Fiscal Year:	FY 2010	**Task Last Updated:**	FY 01/20/2011
PI Name:	Lam, Chiu-wing Ph.D.		
Project Title:	Pulmonary Toxicity Studies of Lunar Dust in Mice and Rats		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Environmental health		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SHFH**:Space Human Factors & Habitability (archival in 2017)		
Human Research Program Risks:	(1) **Dust**:Risk of Adverse Health and Performance Effects of Celestial Dust Exposure		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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City:	Houston	**State:**	TX
Zip Code:	77058	**Congressional District:**	22
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Project Type:	GROUND	**Solicitation / Funding Source:**	Directed Research
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No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/2013 per John James (Ed., 8/24/2012) NOTE: Project extended to 9/30/2011 (from 12/31/2010), per B. Woolford/JSC (1/2011) NOTE: Start/end dates changed to 10/2/2006-12/31/2010 (previously 4/30/2006-1/31/2011) per B. Woolford/JSC via S. Steinberg-Wright/JSC (9/2009)		
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Task Description:	NASA will build an outpost on the lunar surface for long-duration human habitation and research. The surface of the Moon is covered by a layer of fine, reactive dust, and the living quarters in the lunar outpost are expected to be contaminated by lunar dust. NASA established the Lunar Airborne Dust Toxicity Advisory Group (LADTAG) to evaluate the risk of exposure to the dust and to establish safe exposure limits for astronauts working in the lunar habitat. Because the toxicity of lunar dust is not known, LADTAG has recommended investigating its toxicity in the lungs of laboratory animals. After receiving this recommendation, NASA directed the JSC Toxicology Laboratory to determine the pulmonary toxicity of lunar dust in exposed rodents. The rodent pulmonary toxicity studies proposed here are the same as those proposed by the LADTAG. Studies of the pulmonary toxicity of a dust are generally done first in rodents by intratracheal instillation (ITI). This toxicity screening test is then followed by an inhalation study, which requires much more of the test dust and is labor intensive. We succeeded in completing an ITI study on JSC-1 lunar dust simulant in mice (Lam et al., Inhalation Toxicology 14:901-916, 2002, and Inhalation Toxicology 14: 917-92, 2002), and are now proposing to do a study with Apollo lunar dust samples. This study will be similar to our study with the lunar dust simulant. Groups of mice and rats will be intratracheally instilled with a suspension of lunar dust. Lung lavage fluid will be assayed for biomarkers of toxicity, and lung tissues will be examined microscopically for pathological lesions. In the study, reference dusts that have known toxicities and industrial exposure limits will be studied in parallel so the relative toxicity of lunar dust can be determined. The ITI results will also be useful for choosing an exposure concentration for the animal inhalation study on the lunar dust, which is included as a part of this proposal. The animal inhalation exposure will be conducted with lunar dust simulant will prior the inhalations exposure study with the lunar dust. The simulant exposure will ensure that the study techniques used with actual lunar dust will be successful. The results of ITI and inhalation studies with real lunar dust are essential for setting limits for human exposure to lunar dust.
Rationale for HRP Directed Research:	This research is directed because it contains highly constrained research, which requires focused and constrained data gathering and analysis that is more appropriately obtained through a non-competitive proposal.
Research Impact/Earth Benefits:	

NASA TASKBOOK AND PROGRESS REPORT
Pulmonary Toxicity Studies of Lunar Dust in Mice and Rats

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Background, Significance, and Relevance to NASA Lunar Missions

NASA has been contemplating returning to the Moon for further exploration and use it as a stepping–stone for future manned trips to Mars and beyond. To meet this objective, NASA will have to build an outpost on the lunar surface for long-duration human habitation and research. The recent discovery of ice in craters of the lunar south pole has added to the great enthusiasm in the space community about further human exploration of the Moon (David, 2009).

The Shackleton Crater area of the lunar south pole is a candidate landing site (Fig. 1) (JPL-NASA, 2010). The crater lies entirely within the immense Aitken basin (~1500 miles in diameter), which is the largest and oldest impact basin on the Moon. This basin is roughly 8 miles deep, and exploration of its properties could provide useful information about the lunar interior (Spudis et al., 2008). The crater rim is illuminated by sunlight almost continuously; besides being subjected to smaller extreme temperature fluctuations than those that occur at the Apollo landing sites, the crater rim provides good access to solar energy. The interior of the crater is perpetually dark and very cold; any water that landed on the crater from cometary impacts would lie permanently frozen on or below the surface. An engineers' concept of a full lunar outpost is shown in Fig. 2 (LPI 2008). Besides having occasionally been hit by large comets, the surface of the Moon has consistently been bombarded by micrometeoroids for more than 4 billion years. During the high-speed impacts of these micro-grain interplanetary dusts, typically 0.05 mm in diameter, the force and heat melt, partially vaporize, and/or crush particles of surface regolith. Cooling welds the particles together into glassy and jagged-edged agglutinates, which are pulverized to fine dusts upon subsequent impacts. In the course of lunar history, these meteoritic activities have created a relatively even particle-size distribution of the regolith over the whole lunar surface. The regolith contains about 10% to 20% fine dust with particle diameters less than 20 μm (Park et al., 2008).

The lunar regolith is made up of minerals derived from anorthositic, gabbroic, and basaltic rocks that are also common in the Earth's crust; aluminosilicate and ferromagnesian silicate minerals including plagioclase feldspar, pyroxenes, and olivine make up the bulk of the lunar regolith (CxP, 2004). High-speed micrometeoroid bombardments fracture the lunar regolith grains into fine dust generating numerous new surfaces. The fractured surfaces on the particles consisting of many broken chemical bonds would be chemically reactive and remain to be so in this vacuum environment; studies on Earth show that when freshly-ground minerals of the types found on the lunar surface were exposed to water, hydrogen peroxide (a reactive oxygen compound formed when reactive dust surfaces come into contact with water and oxygen) was detected in concentrations raging from 1 to 25 nmol/m2 mineral (Hurowitz et al., 2007). It is well known that freshly-fractured silica produces more reactive oxygen species (ROS) and is more toxic then aged silica dust (Vallyathan et al., 1995). The lunar surface regolith residing in a near-vacuum environment further subjected to constant irradiation from solar ultraviolet light and x-rays in the daytime and solar wind at night; these solar radiations alternately impart positive and negative charges to the dust. The surfaces of the charged lunar fine dust are expected to be populated with "unsatisfied" chemical bonds, making them very reactive (CxP, 2004; Stubbs et al., undated). According to Dr. Lawrence Taylor, a member of the NASA Lunar Geology Team, the samples of lunar surface soil collected during the Apollo program show that the regolith contains about 1% to 2% very fine dust (</= 3 μm; Taylor, 2007, personal communication, 2009), which is respirable (can penetrate deep in the lung) by humans; about 80% of the mineral in the fine-dust portion of lunar regolith is silica-rich glass. While astronauts are living on the Moon, as they go in and out of the habitat (including bringing instruments, hardware, and spacesuits in for servicing or refurbishing) they will bring dust, which is very adherent, into the living quarters of the lunar outpost. The potential for dust contamination of the lunar habitat can easily be inferred and visualized by examining Apollo 17 astronaut Dr. Jack Schmitt's soiled suit (Fig. 3) and reading the Apollo crews' comments about exposure to lunar dust in the Command Modules during their return journeys to the Earth. Respiratory tract irritation resulting from lunar dust exposure was reported by crewmembers of Apollo missions 12, 16, and 17 (Wagner, 2006).

Task Progress:

Therefore, fine reactive lunar dust entering the habitat, it can be expected to produce toxicity in the lungs if it is inhaled and could pose a health risk to astronauts living on the Moon. NASA has established a Lunar Airborne Dust Toxicity Assessment Group (LADTAG), which includes national experts in toxicology and lunar geology, to evaluate the risk of exposure to the airborne dust and to establish safe exposure limits for astronauts working in the lunar habitat; NASA has also directed its toxicology laboratory at the Johnson Space Center (JSC) to investigate the pulmonary toxicity of lunar dust in experimental animals to obtain the needed data. The NASA JSC Toxicology Laboratory has invited the National Institute for Occupational Safety and Health and other academic institutes to participate in these important toxicity studies.

General Design of Experiments

Toxicity of a dust in the lungs of exposed subjects may manifest as inflammation, necrosis, fibrosis, etc. It is believe that reactive oxygen species generated by the dust may play the key role of initiation and progression of lung diseases (Vallyathan et al, 1998). When subjected to toxic insults, such as exposures to a toxic dust, the resident cells in the lungs, chiefly macrophages, release cytokines and other chemotactic factors to recruit white blood cells (chiefly neutrophils) into the lungs. Serum proteins also enter the lung as a result of irritation-induced increase of vascular permeability. Tissue injury or cell death (necrosis) will release cellular enzymes such as lactate dehydrogenase into the lung fluid. These biomarkers of toxicity can be assessed in bronchioalveolar lavage fluids (BALF) of dust-exposed animals. Persistent irritation, inflammation, and tissue injury in the lung can lead to necrosis and fibrosis. Microscopically examination of lung tissues of dust-exposed animals could review the presence of histopathological lesions (Fig. 4). Animal study to evaluate toxicity or toxicological potency of a dust in the lung is generally done first by an intratracheal or intrapharyngeal instillation (ITI/IPI) study, in which the dust of interest can be compared with reference dusts of known toxicity (Driscoll et al., 2000). In ITI/IPI study, test dusts are suspended in saline or another nontoxic medium, and are instilled directly into the lungs of rodents at same dose levels so relative toxicity of these dusts can be compared (Fig 5). This toxicity screening study using unnatural exposure route is then followed by an inhalation study. Thus, lunar dust samples in our ITI/IPI study will be tested simultaneously with two common reference dusts, titanium dioxide (Retile R-100, Du Pont) and crystalline silica (quartz or Min-U-Sil 5, U.S. Silica). The pulmonary toxicities of these two reference dusts are well characterized: titanium dioxide is low in toxicity, while quartz is a fibrogenic dust that can produce a spectrum of lung lesions. The Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have set occupational exposure limits (permissible exposure limit [PEL] and threshold limit values [TLV], respectively) on both dusts. The data of comparative toxicities of the test dusts in these instillation studies will be useful for LADTAG to establish limits for astronaut exposure to the lunar dust (Fig. 5).

As discussed above, the surface dust is expected to be chemically reactive in the high-vacuum lunar environment on the Moon (CxP, 2004; Stubbs et al., undated). The lunar soil samples collected during the Apollo missions were exposed to air and moisture on their journeys to Earth and also exposed to trace levels of oxygen and water molecules during their prolonged storage on Earth. The NASA Geology Team believes that the Apollo lunar dust has been “chemically passivated” by these atmospheric components (McKay, 2009, personal communication). The NASA Geology Team therefore believes that grinding will “restore” the chemical reactivity of the passivated lunar dust. They will aerodynamically separate a very fine fraction (mass median diameter ~ 2 μm) from an unground lunar dust sample; the remaining coarse dust will be ground, and a very fine portion will again be isolated in the same manner. The Geology Team will provide the unground and ground dust samples of respirable sizes to the JSC Toxicology Group for evaluation of their toxicity in the lungs of exposed animals.

Intratracheal / Intrapharyngeal Instillation The NASA Geology team obtained two small samples of Apollo 16 highland lunar dusts of different geological maturity (#61501 and #62241) for preliminary study. Because only very small samples of dust particles in respirable sizes were aerodynamically isolated, a pilot study was conducted on these two unground lunar samples in mice. Groups of 5 mice (C-57 male) were each intrapharyngeally instilled at doses of 1, 0.3, or 0.1 mg/mouse of lunar dust or reference dust and BALF biomarkers of toxicity were assessed on 7 and 30 days after the dust instillation. The results assessed on either time point showed that both lunar dust samples at the high doses were able to induce pulmonary inflammation and cellular injury; Figures 6 and 7 illustrate increase levels of BALF biomarker indices including inflammation cytokines in comparing control animals given saline (data on 7-day not shown). The overall results showed that lunar dust was more toxic than titanium dioxide, but less toxic than quartz. The two lunar dust samples showed similar toxicity.

Nose-Only Inhalation Studies

The data from the ITI studies in rats will be useful for determining the exposure concentrations for the rat inhalation toxicity study with lunar dust. From these toxicity data, we will choose three exposure concentrations (high, middle and low) that would be likely to produce moderate, mild, and no effects in the lungs of exposed rats. Because of the limited quantity of lunar dust, the inhalation exposure will be carried out in nose-only exposure chambers. We are planning to carry out a 4-week inhalation. We have set up two dust generation-exposure systems (Fig. 8), each consisting of a Vilnius Dry Aerosol Generator (VAG), a cyclone, and an NYU-Jaeger nose-only inhalation exposure chamber (CH Technologies, Westwood, NJ) similar to one tested by Battelle (Columbus, OH). The Battelle Group (Shawn et al. , 1995) concluded that “aerosolization of small quantities of dry powders with VAG is controllable, consistent, repeatable and predictable.” We tested the performance of our two exposure systems using a simulated lunar dust (JSC-1Avf, a fine dust sample isolated from a volcanic ash and provided by Dr. James Carter of the University of Texas at Dallas, Dallas, TX). The concentration profile of dust in each chamber was monitored by a Cassella Microdust Pro Real-time Dust Analyzer (Casella USA, Amherst, NH). Simultaneously, the dust in a known volume of chamber atmosphere was collected (1 L/min) for 5 hours continuously on filter paper for quantitative determination of the average dust concentration in the chamber for confirmation.

Fig. 9 shows the concentration profile of one 5-h run recorded using the Casella dust monitor. The aerodynamic diameter of the dust particles was determined by an Aerodynamic Particle Sizer Spectrometer 3321 (TSI Incorporated, Shoreview, MN). Since the TSI 3321 could not give a mass median aerodynamic diameter (MMAD) of our test dust directly, the MMAD was estimated. The analytical results from the TSI 3321 allow us to roughly estimate the MMAD to be 2.8 μm and the geometric standard deviation of the dust in the chamber to be 1.5; these results indicate that the dust generated into the chamber was respirable in size. From the results of the five 5-hour dry runs in each chamber system, we concluded that these systems are suitable for our lunar dust exposure. However, to further improve or refine our dust monitoring capability, we have acquired a Quartz Crystal Microbalance (QCM) Cascade Impactor Real-Time Air Particle Analyzer (California Measurements, Inc., Sierra Madre, CA) that could be used to obtain real-time chamber concentration and aerodynamic particle size information. We are in the process acquiring another nose-only chamber for exposure of control animals to air. We will carry out a pilot inhalation study with simulated lunar dust in rats exposed for 4 weeks (5 h/d, 5 d/wk). After a successful study with lunar dust simulant, we will conduct an inhalation study with lunar dust in rats. BALF will be obtained from the rats after 7 and 30 days, while lung tissues will be harvested 1 and 3 months after the inhalation exposure for pulmonary toxicity assessment. The results of both the ITI and inhalation studies will provide toxicity data needed to assess the health risk of dust exposures on the Moon and data needed for LADTAG to set safe exposure limits of lunar dust.

(Figures will be provided upon request; Chiu-wing.Lam-1@nasa.gov)

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Bibliography Type:	Description: (Last Updated: 07/16/2023)
Articles in Peer-reviewed Journals	Park JS, Liu Y, Kihm KD, Greenberg P, Taylor LA. "Characterization of lunar dust for toxicological studies. I: Particle size distribution. " Journal of Aerospace Engineering, 2008 Oct;21(4):272–9. http://dx.doi.org/10.1061/(ASCE)0893-1321(2008)21:4(266) , Oct-2008