also include a new series of ground experiments to determine the dose response relationship for various energy spectra as well as to determine the effects of mechanical stress on the DNA.

The authors' goals are not only to support future technical needs of the manned space program, but also to support education. Scientists as well as students from fields as diverse as Chemistry, Biochemistry, Physics, Engineering, Astronomy, and Environmental Science benefited from involvement in every level of design, fabrication, testing, and data analysis. The design and fabrication of the TAMSE and ASPR-GRaDEx-I payloads represent several years of time and learning invested by students and their mentors. The investigators and authors of this paper believe that interdisciplinary technical training of American youth is critical to the long-term interests of the United States. Only by maintaining a competitive technical edge will the United States continue to be a world economic and space leader. We are both grateful and proud to live in a country where opportunities like NASA's GAS program are made available to educators, students, and other researchers. In the same spirit, data from the TAMSE and ASPR-GRaDEx-I payloads will be made available to educators who contact the authors (Joe.Ritter@msfc.nasa.gov and rolando@nsu.acast.nova.edu.). Those interested may visit the ASPR website at http://fs.broward.cc.fl.us/north/ecs/nasa/ .

The authors believe that this technique can eventually be improved to a point where radiation dosimetry from very negligible to lethal doses can be accomplished in a single small lightweight inexpensive package suitable for use on both robotic flights, and in support of manned space flight.

## Acknowledgments

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