Evidence Report:

Risk of Adverse Health & Performance Effects of Celestial Dust Exposure

Human Research Program
Space Human Factors and Habitability (SHFH) Element

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Crew members can be directly exposed to celestial dust in several ways. After crew members perform extravehicular activities (EVAs), they may introduce into the habitat dust that will have collected on spacesuits and boots. Cleaning of the suits between EVAs and changing of the Environmental Control Life Support System filters are other operations that could result in direct exposure to celestial dusts. In addition, if the spacesuits used in exploration missions abrade the skin, as current EVA suits have, then contact with these wounds would provide a source of exposure. Further, if celestial dusts gain access to a suit's interior, as was the case during the Apollo missions, the dust could serve as an additional source of abrasions or enhance suit-induced injuries. When a crew leaves the surface of a celestial body and returns to microgravity, the dust that is introduced into the return vehicle will "float," thus increasing the opportunity for ocular and respiratory injury.

Because the features of the respirable fraction of lunar dusts indicate they could be toxic to humans, NASA conducted several studies utilizing lunar dust simulants and authentic lunar dust to determine the unique properties of lunar dust that affect physiology, assess the dermal and ocular irritancy of the dust, and establish a permissible exposure limit for episodic exposure to airborne lunar dust during missions that would involve no more than 6 months stay on the lunar surface. Studies, with authentic lunar soils from both highland (Apollo 16) and mare (Apollo17) regions demonstrated that the lunar soil is highly abrasive to a high fidelity model of human skin. Studies of lunar dust returned during the Apollo 14 mission from an area of the moon in which the soils were comprised of mineral constituents from both major geological regions (highlands and mares regions) demonstrated only minimal ocular irritancy, and pulmonary toxicity that was less than the highly toxic terrestrial crystalline silica (Permissible Exposure Limit [PEL] 0.05 mg/m3) but more toxic than the nuisance dust titanium dioxide (TiO2 [PEL 5.0 mg/m3]). A PEL for episodic exposure to airborne lunar dust during a six-month stay on the lunar surface was established, in consultation with an independent, extramural panel of expert pulmonary toxicologists, at 0.3 mg/m3.

The PEL provided for lunar dust is limited to the conditions and exposure specified therefore additional research remains to be accomplished with lunar dust to further address the issues of activation, address other areas of more unique lunar geology (Glotch et al., 2010; Greenhagen et al., 2010), examine potential toxicological effects of inhaled or ingested dust upon other organ systems, such cardiovascular, nervous systems, and examine effects of acute exposure to massive doses of dust such as may occur during off-nominal situations. Work to support the establishment of PELs for Martian dust and dusts of asteroids remains to be accomplished.

The literature that describes health effects of exposure to toxic terrestrial dusts provides substantial basis for concern that prolonged exposure to respirable celestial dust could be detrimental to human health. Celestial bodies where a substantial portion of the dust is in the respirable range or where the dusts have large reactive surface areas or contain transition metals

or volatile organics, represent greater risks of adverse effects from exposure to the dust. It is possible that in addition to adverse effects to the respiratory system, inhalation and ingestion of celestial dusts could pose risks to other systems.

III INTRODUCTION

NASA has adopted a multi-destination human exploration strategy and is developing a core set of capabilities that will allow increasingly complex missions to near-Earth asteroids, the Moon, and Mars and its moons (NASA 2011). It is anticipated that a return to the Moon or missions to Mars will involve construction of habitats on the surface in order to support long-duration human habitation and research. Therefore the potential opportunities for exposure of crews to celestial dusts, which could be returned to habitats after surface activities, would far exceed those occasioned by the limited number and duration of extravehicular activities (EVA) that occurred during the Apollo lunar landings. Therefore the risk of exposure to celestial dusts and the potential risk of adverse health effects will be elevated well beyond that experienced by the Apollo crews. During the Apollo missions, lunar dust, which had adhered to space suits during EVAs, gained entry to the interior of the lunar modules where, after becoming dislodged, it became airborne with the loss of lunar gravity upon ascent of the vehicle from the surface. The airborne dust irritated the eyes and throats of Apollo crews (Wagner, 2006). A flight surgeon, who was exposed to lunar dust during post-mission handling of EVA suits, reported symptoms consistent with an allergic response, which worsened with each exposure (Scheuring et al., 2008). During the Apollo era, this anecdotal evidence of the possible toxicity of lunar dust was followed by an effort to experimentally assess its toxicity, but the effort produced little useful information because interpretation was complicated by spontaneous pathology in control animals (Holland and Simmonds, 1973). Later studies (Batsura, et al., 1981; Kustov et al., 1974 and 1989) also suffered from limitations that compromised the quality of the toxicity assessments they provided. These assessments ranged from no effects (Kustov et al, 1974), when indices were assessed after animals were exposed to air that had passed over lunar dust, to findings of fibrosis after intratracheal instillation of massive amounts (50 mg) of dust (Kustov et al., 1989). Therefore at the time that new missions to the moon were being planned, the toxicity of lunar dust remained to be determined.

The importance of particulates in air as a hazard to health has received increasing attention since the time of the Apollo missions. During the 1980s and ’90s, understanding of the mechanisms involved in the hazards posed to humans by exposures to the silicate mineral asbestos matured. There was tremendous concern when large-scale epidemiological studies, which had become feasible with advances in methods for processing large data sets, demonstrated the relationships between airborne particulate matter (PM) and impacts on human health (Bhatnagar, 2006; Cassee, 2007; Maynard, 2015). A substantial volume of research was begun that contributed to paradigms, which continue to evolve, that relate physiochemical characteristics of particulates to pulmonary toxicity. Prominent among the physiochemical features of mineral dusts that affect or may affect toxicity are size, morphology (shape, sharp edges, fractured surfaces, surface defects), surface area, surface reactivity, and solubility (Castranova et al.,1996; Donaldson and Borm 1998; Donaldson et al., 2001; Fenoglio et al., 2000; Fubini 1998, 2002; Ghaizza et al., 2010; Gutherie, 1997; Jones and BéruBé 2007;

Kajiwara et al., 2007; Napierska et al., 2010; Ovrevik et al., 2005; Pauluhn 2011, 2012; Sager et al., 2008; Schoonen et al., 2006; Schwarze et al., 2007; Warheit et al., 2006. 2007).

Unlike Earth, on which atmospheric and water erosion of the surface has caused most rock to be formed by sedimentation, these processes are absent on the moon. Rather, the lunar rocks are volcanic in origin (igneous). The lunar surface is comprised principally of two geological regions, the mares and highlands regions. The mares region is composed of dark basalts, which form from rapid cooling of molten rock from massive lava flows. The highlands region is composed mostly of anorthosite that forms when igneous cools more slowly than basalts. These regions comprise 17 and 83%, respectively, of the exposed crust of the lunar surface. The vast majority of the mares are on the near side (Pieters, 1986). Evidence from the Lunar Reconnaissance Orbiter Diviner Lunar Radiometer Experiment revealed the presence of highly evolved, silica-rich lunar soils in kilometer-scale and larger exposures, which indicates that the moon has experienced a diverse set of igneous processes (Glotch et al., 2010; Greenhagen et al., 2010). Recent results, reviewed by Jaumann et al. (2012) also indicate OH- and H2O are formed and retained even outside of polar regions. H2O and OH- are thought to be formed "by solar wind protons interacting with oxygen-rich rock surfaces produced during micrometeorite impact on lunar soil particles" (Jaumann et al., 2012).

Lunar regolith is the layer of unconsolidated rocks, pebbles, and dust over lunar bedrock (Colwell et al., 2007). Lunar dust, defined as particles less than 20 microns (μm) (Liu and Taylor, 2011) comprises about 20%, by weight of the lunar soil (Park et al., 2008). The respirable fraction of the dust (less than 2.5 μm) comprises 1-3% of the mass fraction of mature lunar soil (Cooper et al., 2010; McKay et al., 2015). Lunar dust possesses properties that have been associated with toxicity of mineral dusts. The native shape of lunar dust is a complicated morphology with glassy beads and irregular, sharp particles with extensive surface area (Liu et al., 2008). The surface of dust on the moon is likely to be reactive due to broken, dangling chemical bonds resulting from commutation due to micrometeoroid bombardment, proton bombardment from the solar wind, and ultraviolet and intergalactic radiation (Liu and Taylor, 2011). Further, nanophase metallic iron (Fe0), which can catalyze the formation of hydroxyl radicals in solution via the Fenton reaction, is present in lunar dust particles (Taylor et al., 2001). The fraction of total iron present as Fe0 increases as the particle size diminishes (Taylor et al 2011), at least as size decreases to 2 μm (McKay et al, 2015). These features suggested that lunar dust may be toxic. Data from recent studies in rodents (James et al., 2013; Lam et al., 2013) confirm that lunar dust is toxic to the respiratory system, but fortunately, these characteristics do not confer toxicity that exceeds that of quartz obtained here on Earth.

Large portions of the surfaces of carbonaceous asteroids are covered in dust (Murdoch et al., 2015). On these celestial bodies the processes that operate on the lunar surface to produce dust and affect its properties (space weathering) are also effective and produce particles with rims containing Fe0 (Matsumoto et al., 2014). However, on asteroids of less than 1 km in diameter, dust produced by comminution resulting from micro meteor impacts likely attains escape velocities and is lost (Delbo et al., 2014). On these bodies thermal fatigue by temperature cycling is likely the more important process for dust formation (Delbo et al., 2014). On these small asteroids, vibrations from impacts, thermal fluctuations, and electrostatic lifting of surface

particles (Garcia et al., 2015; Renno and Kok, 2008) cause the smallest particles to migrate to gravitationally stable areas so that dust ponds, craters with flat floors filled with dust, result from migration of fine particles to low areas. Near sunset, oscillating canopies of dust will form over negatively charged craters on surfaces of airless bodies (Collier et al., 2012). On asteroids such as Vesta that are located where impact velocities of micrometeors are significantly less than they are at one astronomical unit from the sun (on the moon), shock dominates over melting and vaporization, and "regolith does not accumulate detectible nanophase opaque particles on rims of grains" (Pieters et al., 2012).

On Mars, measurements made by the Spirit rover identified differences between the textures of surface and subsurface particles in Gusev crater. Wind alters the grain size distribution, angular shapes, and agglutinates caused by comminution in grains on the surface. Therefore, among grains ~ 100 μm (detection limit for Spirit), the agglutinates that are typical of lunar soils in this particle size range are absent, and most grains are rounded (McGlynn et al., 2011). The fine-grained surficial dust, because of global mixing by wind (Yen et al., 2005), is very similar at the various landing sites (Schuerger et al., 2012). Indeed, "chemical analyses conducted with the Alpha Particle X-ray Spectrometer on the rover Curiosity corroborate the earlier compositional measurements of fine-grained wind-transported materials", and confirm the remarkably homogeneous chemical composition of the dust (Downs, 2015). It is highly oxidized and contains basaltic materials, nanophase iron oxides (npOx), and extremely high salts levels (e.g., MgCl2, NaCl, FeSO4, and MgSO4 [Morris et al., 2006]), high concentrations of heavy metals and perchlorate, and is likely acidic (Schuerger et al., 2012). The perchlorate content, which ranges between 0.5 and 1%, is 3 to 4 orders of magnitude greater than in soils on Earth (Davila et al., 2013). On Mars large-scale electrostatic fields generated by charged sand and dust in the dust devils and storms, as well as during normal saltation, can induce production of H2O2, which can condense, precipitate and be adsorbed into the regolith (Atreya et al., 2006). These features suggest that the Martian dust is likely toxic.

Because evidence with which to assess the toxicity of lunar dust was unavailable when the planning of crewed missions to the moon was resumed in the last decade, NASA recognized a risk of adverse health effects associated with exposure to lunar dust and identified gaps in our knowledge about features of the dust and its toxicity that needed to be filled in order to establish safe exposure limits that would inform the design of habitats and vehicles so that exposures of crews to lunar dust would be limited to safe levels. Because the human exploration strategy now envisions multiple destinations, the scopes of these gaps have been extended beyond lunar dust to include the need to obtain evidence with which to assess the toxicity of dusts that will be encountered at other celestial destinations.

IV EVIDENCE

A Spaceflight Evidence

All spaceflight evidence pertaining to the effect of lunar dust on astronauts is anecdotal (Category III). The post-flight debriefing reports of the Apollo astronauts serve as a base of evidence (Armstrong et al., 1969; Cernan et al., 1973; Conrad et al, 1969; Scott et al., 1971;

Shepard et al., 1971; Young et al, 1972). In these reports, the Apollo crews provided several accounts of problems associated with their exposure to lunar dust. The following are excerpts from the reports of their debriefings:

1. During Apollo 11, despite attempts by each astronaut to remove dust from his suit and equipment before entering the lunar module (LM) a large amount of lunar dust and grains was brought into the cabin. When the crew removed their helmets, they noticed a distinct, pungent odor, like that of gunpowder, emanating from the lunar material. Both crewmen discovered after they removed their helmets, overshoes, and gloves that they were very dirty. One grain of material got into the Commander's eye, but was easily removed and caused no problem. Crewmembers reported sleeping with their helmets on, in part, so they "wouldn't be breathing all that dust" (Armstrong et al, 1969; Sheehan, 1975). The concern that particles remaining in the lunar module would float in the cabin atmosphere at zero-g after ascent caused the crew to wear their helmets to prevent eye and breathing contamination. Precautions were taken to prevent the command module (CM) from becoming contaminated with dust contained in the LM. Despite these precautions some dust entered the CM. The CM was cleaned during the return to earth at 24-hour intervals using the vacuum brush and towels. In addition, the circulation of the cabin atmosphere through the lithium hydroxide filters continued to remove traces of particulate material (Armstrong et al., 1969).
2. During Apollo 12, the crew members reported that both LM and CM were contaminated with lunar dust; "The LM was filthy dirty and had so much dust that when I took my helmet off, I was almost blinded. Junk immediately got into my eyes"; and "[t]he whole thing was just a cloud of fine dust floating around in there." After the LM docked to the CM, dust infiltrated the CM. Crewmembers gave the following account of this period of contamination: "On the way back in the CM the system could not handle the dust, so it was continuously spread inside the spacecraft by the system"; "[w]e chose to remain in the suit loop as much as possible because of the dust and debris floating around"; and "[t]o keep our eyes from burning and our noses from inhaling these small particles, we left our helmet sitting on top of our heads"; "When you took your helmet off, you could smell the lunar dirt. It smelled like – the nearest analogy I can think of is gunpowder." The dust was so fine that it was not removed by the filters "the system is not doing the cleaning, the dust is too fine" (Conrad et al., 1969).
3. By contrast, the Apollo 14 crewmembers stated: "The cabin dust kind of swirled around. A lot of that went out through the relief valve at that point, which might have reduced it somewhat". Dust was not a problem for us in the cabin"; and the dust control procedures were effective; we got very little dust back in the command module; I thought the command module was remarkably clean"; "there was no loose dust coming off the suits"; "We felt the procedures… certainly reduced the dust to a minimum" (Shepard et al., 1971). (It should be noted, however, that in the Apollo 14 mission the crew did not experience any interference by dust with visualization of the surface during approach as was the case in earlier and later missions. This may have

been due in part to the Apollo 14 landing site being intrinsically less dusty than other Apollo landing sites, (Shepard et al., 1971)).

4. The Apollo 15 crewmembers stated: “Our legs from about thigh down were just about completely covered with dirt”; “Cabin smelled like gunpowder when we first came into LM from EVA”; “you could see particulate matter floating around”; the vacuum cleaner did a good job of clearing the dust from the LM”; “When we woke up the next morning, I was surprised how clean the spacecraft was. I think most of the dust had been removed”; “Yes, the ECS (Environmental Control System) does a pretty good job of cleaning the place out. The smell was gone” (Scott et al., 1971).

5. Apollo 16 crewmembers provided the following accounts: “The LM was extremely clean until the first EVA and then it was extremely dirty”; “I question whether the vacuum cleaner ever worked properly”; and “I thought it was quite a hazard over there floating through the LM with all the dust and debris. A number of times I got my eyes full of dust and particles. I felt like my right eye was scratched slightly once” (Young et al., 1972; Wagner, 2006).

6. The Apollo 17 crewmembers recalled: “After the first EVA, we found out what the dust problem really was”; “Really, the dust was so deep and soft...”; “Probably the most difficult job of all the closeouts was trying to dust the suits”; “I had the sinus irritation on the surface”; “As soon as we were hard docked, the commander took off his helmet”. “…because of the dust debris in the LM spacecraft, I’m sorry I did. I could have left the helmet on, and I would have had a lot less eye and mouth type of irritation”. “You knew [that] you were in a very heavily infiltrated atmosphere in the LM because of the lunar dust”; “[t]he dust clearing was remarkable considering the amount of dust we had”; “[a]lthough there was a lot of irritation to my sinuses and nostrils soon after taking the helmet off, by 2 hours that had decreased considerably”; “I did all the transfer with my helmet off and I am sorry I did because the dust really bothered my eyes and throat. I was tasting it and eating it”; and “[w]hen I climbed in the tunnel I could tell there was a lot of dust in the LM and you could smell it” (Cernan et al., 1973). “You have to live with it but you're continually fighting the dust problem both outside and inside the spacecraft. Once you get inside the spacecraft, as much as you dust yourself, you start taking off the suits and you have dust on your hands and your face and you're walking in it. You can be as careful in cleaning up as you want to, but it just sort of inhabits every nook and cranny in the spacecraft and every pore in your skin. Although I didn't have any respiratory problems, I think the LMP (lunar module pilot) which he can comment on later, had some definite local respiratory problem immediately after the EVAs due to the dust in the cabin”. “Almost immediately upon removing my helmet, I started to pick up the symptoms that you might associate with hay fever symptoms. I never had runny eyes or runny nose. It was merely a stuffiness in the nose and maybe in the frontal sinuses that affected my speech and my respiration considerably. After about 2 hours within the cabin, those symptoms gradually disappeared. By morning of the next day, they were gone completely. After the second and third EVAs, although I'm sure the dust was

comparable, the symptoms were not nearly as strong as after the first EVA. That was as if I either developed a mucous protection of the affected areas or had some way or another very quickly developed an immunity to the effects of the dust" (Cernan et al., 1973).

Although no substantive evidence exists that astronaut performance was impaired by lunar dust (Wagner, 2006), one can imagine that if a crew member were "almost blinded" and had to "remain in the suit loop as much as possible because of the dust and debris floating around," the dust did have some impact on performance.

Dust from the lunar soil that was carried into the spacecraft during the Apollo missions proved to be a significant, intermittent problem. With the return to the moon and planned long-duration stays on the lunar surface, contamination problems and toxicity of the dust are potentially much more serious than those that were experienced during the Apollo missions. Gravity at one-sixth that of the gravitational force of the Earth increases the time in which dust remains airborne, thereby increasing the probability that these dust particles will be inhaled. Some examples of lunar dust grains are provided in Figure 1.

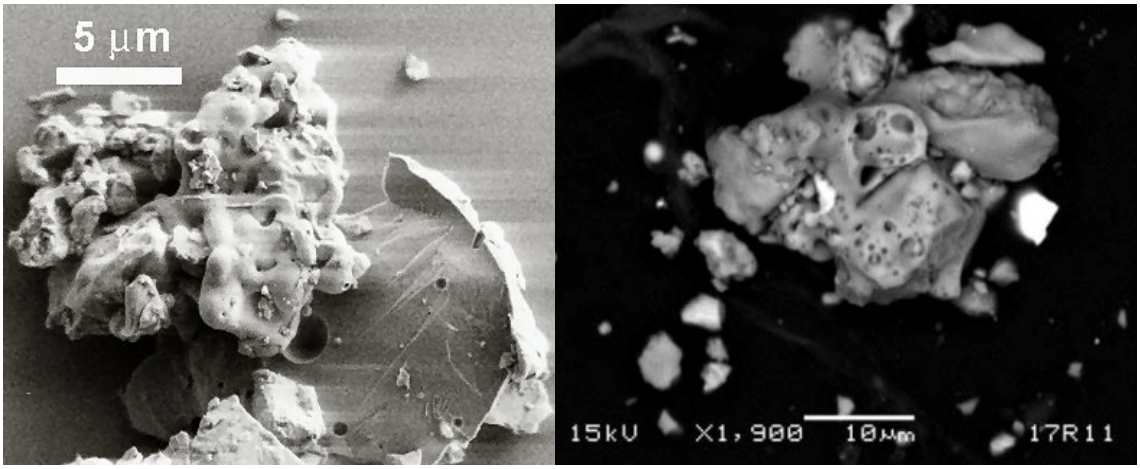

Figure 1. Examples of lunar dust grains. – LEFT: Scanning electron microscope (SEM) image of a typical lunar agglutinate. Note the sharp edges, reentrant surfaces, and microcraters. Smaller grains, which are less than 1 µm in diameter, are attached to this particle, and are also seen as loose grains in the upper portion of the image. RIGHT: SEM image of a lunar agglutinate fragment that was removed from the outer surface of Harrison Schmitt's EVA suit.

B Ground-based Evidence

Humans are typically exposed to respirable dust "of minerals and geomaterials during activities such as mining, quarrying, and sandblasting, building demolition, and natural processes such as volcanic ash eruption and dust storms" (Hurowitz et al., 2007). Ground-based evidence includes data that are derived from people who are exposed occupationally to mineral dusts in industrial settings, from people who live in close proximity to active volcanoes and have been exposed to volcanic ash, people who have live in regions exposed to windborne dusts from deserts, and from controlled laboratory experiments performed with humans, animals and cells. Mechanistic insights also guide our thinking concerning the potential toxicity of celestial dusts.

1. Evidence from Human Exposures during Industrial Operations

Workers in the mining industry are often exposed to dust from freshly fractured mineral deposits. When these workers do not utilize respiratory protection or use inadequate protection the consequences are devastating. A prime example of this is the Hawks Nest mining disaster that occurred in West Virginia. In 1927, during the boring of a tunnel, deposits of silica were identified and mined; however, the workers did not use respiratory protection during the operations. Estimates of the proportion of workers who died, many within a few years of the exposure, are typically about 30% of the 2000 exposed workers (Cherniack, 1986). Silicosis has also caused fatalities among sandblasters (Abraham and Wiesenfeld, 1997). This rapidly lethal form of silicosis, "acute silicosis," is characterized by alveolar proteinosis and interstitial inflammation (Driscoll and Guthrie, 1997). The respiratory effects are not exactly like those one would expect from simple or chronic silicosis, a disease that usually requires decades to develop after prolonged exposure to lower concentrations of silica dust. The latter disease is characterized by silicotic nodules that are clearly distinct from surrounding tissue and often surrounded by an inflammatory response (Driscoll and Guthrie, 1997).

In addition to increased risk of silicosis, individuals with detectable exposure to crystalline silica (CS) were found by Calvert (Calvert et al., 2003) to have significantly increased risk for silicosis, chronic obstructive pulmonary disease, pulmonary tuberculosis, and rheumatoid arthritis. Although the International Agency for Research on Cancer released a monograph in 1997 (IRAC 1997) that classified crystalline silica as a carcinogen, the dose-risk relationship, pathogenic mechanisms and the carcinogenic role of silica per se in absence of silicosis remains unclear (Erren et al., 2009; Pelucchi et al., 2006). However, substantial empirical evidence reviewed by Cox et al. (2011) supports an exposure-response relation between CS and risk of chronic inflammation, silicosis, fibrosis, and lung cancer, in a scenario in which exposures provoke inflammation, which produces increased reactive oxygen species (ROS) and reactive nitrogen species (RNS), pro-inflammatory mediators such as TNF-alpha, and eventual damage to lung tissue and epithelial hyperplasia, resulting in fibrosis and increased lung cancer risk among silicotics.

2. Evidence from Animal and Human Exposure to Urban Air Pollution

Urban air pollution is comprised of a mixture of contaminants. These include PM, ozone, carbon monoxide, nitrous oxides, sulfur oxides, heavy metals like lead and mercury, polycyclic aromatic hydrocarbons, and toxic chemicals. PM is a mixture of solid particles and liquid droplets that vary in size, shape, surface area, chemical composition, solubility and origin (Pope and Dockery, 2006). The size distribution of PM in air is trimodal within aerodynamic size ranges that are related to inhalation and depth of penetration within the respiratory system. Coarse particles, having aerodynamic diameters 10 – 2.5 μm (PM10 – PM 2.5), are derived primarily from dusts of soil, crustal materials, produced by farming, mining, windstorms, volcanos, as well as sea salts, pollen, mold, and spores. Fine particles (PM2.5 – PM0.1) are derived primarily from abrasive wear from tires and brakes, combustion processes, industrial processes, (e.g., smelters, cement plants, paper mills, and steel mills), and sulfate and nitrate particles generated by conversion from primary sulfur and nitrogen oxide emissions and

secondary organic aerosol from volatile organic compound emissions (Gasser et al., 2009; Pope and Dockery, 2006; Schettler, 2005). Ultrafine or nanoparticles are those with aerodynamic diameter < 0.1 μm (PM0.1). They are derived from abrasive wear from tires and brakes, combustion sources and atmospheric photochemical reactions and usually persist for minutes to hours before coagulating or condensing to form larger complex aggregates in the range of fine particles (Gasser et al., 2009; Pope and Dockery, 2006; Schettler, 2005).

Shins et al. (2004) found PM10 from a rural setting produced greater inflammatory effects in rat lungs than PM2.5 from an industrial area and attributed the response to endotoxins or related contaminants. Others, however, have demonstrated that coarse particles have a toxicological capacity equivalent to fine particles on a mass basis (Sandstrom et al., 2005). The oxidative reactivity (OR) of size segregated PM was tested at a traffic site (Price et al., 2014). PM2.5 and PM0.1 caused more DNA damage than coarse PM10. PM exhibited more OR compared to manufactured carbon black particles. Size, surface area and metals (Zn and Fe) are important particle characteristics for OR (Price et al., 2014; Verma et al., 2010). On the other hand, Miller et al. (2012) claim that unpublished data demonstrate that "clean carbon particles with significantly lower levels of the surface chemicals and metals can generate similar, if not greater, levels of free radicals than that of urban particulates of a similar size". Miller et al., (2012) argue that surface components alone may not be able to predict the toxicity of different PM and that in biological systems PM induce the cells/tissues to synthesize biologically-derived free radicals, adding to that produced directly by PM. There are several mechanisms by which different components of PM can generate free radicals and contribute to oxidative stress in biological systems. The pathways underlying effects on the cardiovascular (CV) system are complex and remain to be fully elucidated; however, PM-induced oxidative stress (Ayres et al., 2008; Donaldson et al., 2013; Miller et al., 2012), and local and systemic inflammation (Donaldson et al., 2013) repeatedly emerge as potential mechanisms in all detrimental effects of PM on the CV system. Therefore, although the particulate constituents of urban air pollution are largely derived from anthropogenic sources, while those of celestial dusts are naturally occurring, the relevance of the finding of studies of the former to assessments of toxicity of the latter is to direct attention to features of celestial dusts that indicate that they may induce oxidative stress and inflammation. Indeed, Rowe (2000, 2007, 2013) suggested that exposure to lunar dust, in susceptible individuals, may lead to cardiovascular effects that are similar to those produced through exposure to air pollution.

3. Evidence from Humans and Laboratory Animals Exposed to Volcanic Ash

Volcanic ash originates from processes resulting in explosive eruptions into the atmosphere or pyroclastic flows from the surface and discharging ash as they cool, or some combination thereof. Basic (e.g. basaltic) eruptions produce no crystalline silica (e.g., quartz), and contain mafic minerals such as calcium-rich feldspar, pyroxenes and olivine, whereas acidic (e.g. rhyolitic) eruptions produce silica-rich (> 69% wt) ash with high concentrations of felsic minerals such as quartz, potassium-rich feldspar and silica glass (Derbyshire et al., 2012). The particle size, mineral composition, and form of the minerals vary considerably from volcano to volcano as well as from one eruption to another eruption of the same volcano. Analysis of 63 ash

samples from around the world showed that the fraction <4 μm varied from 0–17 vol% (Horwell, 2007). Usually, the ash will have had hours to days to react with the oxygen and water vapor of the atmosphere to passivate surfaces before being inhaled by humans.

Shortly after Mount St. Helens erupted in 1980, a number of experts began to investigate the effects of volcanic ash on those who had been exposed to the dust (Bernstein et al., 1986). The crystalline silica content of this dust ranged from 3% to 7%. The primary acute effects were re¬flected in increased emergency room visits for asthma, bronchitis, and eye discomfort (Baxter et al., 1981). The ash was noted to exacerbate chronic respiratory conditions. The increase in hospital admissions lasted approximately 3 weeks (Nania and Bruya, 1982), and immune parameters were affected even 1 year later (Olenchock et al., 1983). The British West Indian Montserrat volcano began erupting in 1995, causing an ash fall from pyroclastic flows that contained 10% to 24% crystalline silica (Baxter et al., 1999). Recorded incidences of childhood wheezing increased as a result of relatively intense exposures to the ash (Forbes et al., 2003). There was a positive association between exposure to volcanic ash from the 2002 eruption of Mount Etna, (Sicily, Italy) and acute health effects in the Catania residents. Similarly, Icelanders exposed to volcanic ash from Eyjafjallajökull had increased prevalence of respiratory symptoms, specifi¬cally asthma and chronic bronchitis, com¬pared with a control population in northern Iceland (Carlsen et al. 2012). Sustained long-term health effects have not been reported in association with exposures to volcanic ash, although there is speculation that the high cristobalite content of the Montserrat ash could lead to silicosis many years later (Baxter, 1999).

Animal studies that focused on the biological effects of chronic inhalation exposure to Mount St. Helens volcanic ash or quartz, under controlled laboratory conditions, indicate significant dose-response to both materials (Wehner et al., 1986). The quartz that came from the volcano was found to be markedly toxic and fibrogenic; by contrast, the volcanic ash was much less toxic (Martin et al., 1984; 1986). Similar results were noted in other animal studies (Beck et al., 1981; Raub et al., 1985; Wiester et al., 1985), suggesting that quartz is a much more potent pulmonary toxicant than volcanic ash (Beck et al., 1981; Martin et al., 1986; Raub et al., 1985). However, the presence of volcanic ash in the inhaled air did increase the “histamine sensitivity” of the epithelial irritant receptors (Wiester et al., 1985) as well as inhibit the ability of alveolar macrophages to protect against infection (Vallyathan et al., 1995). Ash from Eyjafjallajökull disrupted pathogen-killing and inflammatory responses of macrophages, increased bacterial replication, and decreased bacterial killing by antimicrobial peptides (Monick et al., 2013).

The toxicity of volcanic ashes has been evaluated in rats that were dosed once by intratracheal instillation (Lam et al., 2002a,b; Latch et al., 2008). Ashes that were obtained from the San Francisco volcano field in Arizona (lunar dust simulant) and from a Hawaiian volcano (Martian dust simulant) were compared to the toxicity of TiO2 and quartz. Lungs of mice that have been harvested 90 days after receiving a dose of 1 mg of lunar simulant showed chronic inflammation, septal thickening, and some fibrosis. No changes were seen at the low dose of 0.1 mg/mouse (Lam et al., 2002a,b). The Martian dust simulant elicited a response that was similar to that of the lunar simulant, except that there was an inflammatory and fibrotic response even at a dose of 0.1 mg/mouse. The response of the mouse lungs to 0.1 mg quartz was comparable to the response to the Martian dust simulant. In another study, the effect of these same simulants

was assessed on human alveolar macrophages (Latch et al., 2008). The lunar dust simulant was comparable in cell viability reduction and apoptosis induction to the TiO2 (negative control). Both were less toxic than the quartz positive control. Both simulants showed a dose-dependent increase in cytotoxicity. Recently, studies by Cervini-Silva et al. (2014) demonstrated that allophane, the main component of respirable dust derived from aged volcanic soils derived from ash and larger sized clasts (Horwell et al., 2015) that was collected from New Zealand, Japan, and Ecuador, induces lipid peroxidation in cell membranes, and cytotoxicity in murine monocyte/macrophage cells. The lipid peroxidation was controlled by, but not restricted to, structural or surface-bound Fe3+. The reactivity of Fe3+ soluble species originating from surface-bound Fe3+ or small-sized Fe3+ refractory minerals in allophane surpassed that of structural Fe3+ located in tetrahedral or octahedral sites of phyllosilicates or bulk iron oxides. Thus the study suggests an adverse effect of dust from volcanic solids may be mediated by oxidation.

Ash samples from Monserrat, Eyjafjallajökull, and Grímsvötn displayed little ability to lower levels of lung antioxidants, caused little haemolysis and low acute cytotoxicity in human alveolar type-1 like epithelial cells (Horwell et al., 2013). However, cellular mediators MCP-1, IL-6, and IL-8 showed chronic pro-inflammatory responses to ash samples from all three volcanos, despite substantial differences in the sample mineralogy and eruptive styles (Horwell et al., 2013). Cell-free tests showed substantial hydroxyl radical generation in the presence of hydrogen peroxide for Grímsvötn samples, as expected for basaltic, Fe-rich ash (Horwell et al., 2013). “The value of the pro-inflammatory profiles in differentiating the potential respiratory health hazard of volcanic ashes remains uncertain” (Horwell et al., 2013).

4. Evidence from Animal and Human Exposure to Airborne Desert Sand

The Chinese desert margins experience high atmospheric dust levels, and pneumoconiosis is thought to affect several million people in these regions (Derbyshire 2001). Nonindustrial silicosis or desert lung syndrome has been recognized in North Africa, the Middle East, China, and India (Morman and Plumlee, 2013). Episodic exacerbation of allergenic respiratory inflammation by sand dust that originates in Asia or Arizona has been observed in East China, the Korean Peninsula, Japan and the United States (Ichinose et al., 2008). Mallone et al. (2011) reported finding increases in mortality for cardiovascular, circulatory and respiratory causes in Rome related to increases in Saharan dust even when the data were adjusted for ozone and temperature. A study in the Canary Islands demonstrated an association with heart and respiratory mortality and both indicators of PM in the inhalable size range (PM10), which is the fraction of airborne material which enters the nose and mouth during breathing, and respirable fraction (PM2.5), which is the fraction that can reach the alveoli, and found rates of respiratory mortality increased 4.9% for each increase of 10 μg/m3 in PM10 (Lopez-Villarrubia et al., 2010). Morman and Plumlee (2013) recently reviewed epidemiological studies that have identified associations between far-traveled inorganic mineral dusts from primary sources and increased morbidity and mortality in Europe and Asia. Interestingly, effects of PM2.5–10 were stronger than those of PM2.5 on cardiac and circulatory mortality during Saharan dust episodes (Mallone et al., 2011; Tobias et al., 2011).

Direct exposures of "Imprinting Control Region" (ICR; [expression of a gene occurs only from one of the two alleles]) mice to sand dusts from Asia (Tengger Desert in north central China) and from Arizona via intratracheal instillation (ITI) have been reported to aggravate ovalbumin-associated eosinophilic lung inflammation (Ichinose et al., 2008). The aggravating effects of the two dusts differed and were related to the mineral content, mainly SiO2 of the dusts (Inchinose et al., 2008). In other studies, in which ICR mice were exposed by ITI to sand dusts collected from the Tengger Desert or from the atmosphere in Japan, after they had been hot air sterilized to remove adhering biological and chemicals substances, Naota et al., (2010) reported finding localized accumulation of dust particles in the bronchioles and the alveoli; acute inflammatory changes characterized by infiltration of macrophages and neutrophils; degenerated alveolar walls and bronchial epithelial cells, as well as a weakened positive immunolabeling for laminin, at 24 hours after a single exposure. Positive immunolabelings for interleukin-6, tumor necrosis factor–α, inducible nitric oxide synthase, and superoxide dismutase were observed mainly in the inflammatory cells in the lesions. Naota et al., (2010) interpreted the results as indicating that sand dust particles caused damage to the lung tissue through a direct physical effect, and cytokines and oxidative stress generated in the lesion contributed to acute toxicity. When the study was repeated and effects observed at longer intervals after exposure it was found that the acute inflammation subsided from one week to one month after installation and at 2 and 3 months after instillation focal infiltration of lymphocytes and accumulation of epithelioid macrophages and formation of some granulomas were observed.

The principal mineral component of windborne desert dusts Asian and North African dust is silica, 61 and 63%, respectively, in the form of feldspar and quartz (<20 – 60% of total mineral content) (Derbyshire, 2007; Naota et al., 2010). In addition to mineral content these dusts may carry bacteria, fungi, and endotoxins, as well as toxic metals and other toxins derived from both natural and anthropogenic sources that adversely affect health (Morman and Plumlee 2013). Therefore determination of specific effects of a particular constituent of these dusts would be difficult. During the Great American Dust Bowl of the 1930s, excessive and prolonged inhalation of dust resulted in inflammation of the alveoli, dust pneumonia, and death. Characterization of the dusts found high silica content, on average greater than 72%, but no pathogenic organisms (Morman and Plumlee 2013). On the other hand, airborne dust samples collected for a year from 15 sites across the Middle East showed that at all sites the WHO guidelines for maximum ambient PM exposure were exceeded. All silicate mineral particles were thinly coated with a silicon-aluminium-magnesium layer and quartz particles were partly rounded, without fractured surfaces, and coated with clay minerals and iron oxides; no asbestos fibers were found (Engelbrecht et al., 2009). These findings would suggest the toxicity of the collected dusts is low but, as noted earlier, the incidence of desert lung syndrome in the Middle East is a health concern.

5. Evidence from Laboratory Animals Exposed to Authentic Lunar Dust

a. Dermal Effects of Exposure to Lunar Dust

Crew members can be directly exposed to celestial dust in several ways. After crew members perform extravehicular activities (EVAs), they may, as Apollo astronauts did, introduce

into the habitat dust that will have collected on spacesuits and boots. Cleaning of the suits between EVAs and changing of the Environmental Control Life Support System (ECLSS) filters are other operations that could result in direct exposure to celestial dusts. In addition, if the spacesuits used in exploration missions abrade the skin, as current EVA suits have, then contact with these wounds would provide a source of exposure. Further, if celestial dusts gain access to the suits' interiors, as was the case during the Apollo missions, the dust could serve as an additional source of abrasions or enhance suit-induced injuries. Severe abrasion could compromise the protective barrier provided by the skin and thereby increase the risk of infection and the risk of fluid loss. The abrasive effect of lunar dust on skin has been evaluated with a transdermal-impedance technique that measured changes in resistivity of pig-skin, a high fidelity surrogate for human skin, after abrasion with lunar soil simulant (JSC-1A), as well as with authentic lunar dust (Jones et al., 2009). The transdermal-impedance technique measures damage to the stratum corneum, the dry, outermost layer, which is important for the barrier function of the skin. The results of these studies show that JSC-1A is abrasive as commercial sandpaper and that authentic lunar dust is similarly abrasive. Classical skin toxicology studies, including chemical irritancy evaluation and sensitization tests remain to be performed (Jones et al., 2009).

b. Ocular Effects of Exposure to Lunar Dust

In accord with recommendations of the Organization for Economic Cooperation Development, the Lunar Dust Toxicity Research Portfolio (LDTRP) group utilized a two-step approach to assess ocular toxicity of lunar dust. In the first step a 100 mg sample of the respirable-sized, jet-milled dust, which had been maintained in dry nitrogen until use, or negative or positive control dusts, were applied to the surface of cultured human keratinocytes and viability was assessed at 3, 30, and 60 minutes after application of the dust (EpiOcularTM Test). As judged by the number of viable cells remaining at each sampling time after exposure, only minimal irritancy was demonstrated by this assay for lunar dust (Meyers et al., 2012).

Because only a minimal irritancy for the dust was demonstrated by the EpiOcularTM Test, lunar dust was not expected to be exceptionally irritating if applied in a study in vivo, and therefore testing was performed, in rabbits, to assess acute irritation in the intact eye (Meyers et al., 2012). In this study 70 mg of non-respirable dust particles (mass mean diameter of 51 μm) were applied to the right eyes and the left eyes served as control. Only slight redness and swelling of the conjunctiva was observed at the first (1-hour) observation time and no adverse effects were noted in the cornea, iris, or conjunctiva at any of the subsequent observation times (24, 48, and 72 hours) (Meyers et al. 2012). The maximum average irritation rating observed at 1-hour corresponded to the Draize scale rating of minimally irritating (Meyers et al. 2012).

In addition to the mechanical irritation, it is possible that the cornea could be adversely effected by molecular changes induced by chronic exposure to low levels of dust that possess surface features that could facilitate oxidative damage. Therefore, to assess this possibility, Theriot et al. (2014), isolated ribonucleic acid (RNA) from corneas of rats that were collected at 1 day and 7 days after exposure expose to filtered air (controls) or 20, or 60 mg/m3 jet mill prepared respirable sized lunar dust for a total of 120 hours (Lam et al., 2103). Microarray analysis performed using the Affymetrix system identified dose-dependent increases in gene

expression in dust-exposed animals in pathways related to oxidative stress response, mitochondrial dysfunction, fibrosis, epithelial healing, TGF-ß signaling, and extracellular matrix remodeling (Theriot et al., 2014). Genes affecting processes related to cell migration, cellular proliferation, and eye development were also found to be altered by exposure to lunar dust. The findings suggest that exposure to lunar dust for 120 hours at a concentration as low as 20 mg/m3 is sufficient to elicit a molecular response in the cornea. As noted by these investigators, "additional studies are required to fully assess the risk of vision impairment and the mechanistic responses initiated in cornea exposed to lunar dust as well as the potential for long-term effects to astronaut health".

c. Pulmonary Effects of Exposure to Lunar Dust

Early efforts to assess the toxicity of lunar dust produced little useful information. In one study interpretation was complicated by spontaneous pathology in control animals (Holland & Simmonds, 1973). Other studies (Batsura et al., 1981; Kustov et al., 1974, 1989) were also inadequate and the range of findings extended from no effects (Kustov et al., 1974) to fibrosis (Kustov et al., 1989). Therefore, when new missions to the moon were being planned in the last decade the toxicity of lunar dust remained to be determined. This deficiency imposed a critical challenge to those responsible for designing ECLSS of the vehicles and habitats that would be used in the lunar exploration missions. This deficiency was addressed by the NASA Office of the Chief Health and Medical Officer who requested "…recommendations for defining risk criteria for human lunar dust exposure and a plan for the subsequent development of a lunar dust permissible exposure limit." The multi-center Lunar Airborne Dust Toxicology Assessment Group (LADTAG) was formed to respond to this request. The LADTAG was comprised of technical experts in lunar geology, inhalation toxicology, biomedicine, cellular chemistry, and biology from within the agency as well as other leading U.S. experts in these fields from other federal agencies, academia, and industry. In an initial LADTAG workshop that was held in 2005, the experts noted that they were unable to reconcile individual expert opinions to set an inhalation standard based on existing data. The array of opinions from these experts spanned a 300-fold range (i.e., 0.01 to 3 mg/m3). The members of the LADTAG concluded that research was necessary to narrow this wide uncertainty range, the lower end of which could not be met by known methods of environmental control, and that there was an urgency to determine the standard so that environmental systems for the then-planned lunar vehicles and habitats could be appropriately designed. Therefore, gaps in knowledge needed to determine the characteristics of lunar dust that contribute to its toxicity, to determine the toxicity of lunar dust to the respiratory system, to the skin and to the eyes, and to establish a permissible exposure limit for airborne lunar dust, were formally documented by the Human Research Program (HRP). Studies to address these gaps were solicited and funded, and a multidisciplinary LDTRP group, consisting of teams of toxicologists, geologists, and chemists, was engaged to conduct studies needed to address the gaps.

It was anticipated that exposures of lunar exploration crews to dust would occur during an interval lasting no more than six hours, which would begin when dust was introduced into a habitat by crewmembers returning from surface activities and end when high efficiency particulate air (HEPA) filters re-established low, baseline, levels of dust. The work schedule of

crews was expected to follow a typical work week with 5 days that included surface activity and 2 days of rest which required no activity outside the habitat, and therefore no introduction of dust into the habitats on those days. Therefore studies were designed to obtain data that would support a recommendation for an exposure limit based upon a time weighted average exposure, as is the basis for an Occupational Safety and Health Administration PEL, and account for episodic exposures to airborne lunar dust for missions involving no more than six months duration.

The sample of authentic lunar dust used to assess toxicity was acquired from the Curation and Analysis Planning Team for Extraterrestrial Materials at the Johnson Space Center (JSC). The sample obtained, 14003,96, had been collected by the Apollo 14 crew. Although in the lunar highlands, the soil at the landing site was comprised of mineral constituents from both major geological regions (Meyer et al., 2011), the highlands and mares regions. Respirable size (PM2.5) lunar dust aliquots for use in toxicological assessments were obtained from the parent 14003,96 sample in several ways by the Geology Team. These are described in detail by McKay et al. (2015). A cyclonic separation method, which utilizes an air vortex and gravity to separate particulates was utilized to separate a few grams of native respirable-size dust from the parent sample. Because of damage to seals of containers caused by the dust when it was collected, it was expected that the surface reactivity of the native dust would have been passivated by exposure to atmosphere during the many years the archived samples were stored (Gaier, 2007). To address this concern, other methods involved grinding a cyclonic-separated fraction by ball mill or jet mill and then separating the respirable sized particles from the ground dusts with the cyclone system, were employed. Grindings and separations were conducted in a dry nitrogen environment to minimize any loss of surface reactivity generated in the dusts by grinding. Grinding was expected to generate silicon- or oxygen-based radicals ("dangling bonds") and expose reduced iron, both of which can react with water to produce ROS (Wallace et al., 2009). Grinding therefore served as a surrogate for processes that activate lunar dust in situ. The volumetric mean diameters of the respirable-size dusts were 1.8, 2.1, and 2.5 μm for the ball-milled, native, and jet-milled dusts, respectively. Fe0 is present in lunar dust particles and the fraction of total iron present as Fe0 increases as the particle size diminishes, at least to 2 μm (Taylor et al 2011). Therefore respirable dust produced by grinding of larger particles contained lower amounts of Fe0 than the native respirable-sized lunar dust. Otherwise, the mineral compositions of the three lunar dusts preparations were similar. (McKay et al., 2015).

Assessment of pulmonary toxicity of lunar dust by LDTRP was accomplished in two phases that utilized different methods of exposure and several systems of analysis. Both phases utilized rats (Fisher 334) for exposure assessment. Rats were chosen because they are more responsive to inhaled particles than other rodents (Bermudez et al., 2004; Maudley, 1997); the rat and human lung responses to inhaled particles are, qualitatively, quite similar (Castranova, 2000); carcinogenesis and risk of tumor from exposure to quartz are similar in man and rats (Kuempel, 2009; Roller, 2009). In the first phase, animals were exposed by ITI to three respirable-sized lunar dusts (one native, and two that had been produced by grinding) and two standard dusts of well-established and widely different toxicities, quartz (PEL = 0.1 mg/m3) and TiO2 (PEL = 5.0 mg/m3) at dosages of 0, 1, 2.5 or 7.5 mg/rat. Cellular and biochemical markers of toxicity were assayed in bronchoalveolar lavage fluid (BALF) collected from lungs at 1 and 4

weeks after instillation (James et al., 2013). Comparative benchmark dose analysis was utilized in which the effects of the various dusts on responsive markers of toxicity were scaled to those of the dusts of know toxicity. The basis of comparison among sensitive biomarkers was the amount of dust predicted from the dose-response curves generated by the Benchmark Dose software (US EPA) that would be required to effect a change equivalent to 1 standard deviation from the control mean. On this basis, the derived PELs for the lunar dust preparations were 1.3 ± 0.4 mg/m3 (jet-milled dust), 1.0 ± 0.5 mg/m3 (ball-milled dust) and 0.9 ± 0.3 mg/m3 (unground, natural dust). This approach indicated that the toxicities of the three preparations of lunar dust were of indistinguishable toxicity and that lunar dust is more toxic than the nuisance dust TiO2 but less toxic than quartz. The lowest PELs among the various endpoints modeled were 0.5 mg/m3 and the average was approximately 1 mg/m3. Therefore, James et al (2013) concluded that a PEL in the range of 0.5 to 1 mg/m3 "was reasonable for the episodic exposures expected inside a lunar habitat during a prolonged mission on the lunar surface".

ITI studies have the benefit that they require far less material than inhalation studies, a very important consideration when the material to be studied is in very limited supply and extremely precious, as is the case with lunar dust, and in this regard is a valuable tool with which to determine the approximate dose range that may be appropriate for later inhalation studies (Driscoll, 2000). As a result, the ITI study was used to facilitate choices of doses for use in an inhalation study, the lower of which was expected to result in a no-observable-adverse-effect level (NOAEL). However, there are a number of widely held concerns with ITI as a means of assessing pulmonary toxicants. The toxicant is introduced to the target organ (lung) in a dose or at a dose rate that substantially exceeds that which would have occurred during inhalation. The toxicant is introduced via an invasive mechanism that may result in a distribution of the instilled material within the respiratory tract that will likely differ from that resulting from inhalation. The vehicle within which the toxicant is suspended or dissolved could influence the distribution, affect the lung directly, or modify the effect of the instilled toxicant. Finally, the anesthetic utilized during the installation could alter the initial effects of the inhaled material (Driscoll, 2000).

Cognizant of the merits and limitation of ITI, the LDTRP conducted a second phase of toxicity in animal studies that involved exposures by nose-only inhalation and where the selection of the dose of the initial exposures was informed by the results of the ITI study (Lam et al., 2013). In phase 2, rats were exposed by nose-only inhalation, for six hours per day, five days per week for four weeks to jet mill ground respirable size lunar dust. Jet milled dust was utilized because ITI studies had demonstrated no significant differences among the toxicities of the lunar dusts preparation and this preparation was available in quantities required for the study. BALF, lungs and lymph nodes (left tracheobronchial and parathymic) were collected at 1 day, 1 week, 4 weeks and 13 weeks post-exposure. Two inhalation sessions were performed. Based on the results of the ITI experiments, animals were exposed to room air or to 21 or 61 mg/m3 respirable sized lunar dust. Slight effects were observed in lungs of rats exposed to 21 mg/m3 and mild to moderate pulmonary toxicity was evident in the group exposed to 61 mg/m3. Because mild effects were observed in the lower-dose group, a NOAEL was not available from this study, and a second inhalation study was conducted, 1 year after the first, which targeted exposure doses of

2 and 7 mg/m3 (Lam et al, 2013). BALF showed concentration-dependent changes in total cell and neutrophil counts (evidence of inflammation), total protein concentrations (a sign of tissue damage), and several cellular enzymes (indicators of cell death) in animals exposed to the two higher concentrations but no significant difference were found in biomarkers measured in BALF of control animals and those exposed to the two lower concentrations of lunar dust. Likewise, inflammation, septal thickening, fibrosis and granulomas were observed in the lungs of animals exposed at the two higher concentrations, but no lesions were detected in rats exposed to the lower doses. Therefore, 6.8 mg/m3 was the highest NOAEL observed in rats after four weeks of intermittent exposure (Lam et al., 2013). Applying an uncertainty factor of 3 for extrapolation from rats to humans and a factor of 6 for extrapolating from 1 to 6 month exposure provided an estimated PEL of 0.4 mg/m3 (Lam et al., 2013).

Although it is a well-recognized method for establishing toxicity factors, a NOAEL has several known limitations. These include the fact that it is highly dependent upon the design of the study. It is influenced by the concentration intervals, the endpoints examined, and limited to the doses actually tested. An alternative to the traditional NOAEL approach is the application of benchmark dose (BMD) analysis (Crump, 1984). The availability of the BMD analysis provided an opportunity to (1) compare and contrast the level of toxicity of lunar dust assessed with this method to that assessed using a NOAEL as a point of departure, and (2) contrast the assessment of toxicity obtained from the inhalation studies with assessments obtained by BMD analysis of dose responses to lunar dust in the ITI study. When BMD was applied to responsive biomarkers and BMDs were extrapolated to humans, using a species factor of 3 and extrapolated from a 1-month exposure to an anticipated 6-month lunar surface exposure (Scully et al., 2013), PELs were 0.6 and 0.9 mg/m3, when less or more restrictive data sets were used, respectively. The less restrictive data set included non-normally distributed data that were successfully modeled. This range was very similar to PEL range (0.5–1.0 mg/m3) derived from the ITI study (James et al., 2013) and to the PEL (0.4 mg/m3) determined from a NOAEL from the same inhalation studies (Lam et al., 2013).

Taken together the results of the ITI and inhalation studies led the JSC Toxicology Group to recommend 0.5 mg/m3 as a safe concentration for periodic exposures during a 6-month mission on the lunar surface. The Group noted that their recommendation was likely to be conservative but it should not be applied to dust from regions of the moon, such as the poles or the dark side, until those dusts are studied to determine their similarity to mare and highland dusts. The results and recommended PEL were presented to the Office of Chief Health and Medical Officer's (OCHMO) staff and to an external Review Committee organized through NASA's Research and Education Support Services in December 2013. The Review Committee produced a report and recommendations in late January 2014. The original recommendation was revised based on input from the Review Committee, and a final 6-month episodic exposure limit for airborne lunar dust of 0.3 mg/m3 was presented to the Medical Policy Board in April 2014 and accepted for incorporation into NASA Standard 3001 (NASA Human Research Roadmap, 2015).

6. Evidence of Potential Risks to Other Organ Systems

The harmful effects to tissues directly exposed to lunar dust (lung, cornea, skin) have been examined, as described above, but an extensive and growing body of literature (for examples, see the epidemiological studies in the sections on effects of exposure to particulate components of urban air pollution (Section B2) and to airborne desert sands (Section B4)) raises substantial concern that exposure to celestial dust could have harmful effects on other directly, or indirectly, exposed tissues. The risk of adverse effects caused by inhalation of celestial dusts to the nose, pharynx, trachea, and larger air conducting areas of the respiratory system and irritation or damage to the mucosa of the gastrointestinal system by ingested dust remains to be assessed. The risk of adverse effects of celestial dusts on systems such as the cardiovascular, nervous systems and immune systems that may be secondarily, or indirectly affected by inhaled or ingested dusts also remains to be characterized.

The residence time of particles depositing in the upper respiratory system, nose, pharynx, trachea, and larger air conducting areas are typically very short due to mechanical clearance provided by nose blowing, sneezing, or the mucociliary escalator (Lippmann et al., 1980). Most particles are removed from the tracheobronchial region within 24 hours. However ultrafine particles may submerge into the mucus of the airway fluid, which may result in their prolonged retention in this region (Schürch et al., 1990; Stahlhofen et al. 1995). Local deposition is important and build-up of concentration on some surfaces can be sufficiently high that the capacity for clearance can be exceeded. Local retentions could account for nasal cancers in furniture workers and for laryngeal cancers in cigarette smokers (Lippmann et al., 1980). Dietz et al. (2004) demonstrated that occupational exposures to cement dust is a risk factor for laryngeal carcinoma. Cement dust contains a mixture of heavy metals that are known human carcinogens (Ogunbileje et al., 2013). These findings are concerning given that Martian dusts contain substantial amounts of heavy metals (Schuerger et al., 2012). The finding of a weak association between silica or silicosis and laryngeal cancer (Chen et al., 2012) also raises concern of possible adverse effects of inhaled celestial dusts in the upper portions of the respiratory system.

Particles cleared from the respiratory tract move to the oropharynx and are then swallowed and thereby transferred from the respiratory system to the gastrointestinal (GI) system (Kreyling and Scheuch, 2000; Lippmann et al., 1980). Thus, ingestion, indirectly by transfer from the respiratory system, or directly, provides another potential route of exposure to celestial dusts. Therefore, potential risk of adverse effects of ingested dust upon the GI system must be considered. A “borderline” association between exposure to dust and the diffuse form of stomach cancer has been found for miners and quarry workers (Santibañez et al., 2012). Lin et al. (2014) recently reported evidence for the association between exposure to chrysotile (white asbestos) mining dust and excess mortality from cancers of the stomach, esophagus, and liver among workers with high cumulative exposure to this mineral dust. García-Pérez, et al. (2015) found excess cancer mortality (colorectal cancer) in the vicinity of Spanish facilities that produce cement. A meta-analysis of studies of occupational exposure to asbestos found that exposure is associated with a moderate increased risk of stomach cancer (Fortunato and Rushton, 2015). The durations of occupational exposures in the studies related above far exceed the acute exposures that are likely to be experienced by crews exploring celestial bodies, but these finding will take

on greater significance and relevance when extended habitation on celestial bodies increases the extent of exposures.

In addition to the consequences to tissues directly exposed to a toxicant, other tissues may be secondarily affected by translocation of the toxicant from the site of initial contact, or by adverse effects propagated to remote tissues by reactions produced in tissue in contact with the toxicant. The American Heart Association (AHA) in 2004 released its first scientific statement on "Air Pollution and Cardiovascular Disease" and concluded that exposure to PM air pollution contributes to cardiovascular morbidity and mortality. Pope and Dockery (2006) reviewed the literature published between 1997 and 2006 and concluded there is "persuasive evidence that exposure to fine particulate air pollution has adverse effects on cardiopulmonary health". In a subsequent statement on this topic the AHA, as noted by Brook et al. (2010), identified several new conclusions:

> *"Exposure to PM 2.5 μm in diameter ($PM_{2.5}$) over a few hours to weeks can trigger cardiovascular disease–related mortality and nonfatal events; longer-term exposure (e.g., a few years) increases the risk for cardiovascular mortality to an even greater extent than exposures over a few days and reduces life expectancy within more highly exposed segments of the population by several months to a few years; reductions in PM levels are associated with decreases in cardiovascular mortality within a time frame as short as a few years"*

According to the World Health Association approximately 3.7 million premature deaths worldwide in 2012 were attributable to urban outdoor air pollution and PM affects more people than any other pollutant (WHO, 2014). Increased acute mortality that is associated with particle "events" is attributed to CV disease (NRC, 2004). PM in air pollution has been shown to impair vascular function, increase blood pressure, promote thrombosis and impair fibrinolysis, accelerate the development of atherosclerosis, increase the extent of myocardial ischemia, decrease heart rate variability, and increase susceptibility to myocardial infarction (Miller et al., 2012; Mills et al., 2007, 2009; Scapellato and Lotti, 2007). The most severe of these effects are found among susceptible populations with preexisting cardiovascular diseases, diabetes, obesity, hypertension or advanced age (Bhatnagar, 2006), but PM could also promote systemic inflammation in young, healthy individuals (Allen et al., 2011; Rich et al., 2012; Riediker et al., 2004; Schaumann et al. 2004; Wu et al., 2012). The association of airborne PM with cardiovascular disease is also evident from observation that in cases of sudden and marked reductions in air pollution (e.g., sudden changes in policy or strikes leading to cessation of emissions), there have been clear reductions in hospital admissions for cardiopulmonary diseases and deaths (Maynard, 2015) and acute reductions in biomarkers of pulmonary and systemic inflammation, oxidative stress, and hemostasis and improvements in measures of cardiovascular physiology in healthy, young adults (Zhang et al., 2013).

In the case of "overloading", where the amount of dust entering the lung exceeds the capacity of the lung to clear (eliminate) the inhaled dust, the movement of inhaled particles from lungs to the lymph nodes has been observed (Dodson et al, 2007; Oberdorster et al., 1988).

Particles that gain access to the post-nodal lymph have the potential to reach any organ of the body (Dodson et al., 2007). Ultrafine particles (UFP) or nanoparticles (NP) with a largest dimension less than 0.1 μm, can transit from the lungs and reach other organs via the lymphatics, by the blood circulatory system (Choi et al., 2010; Geiser and Kreyling, 2010; Nel et al., 2006; Oberdorster et al., 2004), or enter sensory nerve endings embedded in airway epithelia and from there be translocated to structures of the central nervous system (CNS) (Elder et al., 2006; Oberdorster et al., 2005; Sarkozi et al., 2009; Wang et al., 2008). Nakane (2012) performed a systematic review of the literature on translocation of inhaled particles and used categorical regression to relate route of exposure, particle size, particle material, and animal species to the site of particle translocation. His analysis demonstrated "effects for particle size and particle material were large, while the effects for animal species and exposure route were relatively small" (Nakane, 2012). A broad relationship between particle size and site of translocation was demonstrated: <10 μm for translocation in lung tissue, <1 μm for translocation to the blood, and < 50 nm for translocation to the brain and remote organs (Nakane 2012). NP composed of heavy metals and salts, metal oxides, inorganic carbons, and plastics have been observed to translocate from the respiratory system to adjacent lymph nodes and organs beyond the lungs. When NP were found in the brain this was almost invariably subsequent to exposure by inhalation or intranasal administration (detected in 8 of 10 studies) and only once (1 of 6 studies) following intratracheal instillation. The later observation, is consistent with the mechanisms observed by others (Elder et al., 2006; Oberdorster et al., 2005; Sarkozi et al., 2009: Wang et al., 2008) that NP deposited in the nasal mucosa are taken up the axons of the olfactory bulb and transported to the brain.

Studies conducted with NP composed of polystyrene or diesel exhaust particles (DEP) injected into the blood of hamsters demonstrate that translocation of NP from the lungs to the blood may increase vascular inflammation, clotting, and the risk of myocardial infarct (Nemmar, 2004). Studies with cultured endothelial cells demonstrated that iron translocated to blood vessels could damage endothelium by increasing its permeability through the production of ROS and remodeling of microtubules (Apopa et al., 2009). DEP-produced ROS caused oxidative stress, which reduced the bioavailability of endothelium-derived nitric oxide, and thereby antagonized acetylcholine-mediated relaxation in exposed preparations of rat aorta (Miller et al., 2009). A number of in vitro studies with a variety of cell types demonstrate that metallic nanoparticles (gold, magnetite) cause dose-dependent disruption of cytoskeleton and defects in the lysosome function that, together, can promote autophagy (Cohignac et al., 2014). Because of the epidemiological association of air pollution with increased morbidity and mortality, and the very rapid rise in use of nanomaterials in the nascent nanotechnology industry, studies of the toxicity of UFP or NP have been conducted with particles of anthropogenic origin, such as combustion-derived NPs (CDNPs), which are carbon centered and derived principally from traffic (Donaldson et al, 2013) and with engineered nanoparticles, such as gold, silver, TiO2, iridium, polystyrene, etc. (Simko et al., 2010). The translocation of these nanoparticles is low; no more than 5% of the particles move from the lungs to remote organs (Donaldson et al., 2013; Simko et al., 2010). The significance of this small translocation fraction to progression of disease at remote sites has been questioned (Donaldson and Poland, 2013). If NP mass is the effective determinant of toxicity in the organs to which they translocate, then it would seem unlikely that

sufficient mass would be accumulated remotely to be effective. However, if NP number and reactive surface area are the principle determinants of toxicity, then chronic exposure to NP may be hazardous (Geiser and Kreyling, 2010; Kreyling et al., 2006; Tran 2000). CDNPs, which include coal fly ash, diesel exhaust particles, welding fumes, and carbon black, contain large surface areas on which catalytic chemistry produce free radicals that promote oxidative stress and inflammation (Donaldson et al., 2005). Nanoparticles, because they contain substantially more surface area than the same mass of fine particles, have a greater capacity than fine particles to induce inflammation and are generally more toxic than larger particles of the same chemistry (Bermudez et al., 2004).

The possibility that ingested dust particles, as with inhaled particles, may be translocated from the site of initial deposition to remote organs has been investigated. While most studies, using TiO2, have found no, or negligible, translocation of nanoscale or pigment grade TiO2 from the gut to the systemic circulation (Warheit and Donner, 2015), a few studies have observed movement of particles from the gut to other organs. After administering nanoparticles of TiO2 (5 mg/kg) by gavage to adult mice Wang et al. (2007) found, two weeks after administration, that particles had translocated from the gut and were principally deposited in the liver, spleen, lungs, and kidneys. Inflammation and hepatic necrosis, and nephrotoxicity were observed but no abnormal changes were seen in the heart, lung, testicle, ovary, and spleen. Tassanari et al. (2014) reported histopathological effects in the thyroid, adrenals, ovaries, uterus, testes, and spleen in mice after oral administration of 1-2 mg/kg body weight for 5 days. On the other hand, MacNicoll et al. (2015) found that “5 mg/kg body weight of TiO2 nano- or larger particles did not lead to any significant translocation of TiO2 (measured as titanium) either to blood, urine or to various organs in rat at any of the time intervals studied over a 96 h post-administration period”. Jones et al. (2015) found that “very little titanium dioxide is absorbed gastrointestinally at 4 days after an oral challenge”. The issue of translocation of ingested nanoparticles remains unsettled.

The relevance of the finding of potential adverse effects caused by anthropogenic nanoparticles translocated from the respiratory system to remote organs to risk of adverse health effects from exposure to celestial dusts depends upon the composition and abundance of nanoparticles present in the celestial dusts. On small asteroids where gravity is insufficient to retain small particle formed by comminution resulting from micro meteor impacts, UF particles may be few. On the moon 2% of the soil is comprised on respirable size lunar dust and a size-distribution study of an Apollo 14 sample showed that the most frequent particle size was in the 0.1 to 0.2 μm range and “there is a smooth decrease in particle size down to 10s of nanometers” (McKay et al., 2015). The presence of nanoparticles and the abundance of highly reactive nanophase iron spheres in lunar dust suggests that risks to other organs from translocation of inhaled dust cannot be completely discounted. The risk posed by translocation of Martian dusts is not assessable because the size distribution of dusts in the UF, NP range at the Martian surface is unknown.

The risk to other organs posed by inhaled toxicants does not require that the particles leave the respiratory system in order to exert adverse effect in other systems. Pulmonary derived mediators (Seaton, 1995) or activation of sensory nerve fibers (C fibers) in response to ingested

toxicants could produce effects at sites beyond the lungs. Seaton et al. (1995) proposed that deposition of particles in the lung provokes a low-grade alveolar inflammation with a secondary systemic inflammatory response emanating from "spill over" release of cytokines and other mediators from the lungs resulting in adverse cardiovascular effects in susceptible individuals. Many finding have been consistent with this hypothesis. Exposure to PM10 was associated with changes in plasma viscosity (Peters et al., 1997), elevated levels of fibrinogen (Ulrich et al., 2002; Gilmour et al., 2005), platelet activation (Nemmar et al., 2003), bone marrow stimulation and an increase in circulating neutrophils (PMN) and monocytes (Bai et al., 2013; Brook 2010), and PMN adhesion with myeloperoxidase deposition and local oxidative stress in systemic venules (Nurkiewicz et al., 2006). While there is ample evidence to demonstrate that PM causes inflammation that could spread from the lung to the circulation there is some uncertainty as to how closely pulmonary and systemic inflammation are related and under what conditions and to what extent systemic inflammation contributes to acute or chronic clinical events observed in compromised or healthy individuals exposed to high levels of PM (Bhatnagar et al., 2006). While large panel studies show increased plasma fibrinogen and C-reactive protein levels and viscosity that correlated with PM exposures, controlled exposure studies have failed to demonstrate concurrent changes of pro-inflammatory mediators in lung and blood (Scapellato et al., 2007). The converse has also been observed – proinflammatory systemic effects occur in the absence of significant pulmonary inflammation (Araujo and Nel, 2009). In the latter case, it is possible that "activation of inflammatory molecular pathways could have occurred without histological evidence of overt pulmonary inflammation" (Araujo, 2011). Variations in the strength and the consistency of the association between PM exposure and systemic inflammation likely reflects differences in PM chemistry, duration and intensity of exposures, and differences in susceptibility of subject populations (Brook et al., 2010). The potential for systemic inflammation in response to inhaled celestial dusts could be expected to be affected by the same variables, except that spaceflight crews are uniformly more robustly healthy than the general population.

Studies of silicosis with animals have regularly found that silica induces pulmonary inflammation (Castranova, 2004). These studies have typically utilized instillation or inhalation exposures that model acute silicosis (Langley et al., 2011). Therefore, extrapolations from findings of studies of human response to airborne PM, and from the vast majority of studies of silicosis conducted with animals, may inform expectations of the consequences of acute exposures of celestial dust that are sufficient to produce pulmonary inflammation. However, in humans, acute silicosis is exceedingly rare and chronic silicosis, which manifests after long term exposure to occupational doses, is the greater hazard (Chong et al. 2006). In studies in which rats were exposed by inhalation to occupationally relevant doses of silica granulomas developed many months after silica inhalation was ended, as in humans (Greenberg et al., 2007), and the granulomas resembled human silicotic granulomas in their structure and histopathology (Langley et al., 2011). However, the granuloma formation was not associated with significant inflammation, cell death or lung injury in early stages but only at late stages (Langley et al. 2004; 2010). In a toxigenomic study of rats exposed to occupationally relevant doses of silica the prototypic proinflammatory cytokines TNFα, IL1β, IL6, and IFNγ did not appear to be significantly involved in the granuloma formation (Langley et al., 2011). These finding are

consistent with those of others who reported that proinflammatory cytokines may not be necessary for fibrosis (Giordano et al. 2010; Lo Re et al. 2010). The finding that silicotic granulomas in humans cannot be prevented with corticosteroids further raises uncertainly about the association of inflammation with that silica-induced endpoint. Therefore, in occupationally relevant exposures to toxic silicates, processes other than inflammation likely contribute more substantially to the biologically significant endpoints. Such processes may be relevant to chronic low levels of exposures to celestial dusts during prolonged periods of habitation on surfaces of celestial bodies.

Oxidative stress is associated with the development of both acute and chronic silicosis (Castranova 2004; Cox et al., 2011; Shi et al. 1998), and oxidative burden has been shown to progressively rise even after exposures to silica were ended and the lungs had cleared most of the silica (Fubini and Hubbard 2003; Rimal et al. 2005). Systemic oxidative stress could be induced by inspired PM if organic chemicals and transition metals are released from the lung to the systemic circulation, or cause release of ROS from the lung secondary to pulmonary inflammation (Araujo et al., 2008; Mills et al., 2007). PM has also been shown to lead to enhanced lipid peroxidation in the lungs resulting in increased levels in the BALF of oxidized phospholipids (Kampfrath et al., 2011). PM can increase lipid peroxidation in the plasma, resulting in low density lipoprotein particles that are either more oxidized or more susceptible to oxidation, and high density lipoproteins with dysfunctional anti-oxidant and anti-inflammatory properties (Yin et al., 2013). These effects may occur in parallel with or function as pro-atherosclerotic effects (Araujo and Rosenfeld, 2015). Because oxidative stress and inflammatory processes are linked, investigating the effect of PM on oxidative stress, per se, in humans, is difficult. Only a few studies have directly investigated the occurrence of systemic oxidative stress in humans in response to exposure to ambient PM (Brook et al., 2010). Studies of young adults conducted in Denmark demonstrated elevations in biomarkers of protein, lipid, or DNA oxidation in relation to PM exposure from traffic sources (Brauner et al., 2007; Sorensen et al., 2003; Vinzents et al., 2005), and in studies conducted in Taiwan, also performed on young adults, increased levels of 8-hydroxy-2-deoxyguanosine adducts in DNA were found after short-term elevations in ambient PM (Chaung et al., 2007). Increases in plasma homocysteine, a circulating mediator of oxidative stress have been seen after exposure to ambient PM (Baccarelli et al., 2007; Park et al., 2008). Systemic oxidative response to elevated air pollutants among elderly women in Mexico City was moderated by supplementing diet with antioxidant omega-3 polyunsaturated fatty acids (Romieu et al., 2008). Systemic oxidative stress is a mechanism by which inhaled toxic celestial dusts could affect other organs.

A role of the central nervous system in mediating effects of PM10 on the cardiovascular system has been identified in numerous studies (Bai et al., 2007). PM can stimulate vagal afferents and enhance the sensitivity and reactivity of neural reflexes that can promote local and/or systemic inflammation and the release of inflammatory mediators and increase heart rate and reduce heart rate variability (Bai et al., 2007). Reduced heart rate variability is associated with increased risk for cardiac events (Ghelfi, 2011; Kleiger et al., 1987; Tsuji et al., 1996). A review of the literature that implicates the autonomic nervous system in the mediation of cardiovascular effects of PM concluded that “the evidence for this link is relatively consistent for

effects upon the heart", such as decreased heart rate variability but the relevancy to vascular effects is less clear (Donaldson et al., 2013). Therefore a potential for inspired celestial dust to affect pulmonary afferent nerve fibers, which produce effects at sites beyond the lungs, should be included in considerations of the potential toxicity of these dusts.

In addition to the adverse effects upon respiratory and cardiovascular systems air pollution negatively affects the brain and central nervous system (Costa et al., 2014). Epidemiological studies and studies with animals have shown that exposure to air pollution may lead to oxidative stress and neuroinflammation, damage to the brain parenchyma and vasculature, and disruption of the blood–brain barrier, (Block e al., 2012; Calderon-Garciduenas et al., 2008; Costa et al., 2014). Effects upon the CNS, could have secondary health consequence if altered CNS functions affect modulation of the pulmonary, cardiovascular and immune systems (Block et al., 2012; Perez et al., 2015). The observation that the effects of the PM on the CNS are attributable to its metallic components (Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2013) may be relevant to assessing risks posed by inhalation of celestial dusts containing heavy or transitions metals.

7. Evidence from Properties of Dusts Associated with Toxicity and Understandings of Mechanisms of Toxicity – Implications for Assessing Potential Toxicity of Celestial Dusts.

Much effort has been expended in attempts to determine the relationships between various physiochemical characteristics of mineral dusts and other particulates and their toxicities in order to elucidate the mechanism(s) that produce their toxic effects. Unfortunately despite much effort, a complete understanding of the mechanism remains elusive. Studies have examined size, shape, density, charge, crystallinity, chemical composition, and dissolution rate, and each of these properties has been shown to affect toxicity (Braakhuis et al., 2014; Brom et al., 2011; Fubini et al., 2007). Physical properties such as size, shape, density, and charge determine a particle's deposition and distribution in the pulmonary system; clearance and translocation are affected by their size, shape and surface characteristics and ability to induce inflammation and oxidative stress is related to surface characteristics (Braakhuis et al., 2014; Brom et al., 2011; Fubini et al., 2007) and these conditions lead to adverse biological endpoints that can include histopathology, granulomas, fibrosis, and cancer (Braakhuis et al., 2014; Brook et al., 2010; Gray et al., 2015; Zhang et al., 2013) in the pulmonary system as well as vascular damage in other systems (Burn and Varner, 2015; Scapellato et al., 2007). The importance of surface properties in driving processes important to the toxicity of the particle is demonstrated convincingly by the repeated observations that "surface modifying agents, such as polyvinylpyridine-N-oxide (PVPNO) and aluminum lactate, inhibit most adverse reactions to silica in vivo and also decrease the generation of ROS and DNA damage caused by silica" (Fubini et al., 2007). Masking reactive surfaces would facilitate clearance of particles and decrease effects due to activation of macrophage, and PVPNO also scavenges particle-generated hydroxyl radicals (Fubini et al., 2007). However, data from inhalation studies conducted with lunar dust having different surface reactivity characteristics indicate little to no difference in all measures of toxicity (unpublished data).

The importance of surface features to particle toxicity is also illustrated by the finding that inhalation of freshly ground quartz, when compared to inhalation of aged quartz, results in a significant increase in animal lung injury (Lam et al., 2002a,b; Shoemaker et al., 1995). Freshly ground quartz has increased reactive silicon-based oxygen radicals, and animals that are exposed to freshly ground quartz have been found to have decreased concentrations of antioxidant enzymes (Dalal et al., 1990; Vallyathan et al., 1995). Activated quartz particles decay with age in ambient air (Dalal et al., 1990). Quartz dusts containing surface iron as an impurity have been shown to deplete cellular glutathione, contributing to the oxidative damage that is caused by particle and cell-derived reactive oxygen species (Fenoglio et al., 2003). Castranova et al. (1997) suggest that freshly ground quartz dust that is contaminated with trace levels of iron may be more pathogenic than quartz dust alone. The enhancement of toxicity in quartz by freshly fractured surfaces has been consistently observed in animal and cellular systems (Castranova, 2004; Ding et al., 1999; Porter et al., 2002; Vallyathan et al., 1991). Fracturing silica cleaves the Si-O bonds, leaving Si· and SiO· radicals, which, in turn, produce ·OH radicals in an aqueous environment. Aged crystalline silica still produces radicals, but at a much lower level, perhaps by the Fenton reaction that occurs between iron and H2O2 that is generated by macrophage phagocytosis of the particles (Castranova, 2004).

Crystalline silica exposure studies indicate that the generation of oxidants and nitric oxide, that play an important role in the initiation of silicosis (Castranova et al., 2002), has been shown to cause pulmonary inflammation in rats (Porter et al., 2002). Other studies indicate that the mode of action of cytotoxicity and pathogenicity lies in the ability of the mineral to induce lipid peroxidation and protein oxidation and DNA damage (Cerevini-Silva et al., 2014; Vallyathan, 1994). Respiratory exposure to freshly ground silica causes greater generation of ROS from macrophages than exposure to aged silica, which demonstrates that freshly fractured silica is more toxic than aged silica (Porter et al., 2002; Vallyathan et al., 1988).

Since surface activation, which is produced primarily by grinding, is known to increase the toxicity of various mineral dusts, it is essential to ask how quickly surface activation disappears once the dust encounters an oxygen and water vapor rich environment. Vallyathan et al. (1988) demonstrated a bimodal decay by measuring the rate of disappearance of hydroxyl radical formation in an aqueous medium from silicon-based radicals on the surface of ground silica, when that ground silica was kept in air until the time of assay. The half-life of the fast decay was approximately 30 hours, whereas even after 4 weeks approximately 20% of the original activity that was induced by grinding was present on the surface of the quartz. This is similar to the ability of the 24-hour half-life in air of freshly fractured quartz to produce ·OH radicals (Castranova, 2004). Implicating particle-generated ROS in toxicity requires the reconciliation of the relatively brief half-life of this ROS generating reactivity to the lengthy time course of the progression of silicosis. The brevity of the time course is inconsistent with a typical catalyst that is not consumed in the reaction that it facilitates. Fubini and Hubbard (2003) postulate that serial progressions of cellular ingestion cycles, accompanied by a continuous recruitment of alveolar macrophages, PMN, and lymphocytes, as the cause of the sustained and chronic inflammation elicited by silica. During the sustained inflammation, bronchiolar and alveolar epithelial cells are affected by products of oxidatively stressed cells and the particle,

resulting in activation and/or cell death. Particle-derived ROS may also react with cell-derived ROS and RNS yielding new toxic moieties, e.g., peroxynitrite (ONOO-) from nitric oxide (NO) and superoxide anion (O2 •-) (Fubini and Hubbard 2003). This postulate is consistent with findings that free radicals and ROS play key roles in the induction of oxidative stress that contributes substantially to the toxicity of the particle (Borm et al., 2011).

It has been recently proposed that an exposure metric that captures the ability of a particle to cause oxidative stress may offer advantages over traditional mass concentration measurements (Weichenthal et al., 2013). While it has been noted that there is little epidemiological evidence is currently available to evaluate the potential benefits of such an approach." and that the conditions of oxidative defenses would need to be taken into account (Weichenthal et al., 2013) assessment of the ability of a high fidelity simulant of a celestial dust to induce oxidative stress in cells and animals may be a more useful step in assessing its toxicity than projections based upon physical features alone. In this regard, however it is important to adhere to the advice offered by Donaldson et al. (2009) to be careful in interpretation of in vitro studies. These investigators noted that "three different conventional pathogenic particle types, PM10, asbestos and quartz, which cause diverse pathological effects, have been reported to cause very similar oxidative stress effects in cells in culture" Donaldson et al. (2009). With this admonition in mind, oxidative stress remains a common effect of pathogenic particles of various types, which cause diverse pathological effects, and in vitro methods may still serve as useful screening tools to refine and reduce animal testing.

V COMPUTER-BASED SIMULATION INFORMATION

This section is not applicable to this risk.

VI RISK IN CONTEXT OF EXPLORATION MISSION OPERATIONAL SCENARIOS

Multiple probable scenarios exist in which crew members could be exposed to celestial dust during both surface sortie and surface outpost missions. Further, there are opportunities for crew members to be directly exposed to celestial dust after they perform EVAs. Post EVA, crew members will introduce into the habitat and surface lander the dust that has collected on their spacesuits and boots if these items are brought into habitat rather than remaining on a suit port exterior to the habitat or vehicle. Cleaning of the suits between EVAs may also directly expose crew members to celestial dust. For crew members, changing of ECLSS filters is yet another potential route of direct exposure to dusts. These episodic periods of increased dust exposure must be taken into account when long-term exposure limits are calculated. As missions become longer, the greater dose and/or duration of dust exposure will increase the potential human health risk. When a crew returns to microgravity, if dust is introduced into the crew return vehicle, there will be an increased opportunity for ocular exposure if particles of dust are floating throughout the cabin. EVA activities cause dermal injuries when suits that are based on the current design are used, and the introduction of celestial dusts may enhance injuries that will be sustained from contact with the EVA suit. In addition, NASA is considering the use of a rover design that will allow shirtsleeve operation of the vehicle. Thus, the rover, which must be kept in an interior

space to be entered without a spacesuit, may also bring dust into the habitat, which then may be inhaled or ingested if food or water are contaminated.

The site at which various sizes of particles are deposited is critical to an understanding of any aspect of their toxic action. Normally, for any dust particles between 10 and 1 μm, the portion of particles that is deposited in the upper airways falls off from 80% to 20% as size decreases, whereas the pulmonary deposition increases from near zero to about 20%. Pulmonary deposition, after falling off near 1 μm, peaks again near 40% for particles of 0.03 μm, whereas upper airway deposition remains low until a new peak deposition is found at less than 0.01 μm. The portion and pattern of deposition can be modified under conditions of reduced gravity. Data collected from humans during flights of the gravity research aircraft show that particles in the 0.5 to 1 μm range are deposited less in the respiratory system at lunar gravity than at Earth gravity. This finding is consistent with the reduced sedimentation of the particles when the gravity is less. However, a larger portion of the particles is deposited peripherally (in the alveoli) in reduced gravity (Darquenne and Prisk, 2008, Darquenne et al., 2014).

VII GAPS

AEH 1 - What are the unique properties of lunar dust that affect physiology? (Closed)

Lunar dust particles are unlike terrestrial dusts. Lunar dusts are known to have a high surface area and other distinctive shape and chemical characteristics.

The following tasks have been completed:

- **Lunar Dust - Geology** - Geology, Geochemistry and Lithology Science Support Activities
- **Lunar Dust - Cell** - Study of Lunar Dust and Lunar Simulant Activation, Monitoring, Solution and Cellular Toxicity Properties

AEH 2 - What is the toxicity of lunar dust in the respiratory system?

During the Apollo missions, anecdotal evidence of respiratory effects of lunar dust were reported by crewmembers. However, there is no scientifically defensible data with which to assess the toxicity of inhaled lunar dusts. The data to be obtained from the studies described below are therefore essential to determining risk criteria and establishing a permissible exposure limit for airborne lunar dust.

Pulmonary toxicity of lunar will be assessed in rodents by intratracheal/intrapharyngeal instillation (ITI / IPI) studies and by inhalation studies. In the ITI / IPI studies, groups of rodents will be instilled with suspensions of lunar dust and reference dusts (TiO2 and quartz). BALF will be assayed for biomarkers of toxicity, and lung tissues will be examined microscopically for histopathological lesions. The results of the instillation studies will provide information on the toxicity of lunar dust relative to reference dusts whose toxicities are known, and for which industrial exposure limits have been established. The ITI / IPI data will also be useful for guiding the choice of exposure concentrations for the inhalation study in which markers of toxicity in BALF, and histopathology specimens, will be examined.

Epidemiological evidence has established associations between exposure to specific types of mineral dusts and particular pulmonary pathologies. A common pathway by which exposure to mineral dusts leads to pathology involves inflammation and fibrosis. ROS are important mediators of inflammation. Therefore cellular toxicity of activated and passivated lunar dusts will be evaluated in studies that examine the ability of the respirable size particles to induce formation and release of ROS by cells of the respiratory system and to affect secretion of mediators of inflammation, such as interleukins 6 and 8 and Tumor Necrosis Factor Alpha, by cultured lung cells. Various assays of cell viability will also be utilized.

The following tasks have been completed:

- **Lunar Dust D/O** - Cellular Studies to Support Pulmonary Toxicology Evaluation of Lunar Dust, Dermal Studies of Lunar Dust and Ocular Studies of Lunar Dust
- **Lunar Dust-ITI** - Pulmonary Toxicity Studies of Lunar Dust in Mice and Rats
- **Lunar Dust - Cell** - Study of Lunar Dust and Lunar Simulant Activation, Monitoring, Solution and Cellular Toxicity Properties
- **Human Lung Low g** - Clearance of Particles Depositing in the Human Lung in Low-Gravity

AEH 4 - What is the dermal and ocular toxicity of lunar dust? (Closed)

During the Apollo missions crews reported irritation of skin and eyes after exposure to lunar dust. However, there are no data with which to establish the dermal and ocular toxicity of lunar dusts. The determination of the dermal and ocular hazards of lunar dust is necessary to predict and prevent any consequence that could result from insults to the integument or cornea originating from contact with lunar dust during mission operations.

The following task has been completed:

- **Lunar Dust D/O** - Cellular Studies to Support Pulmonary Toxicology Evaluation of Lunar Dust, Dermal Studies of Lunar Dust and Ocular Studies of Lunar Dust

AEH 5 - What are the permissible exposure limits for inhalation of lunar dust? (Closed)

Data collected from AEH Gaps 1and 2 will be analyzed to develop a time-based concentration exposure limit for airborne lunar dust. The standard will cover 6-month episodic exposures, but may include other time-based exposure limits (acute and chronic) contingent upon the availability of data.

The following tasks have been completed:

- **Lunar Dust D/O** - Cellular Studies to Support Pulmonary Toxicology Evaluation of Lunar Dust, Dermal Studies of Lunar Dust and Ocular Studies of Lunar Dust
- **Lunar Dust - Geology** - Geology, Geochemistry and Lithology Science Support Activities
- **LDHS** - LADTAG Lunar Dust Health Standard

- **Lunar Dust-ITI** - Pulmonary Toxicity Studies of Lunar Dust in Mice and Rats
- **Lunar Dust - Cel**l - Study of Lunar Dust and Lunar Simulant Activation, Monitoring, Solution and Cellular Toxicity Properties
- **Human Lung Low g** - Clearance of Particles Depositing in the Human Lung in Low-Gravity

AEH 11 - What is the potential for acute or chronic cardiovascular toxicity of lunar dust?

This gap has been superseded by the gap DUST 11

DUST-11 - What is the potential for acute toxicity of lunar dust (all relevant endpoints), and acute/chronic cardiovascular toxicity of lunar dust?

Extensive research was performed to establish a permissible exposure limit (PEL) for average exposure to lunar dust over the course of a 6-month mission. The gap that remains is to determine the acceptable short-term excursions that do not violate the average but may cause acute toxicity.

Specifically, much evidence has been accumulated that demonstrates that the respiratory system is not the only system that experiences deleterious effects as a result of inhalation of PM. A link between PM in air and cardiovascular morbidity and mortality has been firmly established (Pope et al, 2004; Scapellato et al, 2007; Walker and Mouton, 2008). Pope (2008)) reported that long-term exposures to PM were most strongly associated with mortality attributable to ischemic heart disease, dysrhythmias, heart failure, and cardiac arrest. Oxidative stress initiated in response to inhaled particles is thought to lead to inflammation which in turn stimulates release from lungs into the circulation of pro-inflammatory mediators that promote systemic inflammation that exacerbates or establishes conditions that promote pathology at sites beyond the lungs (Seaton et al, 1995). More recently, studies with nanoparticles have indicated that effects distal to the lungs may originate locally in response to inhaled PM that has translocated from the lungs (Oberdorster et al., 2002, 2004; Nemmar et al., 2004; Borm et al., 2006; Mossman et al., 2007; Rothen-Ruthishauser et al., 2007), or by PM affecting respiratory reflexes or the autonomic nervous system (Gwin et al., 2006). The observation that inhalation of PM produces adverse effects at sites distal to the lungs, and the possibility that some of these effects may be caused by translocation of PM, particularly ultrafines, from the lungs, suggests additional gaps in the knowledge that we will require to fully assess the risk of adverse health effects posed by lunar dust.

In the LADTAG final report of February 7, 2014 cardiovascular risks were highlighted as an area for further risk assessment/research. This assessment was seconded by a recommendation received from the Standing Review Panel that AEH include a new gap to assess the potential for acute or chronic cardiovascular toxicity of lunar dust. Gap Dust 11 acknowledges those recommendations.

In addition, there is evidence to suggest that celestial dusts have potential allergenic properties that may be exacerbated by spaceflight. Several recent studies have provided evidence of immune dysregulation during spaceflight (Mehta et al., 2007, 2013; Crucian et al., 2008, 2009, 2011, 2013), which may contribute to an increased potential for acute hypersensitivity

reactions. Symptoms suggestive of an allergic response, which worsened with each exposure, were documented in a flight surgeon, who was exposed to lunar dust during post-mission handling of EVA suits (Scheuring et al., 2008).

The following task is *Planned-Unfunded:*

- **Acute LD Tox -** Acute Lunar Dust Toxicology

DUST-12: We need to determine if technologies are available or need to be developed to evaluate celestial dust toxicity and/or volatile composition in situ.

The current toxicology evaluations for spaceflight use traditional technologies to identify toxicants associated with the spaceflight environment. In the toxicology field there are emerging "organ on a chip" methodologies that may have merit as a tool in addressing materials toxicity (e.g., at the location of an asteroid or on the surface of Mars) rather than in returning samples. These new technologies will be critical for the evaluation of spaceflight environments exploration class missions provided increased technology with decreased time and mass. If sample return is possible, these technologies may also be employed as a relative toxicity screening tool to help guide the research needed to establish a PEL and possibly reduce the scope of animal testing.

The following tasks are *Planned-Unfunded:*

- **Robotic Precursor** - Robotic Precursor
- **Organ on a chip** - Organ on a chip (Pilot)

DUST-13: We need to determine if there are significant differences in respiratory, cardiovascular, ocular, or dermal toxicity of dusts from different exploration targets or if existing permissible exposure limits can be applied.

Previous research and data mining efforts have focused on the development of the Lunar Dust Permissible Exposure Limit (PEL). The Lunar Dust PEL is not meant to be broadly applicable to dusts/surface materials at other exploration targets. Once these targets are identified and exposure conditions are understood, a risk assessment will likely be required to more fully assess how inherent toxicity/exposure conditions necessitate a different PEL (if a PEL is determined to be necessary for that location).

The following tasks are *Planned-Funded:*

- **Dust Data Mining** - Dust Data Mining
- **Martian Dust TIM** - Martian Dust Technical Interchange Meeting (TIM)

The following tasks is *Planned-Unfunded:*

- Celestial Dust Ground **- Celestial Dust Ground**

DUST-14 - If relative toxicity is unknown and/or significant differences in toxicity do exist, we need to understand the acute and chronic toxicities of the celestial dust and/or volatiles in order to establish permissible exposure limit.

The applicability of the Lunar Dust permissible exposure limits (PEL) will be evaluated for each mission scenario. If the lunar dust PEL is not appropriate for the mission then a comprehensive risk assessment work (potentially on the scale of Lunar Airborne Dust Toxicity Advisory Group - LADTAG) may be warranted if significant potential differences in toxicity exist such that a target-specific PEL is necessitated.

There are no tasks currently planned for this gap.

Addressing these Gaps in our knowledge about features of the dust and its toxicity, will allow NASA to establish safe exposure limits to inform the design of habitats and vehicles so that exposures of crews to celestial dusts would be limited to safe levels.

VIII CONCLUSION

The evidence literature provides substantial basis for concern that prolonged exposure to respirable celestial dust could be detrimental to human health. Celestial bodies where a substantial portion of the dust is in the respirable range or where the dusts have large reactive surface areas or contain transition metals or volatile organics, represent greater risks of adverse effects from exposure to the dust. It is possible that in addition to adverse effects to the respiratory system, inhalation and ingestion of celestial dusts could pose risks to other systems.

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X TEAM

Current Authors

Robert R. Scully, Ph.D. Wyle

Cell Biologist, member of the pulmonary toxicity assessment team. Together with CWL performed the inhalation studies, analyzed data of the inhalation and ITI studies and co-authored several publications from the pulmonary studies (lead author on one). Coordinated meetings of LADTAG and prepared reports of LADTAG meetings. Together with JTJ and CWL presented evidence to support a recommendation for a PEL for episodic exposure to airborne lunar dust to extramural panel of expert toxicologists (EPET) that was convened by OCHMO to review the recommendation, and assisted VEM and TM with providing responses to EPET and formulating their final recommendation to OCMO for the PEL.

Valerie E. Meyers, Ph.D. NASA

Toxicologist and lead investigator for studies of ocular effects of airborne lunar dust and coauthor of several publications resulting from the lunar dust toxicity studies (lead author on one), currently NASA's lead toxicologist; principally responsible, in collaboration with Environmental Sciences Branch Chief, for responding to the recommendations of the EPET and formulating the final recommendation to OCHMO for the PEL for airborne lunar dust.

Past Authors

John T. James, Ph.D. NASA (Retired)

Principle Investigator, LDTRP, responsible for leading and integrating the efforts of all research teams. Chaired meeting of LADTAG, co-authored several publications (lead author on one). Responsible for providing the recommendation of PEL for airborne lunar dust to EPET.

Noreen Khan-Mayberry, Ph.D. NASA

Toxicologist, in collaboration with JTJ, responsible for managing project during its early phases.

Lunar Dust Pulmonary Toxicity Team

Chiu-wing Lam, Ph.D. Wyle

Toxicologist and pulmonary toxicity team lead, principally responsible for establishing the inhalation exposure laboratory at JSC. Performed ITI and inhalation studies, analyzed data and co-authored several publications from the studies (lead author on one). Together with JTJ and RRS presented evidence to

support a recommendation for a PEL for episodic exposure to airborne lunar dust to EPET and assisted VEM and Environmental Sciences Branch Chief with providing responses to EPET and formulation of final recommendation to OCHMO for PEL.

Stephanie Bassett, Wyle

Animal Care Facility Manager, responsible for providing care for all experimental animals. Assisted in collection of tissue samples.

Geology Team

David S. McKay, Ph.D. NASA (Deceased)

Geologist and team lead, primarily responsible for providing the respiratory size lunar dust needed for the toxicity studies. Performed particle size distribution and other physical characterizations of the lunar dust, lead author of a publication that describes physicochemical properties of respirable-size lunar dust.

Bonnie Cooper, Ph.D. Oceaneering (now Hanyang University, South Korea)

Geologist, principally responsible for the development of new techniques that allowed respirable sized dust particles to be separated from the bulk sample by a dry method, which insured that the properties of the dust would not be affected by the isolation method. Coauthor of publication that resulted from these efforts.

Dermal Toxicity/Cell Biology Team

David Loftus, M.D. Ph.D., NASA

Performed preliminary dermal abrasion studies with lunar dust and together with JR performed functional assessments of cells obtained from lungs of animals exposed to lunar dust

John Rask, NASA

Performed, with DL, functional assessments of cells obtained from lungs of animals exposed to lunar dust

Chemistry Team

Antony Jeevarajan, Ph.D., NASA

Physical Chemist, former Chemistry team Lead, current Deputy Chief of the Biomedical Research and Environmental Sciences Division, coauthor of publications that describe activation of lunar dust simulants and authentic lunar dust, and characterization of nanophase iron-enhanced chemical reactivity of ground lunar dust.

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Physical Chemist – Surface Chemistry, performed activation and dissolution physiochemical characteristics of lunar dust.

Molecular Biology Team

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Molecular Biologist, NASA Core Lab Lead, performed studies to profile gene expression in lungs of rats exposed to lunar dust, principle author of publications that are in preparation.

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Final Recommendation for PEL

Torin McCoy, NASA

Toxicologist, Chief of NASA's Environmental Sciences Branch, together with VEM principally responsible for providing response to the recommendations of the EPET and formulating the final recommendation to OCHMO for the PEL for airborne lunar dust.

Lunar Airborne Dust Toxicity Assessment Group – Non NASA Members

Vince Castranova, Ph.D, NIOSH (now University of West Virginia), Toxicologist

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XI ACRONYMS

BALF	Bronchoalveolar Lavage Fluid
BMD	Benchmark Dose
CDNPs	Combustion-derived Nanoparticles
CM	Command Module
CNS	Central Nervous System
CS	Crystalline Silica
CV	Cardiovascular
DEP	Diesel Exhaust Particle
ECLSS	Environmental Control Life Support System
EPA	Environmental Protection Agency
EVA	Extravehicular activities
Fe^0	Nanophase metallic iron
GI	Gastrointestinal
HEPA	High Efficiency Particulate Air
HRP	Human Research Program
ICR	Imprinting Control Region
IPI	Intrapharyngeal instillation
IRAC	International Agency for Research on Cancer
ITI	Intratracheal Instillation
JSC	Johnson Space Center
LADTAG	Lunar Airborne Dust Toxicology Assessment Group
LDTRP	Lunar Dust Toxicity Research Portfolio
LM	Lunar Module
LMP	Lunar Module Pilot

NASA	National Aeronautic and Space Administration
NO	Nitric Oxide
NOAEL	No-Observable-Adverse-Effect Level
NP	Nanoparticle
npOx	anophase iron oxides
NRC	National Research Council
O2	Superoxide anion
$ONOO^-$	Peroxynitrite
OCHMO	Office of Chief Health and Medical Officer
OR	Oxidative Reactivity
PEL	Permissible Exposure Limit
PM	Particulate Matter
PMN	Polymorphonuclear leukocytes (neutrophils)
PVPNO	Polyvinylpyridine-N-oxide
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscope
SHFH	Space Human Factors and Habitability Element
TIM	Technical Interchange Meeting
TiO_2	Titanium dioxide
UFP	Ultrafine particle
µm	Micron