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RESEARCH ARTICLE

Estimating safe human exposure levels for lunar dust using benchmark dose modeling of data from inhalation studies in rats

Robert R. Scully[1], Chiu-Wing Lam[1], and John T. James[2]

[1]*Wyle Science, Technology & Engineering Group, Houston, TX, USA and* [2]*Space Toxicology Office, NASA Johnson Space Center, Houston, TX, USA*

Abstract

The pulmonary toxicity of airborne lunar dust was assessed in rats exposed by nose-only inhalation to 0, 2.1, 6.8, 20.8 and 60.6 mg/m3 of respirable size lunar dust. Rats were exposed for 6 h/d, 5 d/week, for 4 weeks (120 h). Biomarkers of toxicity were assessed in bronchial alveolar lavage fluid (BALF) collected at 1 d, 1 week, 4 weeks or 13 weeks post-exposure for a total of 76 endpoints. Benchmark dose (BMD) analysis was conducted on endpoints that appeared to be sensitive to dose. The number of endpoints that met criteria for modeling was 30. This number was composed of 13 endpoints that produced data suitable for parametric analysis and 17 that produced non-normal data. Mean BMD values determined from models generated from non-normal data were lower but not significantly different from the mean BMD of models derived from normally distributed data. Thus BMDs ranged from a minimum of 10.4 (using the average BMD from all 30 modeled endpoints) to a maximum of 16.6 (using the average BMD from the most restricted set of models). This range of BMDs yields safe exposure estimate (SEE) values of 0.6 and 0.9 mg/m3, respectively, when BMDs are extrapolated to humans, using a species factor of 3 and extrapolated from a 1-month exposure to an anticipated 6-month lunar surface exposure. This estimate is very similar to a no-observable-adverse-effect-level (NOAEL) determined from the same studies (0.4 mg/m3) and a SEE derived from a study of rats that were intratracheally instilled with lunar dusts (0.5–1.0 mg/m3).

Keywords

Benchmark dose, biomarker, inhalation, lunar dust, mineral dust, toxicity

History

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Introduction

The vision for space exploration articulated by President George W. Bush in 2004 called for NASA to return crews to the surface of the moon. It was anticipated that the missions would involve extensive operations on the surface, including construction of habitats and deployment of technologies to permit *in situ* utilization of lunar resources, and would extend to 6 months in duration. Therefore, the number of potential opportunities for exposure of crews to lunar dust, which could be returned to habitats after surface activities, would far exceed those occasioned by the limited number and duration of extravehicular activities (EVAs) that occurred during the Apollo lunar landings. The duration of EVAs during the Apollo missions ranged from about 2.5 to 22 h (Lunar and Planetary Institute). The extended durations of proposed new missions would elevate the risk of exposure to lunar dust and the potential risk of adverse health effects well beyond those experienced by the Apollo crews. During the Apollo missions, dust, which had adhered to space suits used 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). The exposure concentrations that caused these reactions in Apollo crews were not estimated. 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 & Simmonds, 1973). Later studies (Batsura et al., 1981; Kustov et al., 1974, 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 1990s, understanding of the mechanisms involved in the biohazard of the silicate mineral asbestos matured. There was tremendous concern when large-scale epidemiological studies, which had become

Address for correspondence: Robert R. Scully, PhD, Mail Code Wyle/HAC/W7, 1290 Hercules Avenue, Houston, TX 77058, USA. Tel: +1-281-212-1264. Fax: +1-281-204-4591. E-mail: robert.r.scully@nasa.gov

feasible with advances in methods for processing large data sets, demonstrated the relationships between airborne particulate matter and impacts on human health (Cassee, 2007). 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 & Borm, 1998; Donaldson et al., 2001; Fenoglio et al., 2000; Fubini, 1998, 2002; Ghiazza et al., 2010; Jones & BéruBé, 2007; Kajiwara et al., 2007; Napierska et al., 2010; Øvrevik et al., 2005; Pauluhn, 2011, 2012; Sager et al., 2008; Schoonen et al., 2006; Schwarze et al., 2007; Warheit et al., 2006, 2007). Lunar dust (particles less than 20 μm) comprises about 20% (by weight) of the lunar soil (Park et al., 2008). The respirable fraction of the dust (less than 2.5 μm for rats) comprises 1–3% of the mass fraction of mature lunar soil (Cooper et al., 2010). 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 comminution due to micrometeoroid bombardment, proton bombardment from the solar wind and ultraviolet and intergalactic radiation. However, such bonds disappear when meteorite impacts melt the dust and spray it into tiny, glassy beads. 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). These features suggest that lunar dust may be 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.

The dose-response assessment of the risk appraisal process anticipated use of the no-observable-adverse-effect-level (NOAEL) as the point of departure (POD) for establishing toxicity of lunar dust. However, a NOAEL has several well-recognized limitations. These include the fact that it is limited to the doses actually tested, and a NOAEL is not always available – as when effects are observed at the lowest dose tested, does not account for shape of dose-response curve, and does not account for the power of the study – so that smaller *n* or greater variance results in a higher, less health-protective NOAEL – which ''inappropriately rewards'' studies with low power (DPR MT-2, 2004; US EPA BMD Technical Guidance, 2012). Thus, despite the fact that an intratracheal instillation (ITI) study was conducted (James et al., 2013), which was intended to facilitate choices of two doses to be used in an inhalation study, the lower of which could result in a NOAEL (only two doses could be tested concurrently because only two exposure chambers were available), slight effects were observed in lungs of rats exposed to the lower concentration. Because the mild effects were greater than anticipated in the low-dose group, a second inhalation study was conducted, 1 year after the first, in an effort to identify a NOAEL (Lam et al., 2013). The inclusion of the second set of exposures not only successfully achieved the objective related to identification of a NOAEL, but also the additional data allowed assessment of the dose response by benchmark dose (BMD) analysis (Crump, 1984). The utility of a BMD analysis, if applied to only the data from the first inhalation study, would have been severely limited because some BMD models for continuous data require more than three dose groups. For example, the minimum number of dose groups for a 2-degree polynomial model is 3 for modeling and 4 for a valid model fit assessment, and the US EPA Hill model requires a minimum of 4 dose groups for modeling and 5 for assessing the model fit (DPR MT-2, 2004). The availability of the BMD analysis provides 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 POD, 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.

Since efforts first began to assess the toxicity of lunar dust, alternative celestial bodies have been identified as more immediate targets for human exploration missions. However, the sustained relevancy of the original need to assess the toxicity of lunar dust was always evident in the expressed intent of other nations to send humans to the surface of the moon (Wikipedia, 2013). The oblique relationship of those intentions to our efforts has perhaps become more direct with the recent statements of NASA's Administrator that although the moon may not be the first destination for US crewed exploration missions, ''if some other country is going it is possible that an astronaut could be a part of the crew'' (Klotz, 2013). The need for an established safe exposure limit for airborne lunar dust could be more proximal than is currently anticipated.

Materials and methods

The dose-response assessments presented here were performed with data collected during studies for which the methods have been described in detail (Lam et al., 2013). The salient features of the methods are reiterated below.

Animals, care, acclimation

Pathogen-free Fischer 344 adult male rats, 8–10 weeks old and weighing 150–250 g at arrival, were purchased from Charles River Laboratories (Portage, MI) and housed in an animal facility at NASA Johnson Space Center (Houston, TX). The guidelines of the NASA Johnson Space Center (JSC) Institutional Animal Care and Use Committee (IACUC) and the IACUC-approved test protocols were followed. Pairs of animals were housed in cages ventilated with high-efficiency particulate air (HEPA)-filtered air and provided water and food *ad libitum*. The animals were allowed to acclimate to the facility for 1 week before being gradually acclimated over the course of a week to the Battelle rat

restraint tubes (CH Technologies, Inc., Westwood, NJ) in which they would reside during inhalation exposures.

Preparation of lunar dust

Because no evidence was obtained in the ITI study that the different preparations of lunar dust (native, jet mill-ground and ball mill-ground) substantially differed in their toxicities, the jet milled dust was used for the inhalation studies. This was the preferred method because there was insufficient native, respirable dust for the inhalation study and the jet-milled dust was prepared by self-collisions of the larger-size particles rather than by crushing by large balls, which is the ball-mill approach.

Exposures

The outcome of an ITI study (James et al., 2013), conducted with dust derived from samples collected during the Apollo 14 mission from an area near the lunar equator (see Lam et al., 2013, for detailed characterization of the samples), provided data that informed the choice of doses utilized in the inhalation studies whose results are reported here.

The first dust inhalation study was conducted with dust exposure concentrations targeted at 0, 20 and 60 mg/m^3. Because the lower dose of the lunar dust tested caused some adverse effects, a NOAEL was not evident, so a second inhalation study was performed with exposure concentrations targeted at 0, 2 and 7 mg/m^3 in an attempt to identify this POD.

In each of two inhalation studies, 66 rats were randomly assigned to one of three groups: a control group exposed to air, a group exposed to a low concentration of lunar dust and a group exposed to a high concentration of lunar dust. All rats, confined within Battelle restraint tubes, which were connected to a Jaeger-NYU nose-only chamber (CH Technologies), were exposed 6 h daily, 5 d a week for 4 weeks, for a total of 120 h.

Portable CO_2 monitors (Industrial Scientific Corp., Pittsburgh, PA) were used to continuously measure chamber CO_2 concentrations. Chamber CO_2 concentrations generally did not exceed 500 ppm.

Filtered and dehumidified house air was generated by a Jun-Air Compressor (Model OF302-4MD2, Benton Harbor, MI). The flow rate of air to each chamber was maintained at 8.0–8.5 l/min. The minute ventilation of Fischer 344 Rat at 3 months is ~54 ml/min (Parent, 1992)). The minute volume of animals, approximately 1.2 l/min was well supported by the flow rate even during intervals in which a volume of 0.5–1.0 l/min that, was withdrawn from each chamber to measure the dust concentration and 0.5 l/min to sample particle size with a cascade impactor. The lunar dust aerosol stream in each nose-only chamber for rat exposure was generated by a Vilnius Aerosol Generator (VAG; CH Technologies). The aerosol generated by the VAG passed through a cyclone that restricted the particles entering the chambers to those in the respirable range (mass median aerodynamic diameter 2.5 μm).

The particle size distribution in inhalation chambers was determined by use of a Mercer Cascade Impactor (InTox Inc., Albuquerque, NM), a Quartz Crystal Microbalance (QCM) cascade impactor (California Measurement, Inc., Los Angeles, CA), and an APS 3321 (TSI Inc., Shoreview, MN) was used for immediate determinations of the particle size profiles. The particle size profile from each chamber was determined with at least two of these instruments daily. Measurements were taken alternately from the upper and lower levels of each chamber. No difference in profiles was noticed between the two levels, and very little daily variation in the particle size profile was noted in either chamber.

The concentrations in each chamber were photometrically monitored, controlled and recorded by a Casella MicroDust Pro (CasellaUSA, Buffalo, NY). Dust concentrations in the inhalation chambers were verified by gravimetric methods in which dust drawn from the chambers at a known rate and for a fixed interval was collected on pre-weighed filters.

Biomarkers

Biomarkers that were assessed in the bronchioalveolar lavage fluid (BALF) provide indices of inflammation, structural damage, cytotoxicity, stimulation of type II cells and markers of oxidative stress (Lam et al., 2013). Indices of inflammation are provided by measures of cellular components of BALF. Evidence of structural damage is provided by elevation in total protein concentration because transudation occurs when capillaries become damaged. Lactate dehydrogenase (LDH) activities rise in BALF when released from cells as the cell membrane is compromised and thus provides a measure of cytotoxicity (Drent et al., 1996). Stimulation of type II cells is indicated by elevated ALP (Henderson et. al., 1995). Markers of oxidative stress include alanine aminotransferase (ALT), aspartate transaminase (AST) and glutamyl transferase (GT) (Lam et al., 2013).

BALF and blood samples were collected from rats at 1 d, 1 week, 4 weeks and 13 weeks after the termination of exposure. Blood samples were collected from the vena cava for serum chemistry and cytokine assays. After the trachea was catheterized, the left lung was tied and the right lung lobes were lavaged with 4 ml of phosphate-buffered saline (PBS), and then washed four more times with 5 ml of PBS. The first lavage was centrifuged and its supernatant was used for measuring the acellular BAL biomarkers. The cell pellets of the first and subsequent lavages were combined and suspended in 1 ml of 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES)-buffered solution for assessment of cell numbers and cell differentials.

The cell-free BALF and serum samples were analyzed for enzymes and proteins by the NASA JSC clinical laboratory using an AU480 Chemistry System (Beckman Coulter, Inc., Brea, CA), and analytes were measured according to Beckman's standard protocols. Cell counts were performed using a Coulter Multisizer 3 (Coulter Electronics, Hialeah, FL). Total cells, white blood cells, nucleated cells, viable cells and dead cells in BAL were measured as total counts, counts per milliliter and percentage of total cells with a Guava Easycyte System assay (Millipore Corp – formerly Guava Technologies, Inc., Hayward, CA). Microscopic slides bearing BAL cells were prepared in a CytoSpin centrifuge (Shandon CytoSpin II, Shandon Inc., Pittsburgh, PA) and stained with Wright-Giemsa dye solution (Hema-Tec 2000,

Table 1. Biomarkers collected and assessed.

Clin. Lab	Coulter	Cytospin	Guava
(ALP)	Cells Tot	AM#	(% Dead)
(ALT)		AM%	(% Viable)
AST		LYM#	Cells/ml Dead
(GT)		LYM%	Cells/ml Nuc
LDH		PMN#	Cells/ml Tot
Prot		PMN%	Cells/ml Via

At each time point, 19 biomarkers were measured in BALF. Biomarkers measured by the clinical laboratory included: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; GT, glutamyl transferase; LDH, lactate dehydrogenase and Prot, protein. A coulter counter was utilized to measure Cells Tot – total white cells collected in the BALF. AM, LYM, PMN, # and % are alveolar macrophage, lymphocyte, and neutrophil, counts and differentials, respectively, which were measured on Cytospin slides. A Guava Viacount system was utilized to determine % dead and % viable cells and number of cells/ml of BALF that were dead (cells/ml Dead), nucleated (cells/ml Nuc), total cells (cell/ml Tot), and viable cells (Cells/ml viable). Of the 19 variables measured 5 could not be successfully modeled by BMD at any time point. The five are identified by enclosure within parentheses.

Bayer Corp., Elkhart, IN). Cell differentials were performed by visually counting 300 cells.

In the first inhalation study, neither the serum chemistry nor the hematology profiles showed meaningful or consistent changes. Therefore, no efforts were made to assess these variables in the second inhalation study. A total of 19 biomarkers were available for assessment from both studies (Table 1).

Statistics and BMD modeling

The data examined were collected in two inhalation studies conducted in the same laboratory 1 year apart. The personnel, procedures and instruments, and conditions, with the exceptions of the doses of lunar dust used, did not differ in the two studies. Aliquots of jet-milled lunar dust that remained unused from the first inhalation study provided the source of dust used in the second study. Because each study had its own control group, the 0-dose group (filtered air breathing group) had 10 animals whereas each of the 4 groups exposed to one of the doses of lunar dust had 5 animals at each of the 4 post inhalation sampling times. The combination of two studies, which in the present case, expands the range beyond those available in either individual study is not an uncommon practice in dose-response assessments (Allen et al., 1996; Murata et al,. 2002; Pauluhn, 2012) and commonality of personnel, methods and suppliers and temporal proximity of the two studies are features that are in accord with those recommended for combined studies by the EPA Cancer Guidelines (Section 2.2.2.1.3; http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF).

The data among all doses for each biomarker at each time point sampled, i.e. an endpoint, were tested for normality, using the Shapiro–Wilk test, and for homogeneity of variance (Bartlett's test and then Levine's test), before they were tested for differences among means. If the data passed these tests, the means of the various treatment groups were tested for differences by one-way analysis of variance (ANOVA). If differences were detected, post hoc testing by the method of Bonferroni was used to identify pairs that differed significantly. If the data were not normally distributed or variances were not homogenous, then the nonparametric Kruskal–Wallis one-way ANOVA by ranks was performed to test for differences between means and a modified Bonferroni test was used to identify pairs that differed significantly. Statistical significance was established when $p<0.05$ in all cases except for the modified Bonferroni testing. In that case, the threshold for statistical significance was set at 0.05 divided by the number of pairwise rank sum tests that had been performed after a Kruskal–Wallis test had indicated a statistically significant difference between means of the treatment groups. Analyses were performed using Stata (version 12.1; Stata Corp., College Station, TX).

At each time point, biomarkers whose values appeared to change monotonically in response to different doses were identified as candidates for BMD modeling. BMD software from the US Environmental Protection Agency (EPA, Version 2.3.1), which has a nested family of related exponential models, Hill, polynomial and power models available for continuous data, was utilized for the modeling. If the EPA BMD modeling software indicated that the variance was nonhomogeneous, then dose-dependent variance was applied by the software in fitting the models. Among models that demonstrated a good variance model ($p>0.1$) and good fit ($p>0.1$) and acceptable scaled residual values of interest ($<|2|$), when the difference among the lower 95% confidence limits (BMDL) of the computed benchmark doses was less than a factor of three, then the model with the lowest Akaike information criterion was selected; otherwise some model effects were assumed and the model chosen in those instances was the model with the lowest BMD (US EPA BMD Technical Guidance). The BMD identified by the models was the concentration of dust that would result in a 1 standard deviation (SD) change from the mean of the control group; this concentration is the default benchmark reference (BMR) recommended by the EPA.

We modeled data that met criteria for evaluation by parametric methods or could be made to satisfy the criteria by application of transformations. Square root transformation was used for counts, which typically exhibit a Poisson distribution, and arcsine or frootp (=sqrt(p) − sqrt(100 − p) transformation (http://fmwww.bc.edu/repec/bocode/t/transint.html) was applied to percentage data. BMD modeling of transformed data was attempted only if the transformed data satisfied criteria for parametric analysis. BMDs computed with transformed data were evaluated without conversion to the original scale because, although the scale of the effect was transposed, the BMD was generated with reference to the original (untransposed) dose scale. Because we wished to explore how resistant the modeling software might be to departures from normality or homogeneity of variance, we compared the BMDs generated from successfully modeled endpoints (met all criteria for modeling) from three sets of endpoints. The first set was comprised of endpoints having data that were immediately suitable for analysis by parametric statistics. The second set was comprised of endpoints whose data were successfully transformed to meet requirements for parametric analysis. The third set of endpoints included

non-normal data that could not be successfully transformed but, non-the-less, were successfully modeled by the BMD software.

Results

Inhalation system

The animal exposure concentrations, determined on the basis of gravimetric samples, were 2.1 ± 0.4, 6.8 ± 0.9, 20.8 ± 2.5 and 60.6 ± 8.1 mg/m^3, and particle sizes were in the respirable range (Lam et al., 2013).

BMD modeling of biomarkers

A total of 19 biomarkers from BALF (Table 1) were measured at 4 time points after inhalation to provide a total of 76 endpoints. After testing for normal distribution and homogeneity of variance, and attempting to model endpoints that appeared responsive to dose, we successfully modeled 7 of 76 endpoints for dose response using the EPA BMD software. An additional six endpoints were modeled with the BMD software after the data were transformed to meet conditions required for parametric analysis. The biomarkers with measures that had normal distributions were principally those of enzyme activity levels, which typically are normally distributed. Non-normal measures were mostly associated with variables involving counts, which typically follow a Poisson distribution, and percentages, which are not normally distributed (Sokal & Rohlf, 1981). To examine the extent to which the BMD analysis may be resistant to departures from standards required for analysis by parametric methods, we compared the BMDs derived from endpoints with normal data to BMDs derived from endpoints with nonparametric measures that could, nevertheless, be modeled successfully according to EPA BMD modeling criteria. Seventeen additional endpoints with non-normal measures were successfully modeled, for a total of 30.

BMDs, based on a BMR of 1 SD from control means, were computed from models providing the best fit for successfully modeled endpoints, as determined by the US EPA BMD software. These BMDs are given, together with the results of tests that assessed the models, in Tables 2(a) (normal measures) and 2(b) (non-normal measures). Average BMD and BMDL values are given in Table 2 for endpoints that have been grouped on the basis of data distribution, that is, normal, transformed or non-normal data distributions. Aggregate averages are given in Table 2(a) from models derived from both normal and transformed measures and in Table 2(b) from all 30 successful models, including those from non-normal data. The average BMD of endpoints whose data were normal (16.6) was not significantly different from those of endpoints whose data were transformed (8.7) or endpoints whose data were non-normal (8.4). When the average BMD from all endpoints amenable to analysis by parametric methods (including those rendered so by transformation) (12.9) was compared to the average BMD from non-normal endpoints (8.4), the difference between average BMDs of the two data sets was not significant ($p < 0.07$).

The average BMD, BMDL and safe exposure estimate (SEE) decreased as the number of models was increased successively to include those generated from transformed data, and those derived from endpoints in which the data did not satisfy requirements for normality and/or equality of variance. With additional models, the BMD, BMDL and SEE became more conservative, reducing the average SEE derived from BMD from 5.5 with the most exclusive set of models to 3.5 with use of all models acceptable by BMD modeling criteria (Table 3). Table 3 also illustrates the change in average BMD, BMDL, and SEE as the number of models was successfully increased by adding those generated from transformed data and non-normal data to those generated from measures that were normally distributed. Table 3 shows the effect at each of the four post inhalation sampling times. Although there seems to be a trend toward lower BMDs when mean BMD at each time was derived from normal and transformed data (middle, Table 3), the differences between the mean BMDs from the 4 sampling times were not significant.

Discussion

As noted earlier, the data used for BMD analyses were collected from two inhalation studies, conducted 1 year apart, the purposes of which were to identify a NOAEL to be utilized as a POD for assessment of the toxicity of airborne lunar dust and to establish a safe exposure limit for episodic exposure to the dust (Lam et al., 2013). The NOAEL has been a traditional POD used in establishing toxicity but it has well-recognized limitations. These include the fact that the NOAEL is very sensitive to experiment design – it is limited to one of the doses that have been tested and it is highly dependent on sample size. A NOAEL dose in one study may, in another with greater n and greater statistical power, demonstrate a significant effect. This characteristic is troubling because a study with less power may produce a higher NOAEL, which is less conservative and therefore less protective of health. Because of the high sensitivity to experimental design, NOAELs do not provide consistent response levels for comparisons across studies, toxicants and endpoints. NOAELs do not consider the shape of the dose-response curve. A POD based on a BMD is advantageous in that it overcomes limitations associated with the NOAEL. It is not limited to one of the doses tested, extrapolation below the lowest observable adverse effect is possible, it depends less on dose spacing than the NOAEL does, it accounts for the shape of the dose-response curve, it is comparable across endpoints, and it appropriately accounts for the power of the study so that smaller n or greater variance results in a lower, more health-protective BMD. Because of the perceived advantages of using BMD as a POD, it was useful, in establishing a SEE for lunar dust, to compare SEEs based on a POD determined by a NOAEL with a POD that was determined by BMD. For these reasons we utilized the five doses from two inhalation studies with lunar dusts, which were conducted by the same personnel with identical instruments and facilities, to determine a POD based on a BMD in which 1 SD from the control group mean was utilized as the BMR.

Combined results of two studies, in which rats were exposed to four concentrations of lunar dust by inhalation,

Table 2. BMDs and BMDLs derived from (a) normal or transformed and (b) non-normal data.

Time (days)	Biomarker	Model	BMD (mg/mg^3)	BMDL (mg/mg^3)	Test 1: Lack dose response? $p<0.05$	Test 2: Constant variance? $p>0.1$	Test 3: Good variance model? $p>0.1$	Fit: does the model for the mean fit? $p>0.1$	AIC	Scaled residual of interest $<\|2\|$
(a) Models derived from normal data[a]										
1	ALT	Linear	35.8	25.9	0.01	0.17	0.17	0.53	80	−0.25
1	Cells/ml via	Expo4	10.3	4.1	0.01	0.35	0.35	0.38	58	0.95
1	LDH	Hill	18.0	7.5	<0.0001	0.17	0.17	0.16	222	0.00
7	ALT	Hill	18.2	4.7	0.01	0.44	0.44	0.58	80	−0.51
7	AST	Expo5	14.1	7.5	< 0.0001	0.08	0.32	0.62	125	0.10
28	AST	Hill	8.6	5.6	<0.0001	0.14	0.14	0.90	113	0.01
91	AST	Expo5	11.1	6.1	< 0.0001	0.26	0.26	0.64	120	−0.02
Set average			16.6	8.8						
Models derived from transformed data[a]										
7	AM #	Hill	5.5	2.7	<0.0001	0.58	0.58	0.86	−60	−0.06
7	Cells dead	Expo5	17.4	7.6	<0.0001	0.03	0.63	0.13	296	0.07
7	LYM #	Expo4	3.4	2.2	<0.0001	0.13	0.13	0.26	−80	−0.33
28	LYM #	Expo5	11.0	6.6	< 0.0001	0.49	0.49	0.25	−80	0.03
28	LYM %	Hill	7.8	6.8	0.00	0.84	0.84	0.12	−6	0.00
91	LYM %	Expo5	6.9	2.4	0.01	0.26	0.26	0.14	110	0.00
Set average			8.7**	4.7						
Aggregate average			12.9	6.9						
(b) Models derived from non-normal data[b]										
1	PMN %	Expo5	7.5	5.6	< 0.0001	< 0.0001	0.39	0.86	58	−0.22
7	AM %	Expo5	7.7	5.8	< 0.0001	0.00	0.33	0.86	102	0.04
7	Cells Tot	Expo5	6.1	4.2	< 0.0001	0.01	0.67	0.19	870	−0.05
7	LYM %	Expo4	5.3	1.9	0.03	0.65	0.65	0.36	56	0.05
7	PMN #	Expo5	8.4	6.3	< 0.0001	< 0.0001	0.49	0.51	−68	−0.34
7	PMN %	Expo5	11.7	6.8	< 0.0001	< 0.0001	0.31	0.27	81	−0.40
28	AM #	Expo5	6.2	3.3	< 0.0001	< 0.0001	0.58	0.80	44	−0.38
28	AM %	Hill	6.9	6.0	<.0001	<0.0001	0.12	0.48	86	−0.05
28	Cells Tot	Expo5	6.2	4.6	< 0.0001	< 0.0001	0.43	0.37	849	−0.34
28	Cells/ml Nuc	Expo5	10.5	5.9	< 0.0001	0.00	0.23	0.69	251	0.25
28	Cells/ml Tot	Expo5	9.2	6.3	< 0.0001	< 0.0001	0.19	0.70	839	0.26
28	Cells/ml Via	Expo5	10.0	6.5	< 0.0001	< 0.0001	0.11	0.56	839	0.28
28	LDH	Expo5	5.3	4.1	< 0.0001	< 0.0001	0.19	0.81	178	0.03
28	PMN #	Expo5	6.5	5.3	< 0.0001	< 0.0001	0.29	0.42	−57	0.42
28	PMN %	Expo5	6.6	5.2	< 0.0001	< 0.0001	0.25	0.42	77	0.09
28	Prot	Expo5	10.5	6.5	< 0.0001	0.01	0.26	0.70	92	0.15
91	AM #	Expo5	18.5	7.0	< 0.0001	0.00	0.14	0.81	64	0.53
Set average			8.4**	5.4						
Aggregate average			10.4	6.0						

(a) [a]BMDs and associated BMDLs computed by EPA benchmark dose software for data meeting criteria for parametric analysis (upper) or data that were successfully transformed to meet criteria for analysis by parametric methods (lower half). **Not significantly different ($p<0.05$) from normal.

(b) [b]Output from the EPA benchmark dose software for data that did NOT meet criteria for parametric analysis and were not successfully transformed by standard methods to meet criteria for analysis by parametric methods. **Significantly different from normal ($p<0.005$).

Table 3. Change in BMD and BMDL with inclusion of models[a].

	Means		SEE (mg/m^3)[b] (1 month)		Endpoints
Time	BMD (mg/mg^3)	BMDL (mg/mg^3)	From: BMD	BMDL	Modeled
Normal data					
All	16.6	8.8	5.5	2.9	7
1	21.3	12.5	7.1	4.2	3
7	16.1	6.1	5.4	2.0	2
28	8.6	5.6	2.9	1.9	1
91	11.1	6.1	3.7	2.0	1
Normal and transformed data					
All	12.9	6.9	4.3	2.3	13
1	21.3	12.5	7.1	4.2	3
7	11.7	4.9	3.9	1.6	5
28	9.1	6.3	3.0	2.1	3
91	9.0	4.3	3.0	1.4	2
All successfully modeled					
All	10.4	6.0	3.5	2.0	30
1	17.9	10.8	6.0	3.6	4
7	9.8	5.0	3.3	1.7	10
28	8.1	5.6	2.7	1.9	13
91	12.2	5.2	4.1	1.7	3

[a]This table illustrates the change in average BMD, BMDL and SEE at each sampling time as the number of models was increased successively to include those generated from transformed data (middle) and finally, those derived from biomarkers in which the data did not satisfy requirements for normality and/or equality of variance (bottom).
[b]SEE are derived by dividing the BMD by a species uncertainty factor of 3. With additional models the average BMD, BMDL and SEE became more conservative, reducing SEE (based on BMD) from 5.5 with the most exclusive set of models to 3.5 with use of all models acceptable by BMD modeling criteria, or from 2.9 to 2.0 when SEEs were derived from BMDLs.

demonstrated a NOAEL of $6.8\,mg/m^3$, both when biomarkers in BALF were assessed and when lung tissue was analyzed for histopathology (Lam et al., 2013). Because rats are the most sensitive species to toxic effects of inspired poorly soluble materials (Mauderly, 1997); sedimentation rates and clearance capacities indicate that clearance kinetics for poorly soluble materials from the lungs is similar for humans and rats; rats respire 3.5 times more frequently than humans (Parent, 1992; Snyder et al., 1975) and the fraction of respirable particles deposited in peripheral areas of the lung is about 3 times greater in humans than in rats, it was argued that a species uncertainty factor (uf) of 3 was appropriate (Lam et al., 2013). Therefore, the NOAEL for humans was assumed to be NOAEL/uf $= 6.8\,mg/m^3/3 = 2.3\,mg/m^3$ for 1 month of episodic exposure (Lam et al., 2013).

BMDs and BMDLs for the endpoints measured in the BALF that appeared to respond to dose are shown in Tables 2 and 3. BMD and BMDL values were computed on the basis of a BMR dose of 1 SD from control means. Average BMDs and BMDLs are computed for data sets that are comprised on the basis of the distribution of data for the dose responses of the endpoints. One set was comprised on endpoints whose data satisfied criteria for analysis by parametric methods, the second set consisted of endpoints whose data could be transformed to satisfy these conditions and the third set was comprised of endpoints with non-normal data. The averaging of values of BMD and BMDLs across endpoints and time points is atypical. Usually in risk assessment the endpoints are segregated within each time point and a final endpoint would be selected based upon lowest BMDL or upon considerations of biological significance. However, there is no biological basis for distinguishing biomarkers as regards to biological significance because all are markers of the processes contributing to the progression of the adverse effects resulting from the exposures. We also have considered the admonition of the creator of the BMD method who cautioned

> . . . for determining the BMDL . . . it should be noted that the issues involved in this selection are different from those when the NOAEL approach is used. In the latter case, the ''most sensitive endpoint'' is recommended, which is often taken to be the one with the lowest NOAEL. However, it would be a mistake to conclude that it is similarly reasonable to select the endpoint providing the lowest BMDL. For one thing, unlike the NOAEL, the BMDL will be smaller when the sample size is smaller, so the most limited study is likely to produce the smallest BMDL. (Crump, 2002)

Given the lack of rationale for choosing most biologically significant endpoints and the small size of the samples, using the averages of the different endpoints for all biomarkers that could be modeled from a particular data set (distribution) seems reasonable.

When only models from data that were found to be normally distributed were utilized, the BMD and BMDL of 16.6 and $8.8\,mg/m^3$, respectively, were obtained. If only the BMD and BMDL from data that were normally distributed, or transformed to a normal distribution, were considered, then the average BMD and BMDL were 12.9 and $6.9\,mg/m^3$, respectively. If all successfully modeled endpoints, including those with non-normal data, were included, then BMD and BMDL were 10.4 and $6.0\,mg/m^3$, respectively. Therefore, the effect of relaxing inclusion standards for data to be modeled and included in this study was to lower the BMD and BMDL to a very slightly more conservative and therefore health-protective level.

Accepting the rationale given by Lam et al. (2013) for a species uncertainty factor of 3, the 1-month-exposure BMDs from the 3 sets of models extrapolated to humans become 5.5, 4.3 and 3.5 mg/m^3, for the normal, normal and transformed, and all successfully modeled data sets, respectively, all of which are less conservative than the NOAEL-derived SEE of 2.3 mg/m^3. Because the experimentally determined NOAEL is limited to one of the doses tested, it is possible that a higher NOAEL could be discovered if additional doses had been used. In the present study, given the closeness of the SEE derived from a BMR of 1 SD above control values, which is not a no-effect level, and that derived from the experimentally determined NOAEL (Lam et al., 2013) it seems that if it had been possible to include additional doses slightly above 6.8 mg/m^3 then a higher NOAEL would not have been very much different from the NOAEL that was identified. If the more conservative approach of using the BMDL rather than the BMD as the POD is implemented, then the SEEs for a human for 1 month of episodic exposure to lunar dust are 2.0, 2.3 and 2.9 mg/m^3, calculated with the largest to smallest (most restrictive) groups of models. The range (2.0–2.9 mg/m^3) brackets the SEE of 2.3 mg/m^3, derived from the NOAEL (Lam et al., 2013). The convergence of the SEE derived from the NOAEL and the BMD approaches provides added confidence in the SEE.

Extension of the estimates of SEE determined from 1-month exposures to a 6-month lunar mission, with the proviso that exposures are intermittent and for no more than 6 hours per day, 5 days per week, would require reducing the values by 6. Therefore, this study suggests a range at the minimum of 0.3 mg/m^3 (using the BMDL of 2.0 from all models including those generated from non-normal data) to a maximum of 0.9 mg/m^3 (using the average BMD of 5.5 from the most restricted set of models). Extension of the 1-month NOAEL-derived SEE (2.3 mg/m^3) (Lam et al., 2013) to 6 months produces a SEE of 0.4 mg/m^3.

The studies reported here followed a study in which rats were instilled with one of three preparations of lunar dusts or with a reference dust with well-established toxicity (James et al., 2013). Quartz was utilized as the highly toxic reference dust and TiO_2 as a nuisance dust. Four concentrations of each dust were utilized – 0, 1, 2.5 and 7.5 mg/rat – and BMD analysis was conducted on biomarkers that exhibited dose response to quartz and the lunar dusts. The approach in this previous study was new in that it used a relative-toxicity approach to determine the SEE for lunar dusts relative to the PELs (Occupational Safety & Health Administration) for quartz and TiO_2. Because the SEEs were established relative to standards for which toxicity is well supported by a large volume of literature, this approach avoided the use of uncertainty factors. This study showed that the lunar dust preparations (native, jet mill-ground and ball mill-ground) were indistinguishable, the lowest SEEs were slightly above 0.5 mg/m^3 and the averages were near 1 mg/m^3. Therefore for workplace-like exposures of 8 h per day, an SEE of 0.5–1 mg/m^3 was recommended (James et al., 2013). The SEE for humans supported by the present study is 0.3–0.9 mg/m^3.

Conclusions

BMD methodology was used to analyze data from studies in which rats were exposed over 4 weeks (6 h/d, 5 d/week) by nose-only inhalation to air and four concentrations of lunar dust, ranging from 2.1 to 61 mg/m^3. Biomarkers were measured in BALF that was collected at 1 d, 1 week and 1 and 3 months after exposure. When a species factor of 3 is applied and the duration of exposure is extrapolated to 6 months with daily exposure not exceeding 6 hours, an SEE for humans of 0.3–0.9 mg/m^3 is supported by the findings of this study. This value is similar to an SEE derived when a NOAEL was determined from the same studies (0.4 mg/m^3; Lam et al., 2013) and to an SEE range derived from a study of rats that were intratracheally instilled with lunar dusts and reference dusts of well-established toxicities (0.5–1.0 mg/m^3; James et al., 2013).

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References

Allen BC, Strong PL, Price CJ, et al. (1996). Benchmark dose analysis of developmental toxicity in rats exposed to boric acid. Fundam Appl Toxicol 32:194–204.

Batsura YD, Kruglikov GG, Arutyunov VD. (1981). Morphology of experimental pneumoconiosis following inhalation of lunar soil. Bull Exp Biol Med 92:1294–7.

Cassee FR. (2007). Foreword. In: Donaldson K, Borm P. (eds.) Particle toxicology. Boca Raton, FL: CRC Press Taylor and Francis Group.

Castranova V, Pailes WH, Dalal ND, et al. (1996). Enhanced pulmonary response to the inhalation of freshly fractured silica as compared with aged dust exposure. Appl Occup Environ Hyg 11:937–41.

Cooper BL, McKay DS, Riofrio LM, et al. (2010). Sub-10-micron and respirable particles in lunar soils. 41st Lunar and Planetary Science Conference; 2010 Mar 1–5; The Woodlands, TX.

Crump KS. (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4:854–71.

Crump K. (2002). Critical issues in benchmark calculations from continuous data. Crit Rev Toxicol 32:133–53.

Donaldson K, Borm P. (1998). The quartz hazard: a variable entity. Ann Occup Hyg 42:287–94.

Donaldson K, Stone V, Duffin R, et al. (2001). The quartz hazard: effects of surface and matrix on inflammogenic activity. J Environ Pathol Toxicol Oncol 20:109–18.

DPR MT-2. (2004). Guidance for Benchmark Dose (BMD) Approach – Continuous Data. Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

Drent M, Cobben NA, Henderson RF, et al. (1996). Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 9:1736–42.

Fenoglio I, Martra G, Prandi L, et al. (2000). The role of mechanochemistry in the pulmonary toxicity caused by particulate minerals. J Mater Syn Proc 8:145–53.

Fubini B. (1998). Surface chemistry and quartz hazard. Ann Occup Hyg 42:521–30.

Fubini B. (2002). V. Chemical reactivity of the quartz surface in relation to its toxicity and genotoxicity. In: Schneider WD. (ed.) 2002 Quarz. Einstufung, Dosis-Wirkungs-Beziehungen. Workshop vom 07./08. März 2002 in Berlin. Dortmund/Berlin/Dresden. Available from: http://www.baua.de/cae/servlet/contentblob/697144/publicationFile/46888/

Ghiazza M, Polimeni M, Fenoglio I, et al. (2010). Does vitreous silica contradict the toxicity of the crystalline silica paradigm? Chem Res Toxicol 23:620–9.

Henderson RF, Scott GG, Waide JJ. (1995). Source of alkaline phosphatase activity in epithelial lining fluid of normal and injured F344 rat lungs. Toxicol Appl Pharmacol 134:170–4.

Holland JW, Simmonds RC. (1973). The mammalian response to lunar particulates. Space Life Sci 4:97–109.

James JT, Lam C-W, Santana P, Scully RR. (2013). Estimate of safe human exposure levels for lunar dust based on comparative benchmark dose modeling. Inhal Toxicol 25:243–56.

Jones T, BéruBé K. (2007). Chapter 2. Mineralogy and structure of pathogenic particles. In: Donaldson K, Borm P. (eds.) Particle toxicology. Boca Raton, FL: CRC Press Taylor and Francis Group, 13–45.

Kajiwara T, Ogami A, Yamato H, et al. (2007). Effect of particle size of intratrachaeally instilled crystalline silica on pulmonary inflammation. J Occup Health 49:88–94.

Klotz I. (2013). NASA Open to Hitching Ride to the Moon, Agency Chief Says. Space News (June 3). Available from: http://www.space.com/21418-nasa-hitch-ride-moon.html [Last accessed: 26 Jun 2013].

Kustov VV, Belkin VI, Kruglikov GG. (1989). Biological effects of lunar soil. Probl Space Biol 61:1–146.

Kustov VV, Ostapvenko OF, Petrukhin VG. (1974). Research on the biological effect of a fine fraction of lunar soil sent to Earth by the unmanned station Luna-16. In: Vinogradov AP. (ed.) Lunar soil from the sea of fertility. Moscow: M. Nauka, 592.

Lam C-W, Scully RR, Zhang Y, et al. (2013). Toxicity of lunar dust assessed in inhalation-exposed rats. Inhalation Toxicol 25:661–78.

Liu Y, Park J, Schnare D, et al. (2008). Characterization of lunar dust for toxicological studies. II: texture and shape characteristics. J Aerosp Eng 21:272–9.

Lunar and Planetary Institute. Available from http://www.lpi.usra.edu/lunar/missions/apollo/apollo_11/surface_opp/; http://www.lpi.usra.edu/lunar/missions/apollo/apollo_17/surface_opp/. [Last accessed: 23 Jul 2013].

Mauderly J. (1997). Relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk. Environ Health Perspect 105:1337–46.

Murata K, Budtz-Jørgensen E, Grandjean P. (2002). Benchmark dose calculations for methyl mercury-associated delays on evoked potential latencies in two cohorts of children. Risk Anal 22:465–74.

Napierska D, Thomassen LCJ, Lison D, et al. (2010). The nanosilica hazard: another variable entity. Particle Fibre Toxicol 7:39–70.

Occupational Safety & Health Administration. Permissible exposure limits. Available from: https://www.osha.gov/dsg/topics/pel/ [Last accessed: 23 Jul 2013].

Øvrevik J, Myran T, Refsnes M, et al. (2005). Mineral particles of varying composition induce differential chemokine release from epithelial lung cells: importance of physico-chemical characteristics. Ann Occup Hyg 49:219–31.

Parent RA. (1992). Treatise on pulmonary toxicology, Volume I: comparative biology of the normal lung. Vol. 1. Boca Raton, FL: CRC Press.

Park J, Liu Y, Kihm KD, Taylor LA. (2008). Characterization of lunar dust for toxicological studies. I: Particle size distribution. J Aerosp Eng 21:266–71.

Pauluhn J. (2011). Poorly soluble particulates: searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation. Toxicology 279:176–88.

Pauluhn J. (2012). Subchronic inhalation toxicity of iron oxide (magnetite, Fe_3O_4) in rats: pulmonary toxicity is determined by the particle kinetics typical of poorly soluble particles. J Appl Toxicol 32:488–504.

Sager TM, Kommineni C, Castranova V. (2008). Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: role of particle surface area. Part Fibre Toxicol 5:17. doi:10.1186/1743-8977-5-17.

Scheuring RA, Jones JA, Novak JD, et al. (2008). The Apollo Medical Operations Project: recommendations to improve crew health and performance for future exploration missions and lunar surface operations. Acta Astronaut 63:980–7.

Schoonen MA, Cohn CA, Roemer E, et al. (2006). Mineral-induced formation of reactive oxygen species. Rev Minerol Geochem 64:179–221.

Schwarze PE, Øvrevik J, Hetland RB, et al. (2007). Importance of size and composition of particles for effects on cells *in vitro*. Inhal Toxicol 19:17–22.

Snyder WS, Cook MJ, Karhausen LR, et al. (1975). International Commission of Radiological Protection (ICRP) No. 23: Report of the Task Group on Reference Man. New York: Pergamon Press.

Sokal RR, Rohlf FJ. (1981). 13. Assumptions of analysis of variance. In: Biometry the principles and practices of statistics in biological research. 2nd ed. New York: WH Freeman Co., 400–52.

Taylor LA, Pieters CM, Keller LP, et al. (2001). Lunar mare soils: space weathering and the major effects of surface-correlated nanophase Fe. J Geophys Res 106:27985–99.

US EPA. (2012). Benchmark Dose Technical Guidance EPA/100/R-12/001. Available from: http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf. [Last accessed: 23 Jul 2013].

Wagner SA. (2006). The Apollo Experience: Lessons Learned for Constellation Lunar Dust Management. NASA Technical Publication TP-2006-213726. Washington, DC: National Aeronautics and Space Administration.

Warheit DB, Webb TR, Colvin VL, et al. (2007). Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. Toxicol Sci 95:270–80.

Warheit DB, Webb TR, Sayes CM, et al. (2006). Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci 91:227–36.

Wikipedia. (2013). List of proposed missions to the Moon. Available from: http://en.wikipedia.org/wiki/List_of_current_and_future_lunar_missions. [Last accessed: 26 Jun 2013].