Lunar Dust Toxicity: Final Report

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In Memoriam

The lunar dust toxicity project unfolded over several years and was fortunate to involve several pioneering, Apollo-era geologists still pursuing their passion for lunar geology. Their passion was born in them during the days of the moon landings when they were young men. Those of us who worked alongside these remarkable scientists in the last years of their careers are grateful for the knowledge and wisdom they brought to the project. Herein we salute their contribution and remember the good days when they infused us with excitement over returning to the moon to stay forever.

David S. McKay, PhD (1936-2013) enjoyed a career that spanned human spaceflight from his days training Apollo 11 astronauts Armstrong and Aldrin to his work with us on the geological properties of lunar dust that affect its toxicity. Perhaps Dave was best known for his controversial publication in 1996 in the journal *Science* in which he provided evidence for microbial life on Mars based on study of a meteorite that originated from that planet. This finding catalyzed the formation of the NASA Astrobiology Institute under which the quest for life on other celestial bodies was focused. We will remember Dave for his gentle, deep intelligence, and his dedication to scientific discovery.

John F. Lindsay, PhD (1941-2008) came to the United States from Australia to earn his PhD from Ohio State University in geology, which he did in 1968. He was one of the original scientists involved with the Lunar Planetary Institute. Recently he had developed interests in the origins of life and was a Senior Research Associate at the Astrobiology Institute at JSC where he worked with Dave McKay. He made many early contributions to the Lunar Dust Toxicity Project, working hard until his illness got the best of him. Personally, I'll miss his resonant Australian accent and our rambling conversations about the possibility of a Creator behind life's formation.

-JTJ

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Acronyms

BMD	Benchmark dose
BMDL	lower 95% confidence level of the BMD
EVA	Extravehicular Activity
ITI	Intratracheal Instillation
LADTAG	Lunar Airborne Dust Toxicity Assessment Group
NIOSH	National Institute of Occupational Safety and Health
NOAEL	No Observed Adverse Effect Level
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Concentration
POD	Point of Departure
SEE	Safe Exposure Estimate
SRP	Safety Review Panel
TLV	Threshold Limit Value
TWA	Time-weighted Average

1. Introduction

This report is formatted to be easily read by executive, non-experts who wish to understand how the Space Toxicology Office and the Lunar Geology Team at Johnson Space Center developed the data needed for an exposure standard for lunar dust. Experts, whether lunar geologists, inhalation toxicologists, risk assessors, or flight surgeons, who wish to thoroughly review the details of how data were obtained and integrated to formulate an exposure standard, are encouraged to read the core papers referenced by this document and marked with an asterisk. Those core papers have been or will be published in peer-reviewed journals.

a. Initial studies after Apollo and Luna sample returns

Immediately after samples of lunar regolith were returned to earth, either aboard Apollo spacecraft or Luna probes, there was concern about harmful microbes that might be present in the material; however, there was much less attention given to the potential toxicity of the respirable-size dust that was a component of the regolith. Limited attempts were made to determine if the lunar dust was unusually toxic, but none of the attempts yielded satisfactory data that could be used to set a human exposure standard (Scully et al. 2013). Exposures of astronauts during their missions did not seem to cause any apparent harm, although the dust was reported to be irritating, especially when large quantities became airborne after liftoff from the lunar surface. Astronaut exposures were brief, and therefore inconclusive as far as setting a standard for long-term exposures. With the intention of NASA to again place astronauts on the lunar surface for long missions, concern was renewed in 2005 that the respirable-size dust would be unexpectedly toxic, thereby requiring exposure limits. Even though that interest waned some years later as the lunar return was set aside, astronauts from the U.S. and many other countries are expected to eventually undertake prolonged missions on the lunar surface that will involve exposures to lunar dust.

b. Formation of the Lunar Airborne Dust Toxicity Assessment Group

In 2005 a meeting was held at Ames Research Center to bring together lunar geologists, toxicologists, and medical doctors to share data and determine to what extent we needed to understand the toxicity of lunar dust. That fall a meeting was held at NASA Headquarters involving many who participated in the Ames meeting as well as inhalation toxicity experts working outside the agency. The data available at that time were presented, and the experts were asked to estimate the value for safe human exposures for missions of 6 months duration. After lively and forthright discussions, it was determined that the uncertainty in setting a lunar-dust exposure standard was 300 fold. Opinions varied depending on how much emphasis an expert placed on general knowledge of mineral dusts and how much was placed on the many uncertainties of lunar dust properties. For some experts, the uncertainty factors were quite large, and according to others, they could be largely discounted.

During this meeting, the Lunar Airborne Dust Toxicity Assessment Group (LADTAG) was formed to provide a structure under which experts could meet to discuss plans and data developed by NASA toxicologists and geologists. Furthermore, members of this group could be called upon as necessary to give their individual opinions regarding data and how it could be applied to development of an exposure

standard. The LADTAG consisted of lunar geologists, inhalation toxicologists, physicians, and one Apollo astronaut/geologist. Members came from within the agency and from institutions with little or no NASA affiliation. In a letter dated 29 November 2005, the Chief Health and Medical Officer of NASA challenged the LADTAG to determine risk criteria for exposure to lunar dust and develop a permissible exposure standard for lunar dust inhalation.

The need for an inhalation standard was clear, and to this the LADTAG added studies that would examine the ocular and dermal toxicity of the dust. The project then fell under the purview of the Human Research Project, which funded the entire project and the core studies reported herein. The studies and progress were periodically reviewed by one of the HRP's Standing Review Panels that had been populated with experts who were not previously associated with the project. Leaders of the project responded to the panelists' expert recommendations and concerns as necessary to improve the research effort. The group of research scientists at Johnson Space Center also partnered with experts from the National Institute of Occupational Safety and Health (NIOSH) in Morgantown, WV to conduct the initial intratracheal instillation (ITI) studies in rats at that institute. Scientists there had an influence, through the LADTAG, on the design and execution of the studies conducted at NIOSH, and later on the inhalation studies conducted at JSC.

c. Goal of the investigations

The primary goal of the project was to develop a database from which experts could set a lunar dust exposure standard based on the risk of respiratory injury caused by exposures to dust for missions up to 6 months in duration. Standards set for exposures to airborne pollutants, which originate *within* a space habitat, assume that the exposures will be continuous as long as an astronaut is inside the vehicle or habitat. Exposures inside a lunar habitat to an *external* pollutant, such as lunar dust, are going to be quite different. After an extravehicular activity (EVA), dust will be brought into the habitat on suits, surface samples, and on hardware brought in for repairs or cleaning. Based on current information, we assume that airborne dust levels will spike after each EVA and high efficiency particulate air (HEPA) filters will remove the dust over a period of no more than 6-8 hours to a low, baseline NASA has learned that astronauts need "weekends" off, so their exposures will be very much like workers in an earth-based industrial workplace. Thus our goal was to recommend, from the database developed, a time-weighted-average dust exposure standard for the period of time when the dust levels have spiked immediately after an EVA. This realistic goal gave us two distinct advantages. One was that we could compare our lunar dust toxicity findings directly to the toxicity of dusts that have well-established industrial exposure standards (TiO_2 and quartz). The second was that we could conduct our inhalation exposures on a 5 days/week schedule, with weekends off, knowing that this is a good model for the exposures that are expected within the lunar-surface habitat. The team also conducted a two-part ocular toxicity study, which will be briefly described in this report. The results of the ocular toxicity study will also be used to inform the interpretation of the instillation and inhalation studies.

2. Approach

a. Preparation and characterization of lunar dust (McKay, et al. 2013)

A parent sample of lunar dust (Apollo 14, 14003) was obtained from the Apollo sample curator at Johnson Space Center. Three methods, which are described in more detail (McKay, et al. 2013), were employed to derive respirable-size lunar dust from the parent sample. Briefly, using a flow and cyclone separation method, a few grams of respirable-size dust were obtained from the bulk sample. The second and third methods involved grinding a larger-size fraction by ball mill or jet mill until the product had a suitable size for flow separation to a respirable size via a cyclone system. All preparations were conducted in an ultra-pure nitrogen environment to minimize any loss of surface reactivity from the dusts. All three preparations were used in the ITI study, which required only fractions of a gram of each dust; however, the jet-milled dust was the only preparation used in the inhalation study. There were insufficient quantities of the unground, respirable-size dust for an inhalation study, and the jet-milled dust was considered superior to the ball-milled dust because the former involved self-collisions between dust particles, whereas the latter involved crushing of the particles between the milling balls and the container walls.

The volumetric mean diameters of the respirable-size dusts used for the ITI study were 2.1, 1.8, and 2.5 μm for the native, ball-milled and jet-milled dusts, respectively. The native, respirable-size fraction of dust has a far greater portion of nanophase iron (tiny beads of metallic iron imbedded in the dust grains) than oxidized iron when compared to the portion in larger particles. This means that the native dust has a relatively higher content of metallic iron than the respirable-size particles obtained by grinding larger-sized dust grains to a respirable size. Dust particles in the native fraction were rare below ~30 nm (McKay et al., 2013). The major mineral components of the three dusts were similar (James, et al. 2013).

b. Ocular toxicity testing (Meyers, et al. 2012)

The ocular toxicity of lunar dust was determined in a two-tier study according to recommendations promulgated by the Organization for Economic Cooperation and Development (OECD). The first procedure involved application of a 100 mg sample of the respirable-sized, jet-milled dust, which had been maintained in ultra-pure nitrogen until the first experimental procedure in the ocular study, directly to the surface of cultured human keratinocytes. The EpiOcular™ procedure we used is employed world-wide as an *in vitro* test to replace or inform *in vivo* testing of many kinds of products. Following the standard protocol, the dust samples were applied in duplicate to six cell samples, which were assayed for viability 3, 30, and 60 minutes after application of the dust. The assay ascertains chemical irritancy by enumeration of viable cells after a standard incubation period in which the cells are in contact with the dust. The test of lunar dust was co-studied with materials known to be negative in the assay and materials known to elicit a positive result.

Once the first tier result had shown only a minimal irritancy for the dust, it was possible to advance to the *in vivo* method with confidence that the dust was unlikely to be exceptionally irritating to rabbit eyes. Although the *in vitro* test showed minimal irritancy, the LADTAG working team decided that an *in vivo* test was needed for completeness of the ocular toxicity study. The second tier involved application of nonrespirable dust particles (mass mean diameter of 51 μm) to the eyes of 3 rabbits to determine primarily mechanical irritancy. The amount applied to the right eye of each animal was 70

mg, with the contralateral eye being the control. The reaction of the eyes was scored using the Draize criteria from 1 hour to 72 hours after application of the dust.

c. Intratracheal instillation of dusts - Comparative benchmark dose (BMD) modeling (James, et al. 2013)

The tracheas of groups of 6 F344 male rats were instilled with 5 different dusts at 3 dosages. The respirable-size dusts were 2 dusts of known toxicity (TiO_2 and quartz) and 3 lunar dusts (native, ball milled, and jet milled). The dusts of known human toxicity were chosen because their OSHA permissible exposure levels (PELs) differ by 50 fold; PEL_{TiO2} = 5 mg/m^3 and PEL_{quartz} = 0.1 mg/m^3, and the American Conference of Governmental and Industrial Hygienists (ACGIH) threshold limit values (TLV) for quartz (https://www.osha.gov/dts/chemicalsampling/data/CH_266740.html) and the National Institutes for Occupational Safety and Health(NIOSH) recommended exposure limit (REL) for TiO_2 (NIOSH, 2011) differ by approximately 100 fold; REL_{TiO2} = 2.4 mg/m^3 and TLV_{quartz} = 0.025 mg/m^3. The dose to each anesthetized rat was 1, 2.5, or 7.5 mg of dust, delivered in 0.4 ml of vehicle consisting of 90% physiological saline and 10% Survanta^{TM} (a synthetic lung fluid). A single vehicle control group was used for comparison to all dust-exposed groups. Animals were euthanized 1 week or 1 month after being dosed; lung lavage fluid was obtained for cellular and biochemical marker assessments. Lung tissue was harvested from rats 1 or 3 months after the ITI for histopathology studies.

The lavage fluid data were compiled and tested for suitability of the dose-response curves for BMD modeling. This required that all 5 dose-response curves (representing the 5 types of dust) for a given endpoint and harvest-time were suitable for modeling. Furthermore, the BMD for TiO_2 and quartz had to be at least 10-fold different for the endpoint to be deemed suitable for comparison. In other words, the endpoint had to be sensitive to the 50-fold difference in the PELs of the standard dusts. Nine parameters and harvest times met the acceptance criteria, and from these a safe exposure estimate (SEE) was made for the 3 lunar dusts by comparison of the optimal BMDs for the lunar dusts to the optimal BMDs of the standard dusts.

d. Inhalation toxicity-direct safe exposure estimates from inhalation data (Lam, et al. 2013)

The initial nose-only inhalation study was conducted in acclimated F344 male rats at target doses of 0, 20, and 60 mg/m^3, with exposures lasting 6 hours/day, 5 days per week for 4 weeks. The jet-milled dust was used in this study; the respirable dust in the chamber was generated from a dust generator and then passed through a cyclone. The size distribution and concentration of the dust in the chamber, precisely at the place where the rats were breathing, was carefully monitored by several methods. The primary method of deducing concentration was gravimetric, by which the weight change in a filter used to trap the dust drawn (at known volume) from the chamber was measured over a period of time. Size distributions were established using cascade impactors and verified with an atmospheric-particle sizing instrument.

Rats were euthanized in groups of 5 animals at 1 day, 7 days, 1 month, and 3 months after the end of exposures. The right and left lungs were isolated from each other. The right lungs were lavaged with phosphate-buffered saline and this lavage fluid, after separation into components, was used to

assay cellular and biochemical indices of toxicity. The left lung was fixed with a formalin drip and kept in formalin until processed for standard histopathology. Likewise, lung lymph nodes were isolated and fixed for histopathology. Tissues were stained with hematoxylin-eosin for general pathology and with Mason's trichrome to better visualize fibrotic areas. The slides were read independently by 3 pathologists.

Because the rats exposed to 20 mg/m^3 exhibited some mild toxic effects, a decision was made to expand the study to include exposures targeted at 2 and 7 mg/m^3 with the intention of identifying a no-observed-adverse-effect-level (NOAEL). Thus, 2 additional groups of rats were exposed to these target concentrations and another group to filtered room air. Except for the reduced concentrations, these exposures were identical to the ones performed at the higher concentrations. The times of tissue harvest and methods of analysis were also identical to the higher-concentration study.

e. Inhalation toxicity-benchmark dose modeling of data (Scully, et al. 2013)

Benchmark dose modeling was undertaken on the data involving biomarkers of toxicity in lung lavage fluid from inhalation-exposed rats by treating the controls from both studies (N=10) as a single group, and then fitting the best possible dose response curve through the data from the low-concentration pair and the high-concentration pair combined. Thus, there were 4 data points on each dose-response curve and 1 control point. Data were analyzed in 3 groups: parametric (normally distributed) data, data that could be converted to parametric data, and nonparametric data. A total of 32 BMDs and BMDLs (lower 95% confidence interval of the BMD) were calculated using best fits to the dose response curves and a reference value 1 SD above the mean of the control value (standard in the U.S. Environmental Protection Agency's BMD software). The BMDs and BMDLs were averaged for parametric data, parametric + transformed, and all 32 successful data fits. The highest and lowest of these averages were selected as the extreme limits for consideration of a POD on which to apply safety factors that were identical to the ones applied to the NOAEL.

3. Summary of findings

a. Ocular toxicity (Meyers, et al. 2012)

The first tier of the ocular study, which involved tissue contact by the test dust for up to 1 hour, showed that the jet-milled lunar dust produced only minimal chemical irritancy, whereas positive controls (sodium hydroxide and sodium dodecyl sulfate) produced severe chemical irritation. The near absence of chemical irritancy caused by jet-milled dust in this assay suggests, but certainly does not prove, that the surface-chemical reactivity of this dust is not going to contribute to toxicity when the dust comes in contact with other cell types, for example, in the respiratory system. The mechanical irritancy test, which involved a much larger-size fraction of dust placed in rabbit eyes, also showed minimal irritancy. Of a maximum possible score of 110 points, the jet-milled dust gave a score of 4 points, due to slight redness and swelling of the conjunctiva after 1 hour. The slight redness and swelling resolved within 24 hours, and the results were consistent in the 3 rabbits.

b. Safe exposure estimates from intratrachael instillation - Comparative benchmark dose modeling (James, et al. 2013)

The results of comparative BMD modeling of the ITI data are shown in Table 1. The results depended on biochemical markers of toxicity (LDH) and cellular markers (counts) at both tissue harvest times. While the comparative BMD approach gave useful results, we note that the approach would have been better optimized if lower doses of quartz and higher doses of TiO_2 were used. This would have produced more useful BMD modeling outcomes and more endpoints on which to base the predicted SEEs.

Table 1. Safe Exposure Estimates (SEE, mg/m^3) for 3 Types of Lunar Dust from Biomarkers that were Sensitive to the Differences in Toxicity of TiO_2 and Quartz

Lavage fluid biomarker	Jet-milled dust*	Unground (original) dust*	Ball-milled dust
Total white cells, 1 wk	-0.50	0.44	0.29
Total white cells, 4 wk	0.54	0.19	0.40
Neutrophils, 1 wk**	0.14	0.28	0.14
Macrophages, 1 wk	0.55	0.43	0.46
Macrophages, 4 wk	0.67	0.20	0.95
LDH, 1 wk	0.50	0.45	0.31
LDH, 4 wk	0.88	0.26	0.41
Macrophage stimulation, 1 wk	0.33	0.33	0.21
Macrophage stimulation, 4 wk	0.47	0.24	0.19
AVERAGE SEE (mg/m^3)	0.51 ±0.21	0.31 ±0.10	0.37 ±0.24

*Mann – Whitney U test gave P=0.02 in comparing jet-milled and unground dust SEEs. Other pair-wise comparisons were P>0.05.

**The neutrophil count at 4 wk is not shown because none of the BMD models for Qz fit the acceptance criteria. Nonetheless, it was clear that there was still a strong response to the neutrophil counts in the lunar dusts 4 wk after dose administration.

c. Safe exposure estimates from inhalation data

i. NOAEL as POD (Lam, et al, 2013)

The average, gravimetrically-determined concentrations from the 1-month, rat inhalation studies were as follows: 2.1, 6.8, 20.8, and 60.6 mg/m^3. The default approach to estimating an exposure level that is safe for humans from rat data is to establish a POD based on the NOAEL in rats. This approach may not be as simple as one might suppose because many observed changes in response to a toxic agent are not adverse; they are adaptive. For example, in the 6.8 mg/m^3 group, there were statistically significant changes in the neutrophil counts in the lavage fluid in the group harvested at 3 months after exposure; however, the magnitude of the increase was very small compared to the next higher group and was in the range others investigators have deemed “not adverse.” In addition, there were no adverse effects detected by histopathology in the group of rats exposed to 6.8 mg/m^3. Thus, 6.8 mg/m^3 was considered the NOAEL and used as the POD; however, the true concentration that would

have caused no *adverse* effect is likely to be somewhat above 6.8 mg/m^3 as the effects at the next-higher concentration of 20.8 mg/m^3 were not marked. Using a rat-to-human extrapolation factor of 3 and time adjustment factors of 1.33 to account for the difference between the 6-hour inhalation exposures and an 8-hour work day, and 6 to extrapolate from 1-month animal exposures to 6-months of potential human exposures, the POD of 6.8 mg/m^3 yields a *minimum* SEE of 0.3 mg/m^3.

ii. BMD as POD (Scully, et al., 2013)

In addition to the default NOAEL approach for establishing a POD, a range of PODs was derived from the extremes defined by the lowest average BMDL (5.8 mg/m^3) and the highest average BMD (16.6 mg/m^3) obtained by the BMD modeling. Using these values as PODs and application of safety factors of 1.33, 3, and 6, as described above, yields a lower bound on the SEE of 0.2 mg/m^3 and an upper bound of 0.7 mg/m^3. The results of BMD modeling are shown in Table 2.

Table 2: *BMD and BMDL Means with Inclusion of Various Numbers of Models of the Inhalation Data

*This table illustrates the change in average BMD and BMDL at each sampling time as the number of models was increased successively to include those generated from transformed data (middle) and finally, those derived from biomarkers in which the data did not satisfy requirements for normality and/or equality of variance (bottom).

Time (d)	BMD	BMDL
Parametric Data		
Mean→	**16.6**	**8.8**
1	21.3	12.5
7	16.1	6.1
28	8.6	5.6
91	11.1	6.1
Parametric and Transformed Data		
Mean→	**12.1**	**6.4**
1	21.3	12.5
7	11.7	4.9
28	9.1	6.3
91	6.5	2.8
All Successfully Modeled		
Mean→	**10.0**	**5.8**
1	17.9	10.8
7	9.8	5.0
28	8.1	5.6
91	9.4	3.9

4. Recommended safe exposure limits to lunar dust

The two approaches used to determine a SEE for lunar dust were essentially independent. One approach, the ITI method, depended entirely on the comparative toxicity of lunar dusts to standard dusts that have well established human PELs, TLVs and RELs. The means of comparison was BMD modeling of data from ITI exposures. In this method the slope and intercept of a line plotting Log $BMD_{(1\ SD)}$ vs. Log TLV or Log REL for the two reference dusts quartz and TiO_2, respectively (use of TLV and REL provides more conservative SEEs than use of PELs) were determined and linear regression was used to determine Log TLV for each Lunar dust from its Log $BMD_{(1\ SD)}$. The TLV (mg/m^3) for each lunar dust was then derived by taking the anti-log of its Log TLV.

This approach has many advantages including the following:

- anchored to human exposure levels widely regarded as safe
- required very small quantities of test dust
- used the entire dose response curve

- incorporated a large set of biomarkers of toxicity.

The average toxicities (SEEs) of the lunar dusts deduced by this method were 0.5 ±0.2, 0.3 ± 0.1 and 0.4 ±0.2 mg/m^3 for jet-milled, native, and ball-milled lunar dust, respectively. Pair-wise comparisons at the 95% confidence level showed only that the jet-milled dust and the native dust were statistically different.

The second approach to discerning a SEE for lunar dust was independent of any industrial exposure limits for terrestrial mineral dusts. This approach required establishing a NOAEL-based POD from the inhalation, dose-response data found by analysis of many biomarkers of toxicity. From the POD, investigators must then decide what safety factors to apply to the result from the rat exposures. Within the group of LADTAG toxicologists, there was considerable debate about what species-extrapolation factor to use to bridge any differences in the susceptibility of rats and humans. Ultimately, we settled on a factor of 3, although some toxicologists thought this could be as low as 1 (i.e. no species-extrapolation factor). Extrapolating from animal exposures of 6 hours per day to 8 hours, which constitute a typical work day, upon which TLVs are based, required a time factor of 1.33. The extrapolation from 1 month of rat exposure to anticipated 6 months of astronaut exposure was readily agreed to as requiring a factor of 6. Thus we applied an overall safety factor of 24 to the NOAEL-based POD, obtaining a minimal SEE of 0.3 mg/m^3.

The third approach to determining a SEE depended on BMD modeling of the inhalation data. Two PODs were deduced – one from the BMDL (5.8 mg/m^3) and the other from the BMD (16.6 mg/m^3). BMDLs tend to be very conservative as a POD and BMDs much less so. By applying the safety factor of 24 to these PODs, the SEE estimate was a minimum of 0.2 mg/m^3 and a maximum of 0.7 mg/m^3.

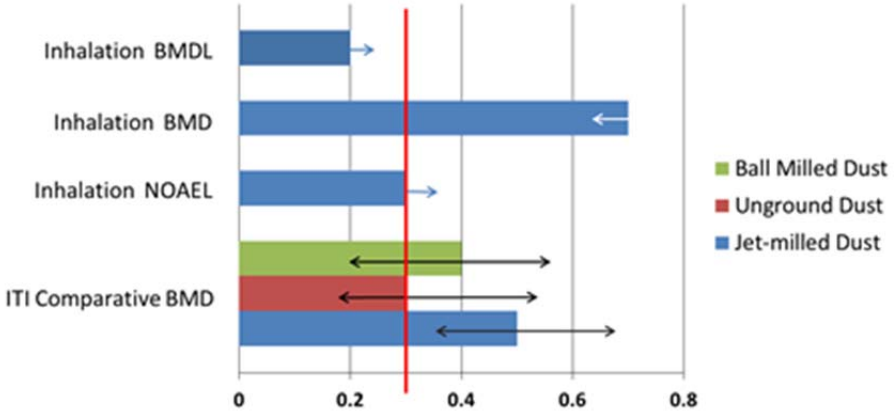
The spectrum of SEE's from the computations above is shown graphically in Figure 1. The SEE derived from the NOAEL of the inhalation study provides the foundation for setting the SEE for lunar dust (0.3 mg/m^3), and is supported by those derived from the comparative benchmark dose analysis of data from the ITI study as well as the those from the application of the BMD methodology to the inhalation study.

The reader will note that the SEEs derived from the ITI data were referenced to a working lifetime of exposure, whereas the other SEEs were estimates from a 1-month exposure in rats, extrapolated to 8 hour working day and to a 6-month exposure in humans. Nonetheless, the "working lifetime" SEEs from the ITI study tend to be higher than the SEEs from the inhalation data. This may be due to our conservative choice of the species-extrapolation factor of 3 for derivation of a SEE from the inhalation data. If we had used a species extrapolation factor of 1, as a few experts in our LADTAG group advocated, then the inhalation-derived SEEs would be 3-fold higher.

Under different scenarios of dust entry into the lunar habitat and times of exposure, one may employ Haber's Rule to estimate time-weighted average exposures that would be safe. Basically, this approach assumes no clearance and that the cumulative amount of dust in the lung is the driver of the toxic effects. Thus, a halving of the dust concentration and doubling of exposure time would yield the same toxicological outcome. For example, 7 days (168 hours) of continuous exposure to a time-weighted

average of 1.6 mg/m^3 would be considered safe if a POD from the 1-month NOAEL (120 hours @ 2.3 mg/m^3) were used (Lam, et al. 2013).

Figure 1. Safe Exposure Estimates (SEE) for a 6-month, episodic exposure to lunar dust based on intratracheal instillation (ITI) data and inhalation data in rats. Arrows indicate the direction from the derived value (bar) that the true SEE should be found (inhalation data) or the SD of the mean SEE (ITI data). Recommended overall SEE is shown by the red line (0.3 mg/m^3).



5. Limitations and Further Research

a. Cardiovascular toxicity

There is a growing body of evidence that particulate matter is associated with cardiovascular effects in humans, especially in urban areas (Pope, et al. 2004; Brook, et al. 2010). Most urban particulate pollution is not mineral dust; however, when dessert-sand dust pollutes the air, an association to cardiovascular effects can sometimes be made (Mallone, et al. 2012; Morman, Plumlee, 2011); however, the association is not consistently found (Karanasiou, et al. 2011) and may vary with particle size (Tobias, et al. 2011). Although we have used a wide array of sensitive endpoints to deduce any adverse effects at the point of entry of the dust (lungs), the potential for adverse effects on the cardiovascular system should be assessed using lunar dust. This is particularly true in light of the attention NASA is gives to the cardiovascular changes during spaceflight (Hamilton, 2008). The LADTAG and the SRP have both suggested a need to post this as a knowledge gap and conduct further research; however, funding for such an investigation was not available. The risk designation is AEH 11: "What is the potential for acute or chronic cardiovascular toxicity of lunar dust?" There are several relatively simple preparations that could be used to explore cardiovascular effects in laboratory animals with lunar dust that was allocated (jet-milled) and unused in our pulmonary toxicity studies. For example, in rats exposed by ITI, endothelins, vasopressors, vasodilators, catecholamines and oxidative stressors could be measured to assess acute cardiovascular effects (Vincent, et al. 2011)

b. Surface reactivity of lunar dust

One of the key problems the LADTAG Working Group attempted to address was the possibility that dust toxicity, as mentioned above, could be increased because of surface reactivity of the dust particles at the surface of the moon. The dust there is subjected to ordinary solar wind radiation (primarily protons), radiation from solar flares (X-rays), and cosmic rays. These radiations have the potential to render the surface of dust reactive, and since there is no atmosphere on the moon, this surface reactivity will persist until a meteorite impacts the lunar surface and melts the dust into larger glassy beads or agglomerates. The geologists on the LADTAG team were unable to settle on which available, earth-based, laboratory processes could adequately simulate the nature of dust activation on the lunar surface. Ultimately, it was decided that grinding of the dust would be the best way to simulate surface activation. Thus, we used jet-milled dust for the inhalation studies and for the *in vitro* ocular testing.

It is the collective opinion of the toxicologists on this report that any enhancement of the toxicity by surface reactivity of lunar dust would be secondary to the primary processes causing toxicity. For example, there is no correlation between the ability of the 3 lunar dusts we tested to produce reactive oxygen species in the terephthalate assay and the respiratory toxicity of the dusts tested by ITI (James, et al. 2013; McKay, et al. 2013). In addition, quartz is highly toxic to the respiratory system, yet it produced low reactivity in the terephthalate assay (Wallace, et al. 2009).

c. Variability of dust upon the lunar surface

We used a single source of lunar dust, from which we prepared various samples for toxicity testing. Samples from Apollo 14 regolith are generally considered to be a combination of mare and highland soils (Wallace, et al. 2009). This means that our test material was well representative of the dust that covers much of the visible surface of the moon. It is unlikely to be representative of dust "mined" from well below the surface or dust from areas at the bottom of polar craters that never see sunlight. Once operations involve the risk that subsurface dust may enter the habitat, a fresh look at the toxicity of lunar dust will be in order.

d. Acute toxicity of lunar dust

From time to time during this project the investigators were asked to consider developing a database for setting a SEE for an acute situation in which a large amount of dust happened to enter the lunar habitat. This would require that human subjects be exposed to lunar dust at various high concentrations to observe their reactions to the material. Ultimately, the credibility of such a scenario was questioned therefore human exposures to lunar dust were never initiated. It should be pointed out that heavy air pollution by volcanic ash, which has properties similar to lunar dust, can cause respiratory irritation (asthma and bronchitis), but no lasting respiratory symptoms unless the ash contains a substantial amount of crystalline silica, particles are very fine (respirable), and exposures are repeated over years (Horwell, Baxter, 2005). Repeated, acute exposures to lunar dust over several years are not considered a credible scenario; however, the immune (allergenic) response of acute exposures may warrant investigation (Lam, et al. 2013).

e. Differences in lung deposition based on reduced lunar gravity

Early in the lunar dust toxicity project, experts weighed the issue of how the moon's 6-fold lower gravity compared to the earth's gravity might affect the toxicity of lunar dust in the lung. One of the major mechanisms of dust deposition in the lung is sedimentation, which is highly dependent on the gravity vector. This possibility has been well studied in human subjects using the lunar gravity that can be created in parabolic aircraft flights. Using particles of 0.5 and 1 μm, it was found that, although deposition is proportionally more distal in reduced gravity, the absolute amount of dust deposition is substantially less (Darquenne and Prisk, 2008). In light of this observation, it was decided that no adjustment or correction factor was needed to deal with differences in dust deposition at earth gravity, where our rat-exposure experiments were performed, when estimating human susceptibility during lunar exploration at $1/6^{th}$ of earth's gravity.

References

Brook RD, Rajagopalan S, Pope CA, Brook JR, et al. Particulate matter air pollution and cardiovascular disease- An update to the scientific statement from the American Heart Association. Circulation 2010; 121:2331-2378

Darquenne C, Prisk GK. Deposition of inhaled particles in the human lung is more peripheral in lunar than in normal gravity. Eur J App Physiol 2008:103:687-695

Hamilton, D. Cardiovascular Disorders, pp 317-359, Ch 16 in *Principles of Clinical Medicine for Space Flight*, eds. Barratt MR and Pool SL, Springer (2008)

Horwell CJ, Baxter PJ. The respiratory hazards of volcanic ash: A review for volcanic risk mitigation. Bull Volcanol, DOI 101007/s00445-006-0052-y

*James JT, Lam CW, Santana P, Scully RR. Estimate of safe human exposure levels for lunar dust based on comparative benchmark dose modeling. Inhal Toxicol 2013:25:243-256

Karanasiou A, Moreno N, Moreno T, Viana M, et al. Health effects from Sahara dust. ETC/ACM Technical Paper 2011/4

*Lam CW, Scully RR, Zhang Y, Renne R, et al. Toxicity of lunar dust assessed in inhalation-exposed rats. Inhal Toxicol 2013 (in press)

Mallone S, Stafoggia M, Faustini A, Gobbi GP, et al. Sahara dust and associations between particulate matter and daily mortality in Rome Italy. EHP 2012; 119:1409-1414

*McKay DS, Taylor LA, Cooper BL, Liu Y, et al. Properties of potentially toxic, respirable-size lunar dust. Meteoritics & Planetary Sci 2013 (submitted)

*Meyers VE, Garcia HD, Monds K, Cooper BL, James JT. Ocular toxicity of authentic lunar dust. BMC Ophthalmology 2012; 12:26-33

Morman SA, Plumlee GS. The role of airborne mineral dusts in human disease. Aeolian Res 2013; 9:203-212

National Institutes for Occupational Health and Safety (2011). Current Intelligence Bulletin 63. Occupational Exposure to Titanium Dioxide. DHHS (NIOSH) Publication No. 2011–160.

Pope CA, Burnett RT, Thurston GD, Thun MJ, et al. Cardiovascular mortality and long-term exposure to particulate air pollution - epidemiological evidence of general pathophysiological pathways of disease. Circulation 2004; 109:71-77

*Scully RR, Lam CW, James, JT. Estimating safe human exposure levels for lunar dust using benchmark dose modeling of data from inhalation studies in rats. Inhal Toxicol 2013 (submitted)

Tobias A Perez L, Diaz J, Linares c, et al. Short-term effects of particulate matter on total mortality during Saharan dust outbreaks: A case-crossover analysis in Madrid (Spain). Science of the Environment 2011; 412;386-389

Vincent R, Kumarathasan P, Goegan P, Bjarnason S, et al. Inhalation toxicology of urban ambient particulate matter: Acute cardiovascular effects in rats. Research Report (Health Effects Institute) 2001; 104:5

*Wallace WT, Taylor LA, Liu Y, Cooper BL, et al. Lunar dust and lunar dust simulant activation and monitoring. Meteoritics & Planetary Sci 2009; 44:961-970

*Papers produced as part of the Lunar Dust Toxicity Project