

## RESEARCH ARTICLE

## Estimate of safe human exposure levels for lunar dust based on comparative benchmark dose modeling

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Brief exposures of Apollo astronauts to lunar dust occasionally elicited upper respiratory irritation; however, no limits were ever set for prolonged exposure to lunar dust. The United States and other space faring nations intend to return to the moon for extensive exploration within a few decades. In the meantime, habitats for that exploration, whether mobile or fixed, must be designed to limit human exposure to lunar dust to safe levels. Herein we estimate safe exposure limits for lunar dust collected during the Apollo 14 mission. We instilled three respirable-sized (~2 μ mass median diameter) lunar dusts (two ground and one unground) and two standard dusts of widely different toxicities (quartz and TiO<sub>2</sub>) into the respiratory system of rats. Rats in groups of six were given 0, 1, 2.5 or 7.5 mg of the test dust in a saline-Survanta<sup>®</sup> vehicle, and biochemical and cellular biomarkers of toxicity in lung lavage fluid were assayed 1 week and one month after instillation. By comparing the dose–response curves of sensitive biomarkers, we estimated safe exposure levels for astronauts and concluded that unground lunar dust and dust ground by two different methods were not toxicologically distinguishable. The safe exposure estimates were 1.3 ± 0.4 mg/m<sup>3</sup> (jet-milled dust), 1.0 ± 0.5 mg/m<sup>3</sup> (ball-milled dust) and 0.9 ± 0.3 mg/m<sup>3</sup> (unground, natural dust). We estimate that 0.5–1 mg/m<sup>3</sup> of lunar dust is safe for periodic human exposures during long stays in habitats on the lunar surface.

**Keywords**

Benchmark dose, inhalation, lunar dust, mineral dust

**History**

Received 19 December 2012

Revised 15 February 2013

Accepted 15 February 2013

Published online 24 April 2013

**Introduction**

As future adventurers explore the lunar surface, they will entrap dust on their suits and episodically bring it into their habitat. Some of the fine dust will become airborne and settle slowly while air filters remove the dust over a few hours, thus limiting inhalation exposures. The health hazards associated with earth-bound mineral dusts have received progressively more attention since the 1970s, when the final Apollo missions were flown to the moon (Horwell & Baxter, 2006). It is not surprising then that in the 1970s, no one asked whether lunar dust, which is pervasive on the lunar surface, could pose a health risk if it were inhaled by astronauts living in a dust-contaminated lunar habitat. The only published study of Apollo dust toxicity involved intratracheal administration of 20 mg of uncharacterized dust into guinea pigs. Spontaneous pathology in controls confounded the results (Holland & Simmonds, 1973).

Anecdotal reports from Apollo astronauts occasionally associated respiratory symptoms with exposure to lunar dust. However, long-term follow-up using spirometry has not shown diminution in respiratory function beyond that expected from aging (Jeffrey A. Jones, MD, personal

communication). The evidence is limited by the small number of Apollo astronauts exposed at the lunar surface ( $n = 12$ ) and the brevity of their exposures. Lunar dust was returned by the six successful Apollo missions and is held in the Curatorial Facility at Johnson Space Center. Samples of dust are precious and experiments with it are constrained to consume as little as possible. Our goal for future missions was to estimate a safe level for long-duration, episodic human exposure to lunar dust using an absolute minimum of dust.

One widely used approach to assessing the pulmonary toxicity of a dust is to instill it into the respiratory system of test animals and quantify cellular and biochemical markers of toxicity present in lung lavage fluid obtained later (Driscoll et al., 2000). Traditionally, one would hope to find a no-observed-adverse-effect level (NOAEL), and then apply large safety factors and a species extrapolation factor to estimate a safe human exposure level. A more sophisticated approach is to fit a dose–response model to the data and select an arbitrary “point of departure”, such as the 1% effect level, on which to apply the same factors one might apply to the NOAEL (Sand et al., 2006). This approach, especially when applied to instillation studies, has its limitations, because instillation of dust suspended in a vehicle is an artificial means of dosing the lungs of test animals. Nonetheless, data from instillation studies can be well correlated with data from inhalation studies that must be performed with much more dust (Henderson et al., 1995).

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We have developed a new approach that avoids direct application of “safety” factors, uses many biomarkers of toxicity, optimizes dose–response modeling, anchors the estimates to widely accepted permissible exposure levels (PELs) for mineral dusts, and requires a minimum of dust for the experimentation. Our basic assumption was that we could find biomarkers in lung lavage fluid from rats given a single dose of dust (measured in mg) with established PELs ( $\text{TiO}_2$  and quartz, measured in  $\text{mg}/\text{m}^3$ ) that were sensitive to the large 50-fold difference in the PELs of these dusts. Given that outcome with dusts of known toxicity, we could then scale the biomarker responses in mg to dusts of unknown toxicity (lunar dusts) to estimate where the “PEL” would be for the dusts of unknown toxicity relative to the two known PELs. Our approach is called “comparative benchmark-dose (BMD) modeling”. Using this approach, we were able to achieve a cluster of safe exposure estimates (SEEs) from  $<0.5$  g of lunar dust, and then discern whether the three types of dust prepared from the parent lunar dust sample were toxicologically distinguishable. The SEEs are meant to be applied just like a PEL would be applied to exposures of industrial workers; they are time weighted averages for the 8 h after lunar dust is brought into the habitat and the crew is exposed until the filters remove the dust to undetectable levels.

## Methods

### Laboratory animals

Fischer 344 male rats (200–250 g) were housed in an AAALAC-approved animal facility at the National Institute of Occupational Safety and Health (NIOSH) in Morgantown, WV. The rats had free access to water and food. The animals were allowed to acclimate for at least 1 week before they were used in experiments. The guidelines of the NIOSH Institutional Animal Care and Use Committee and the approved test protocol were followed.

### Samples of test dusts

To obtain a respirable-sized fraction of unground lunar dust (LDug) from the parent sample, a fine dust fraction was aerodynamically separated from 200 g of Apollo 14 lunar regolith from the Fra Mauro formation (14003,96) in ultrapure nitrogen (up-N) (Cooper et al., 2010). The dust stream was allowed to pass through a cyclone separator designed for rodent studies (CH Technologies, Westwood, NJ). Proper choice of nitrogen flow rate (10 L/min) caused the coarser dust grains to be retained in the cyclone. The effluent aerosols of fine dust of respirable sizes were captured on a  $0.05 \mu\text{m}$  Nuclepore filter. The particle size distribution was determined by the Microtrac laser-scattering technique. The mass median diameter (MMD) was  $2.1 (+0.6/-0.9) \mu\text{m}$ .

A portion of a coarser lunar dust that was captured in the cyclone was jet milled (LDjm) or ball milled (LDbm) to smaller size in up-N. Coarse particles in the ball mill were subjected to violent and rapid crushing by the zirconia balls. In the jet mill, the collisions between the particles in the ultrapure nitrogen stream led to formation of finer dust particles. The resultant products were then subjected to further size separation in up-N as described above. The MMD

of the jet milled dust was  $2.5 (+1.0/-0.7) \mu\text{m}$ ; the MMD for the ball milled sample was  $1.8 (+0.5/-0.9) \mu\text{m}$ . The purpose of grinding the dust was to create freshly activated surfaces on the dust as a means of simulating the effects of micrometeorite impacts causing dust fragmentation, with its possible formation of numerous “dangling bonds” from the formation of unsatisfied valence shells and presumed activation of dust at the lunar surface (McKay et al., 1991; Papike et al., 1991). Because the moon has no atmosphere, surface activation can persist indefinitely until further impacts melt and agglutinate the dust. The mineral composition of all three dusts was similar, having major components as follows:  $\text{SiO}_2$  (48%),  $\text{Al}_2\text{O}_3$  (19%),  $\text{CaO}$  (12%),  $\text{FeO}$  (8%),  $\text{MgO}$  (7%) and  $\text{TiO}_2$  (2%).

The toxic reference dust, quartz (Qz), crystalline silica (Min-U-Sil 5), was obtained from U.S. Silica (Berkeley Springs, WV). The dust had an MMD of  $1.6 \mu\text{m}$ , and particles with diameter less than  $5 \mu\text{m}$  account for 96% of the dust by weight. The low-toxicity reference dust,  $\text{TiO}_2$  (rutile pigment R-100, consisting of  $\sim 99$  wt%  $\text{TiO}_2$ ,  $\sim 1$  wt% alumina), was a product of DuPont Company (Newark, DE) and a gift from David Warheit of DuPont. The average primary particle size of this dust was about  $0.3 \mu\text{m}$ , which is smaller than the other dusts; however, this is a size upon which the PEL for  $\text{TiO}_2$  is applied. Thus, this dust fits the needs we have for this experiment – it is tied to a specific PEL. Reference dusts were used without further treatment.

### Intratracheal instillation of dusts

All dust preparations were suspended in a saline-10% Survanta<sup>®</sup> medium (Abbott Nutrition, Columbus, OH), which was essential for effective suspension of the hydrophobic lunar dusts. Groups of six anesthetized rats were each instilled with 0.4 ml of the vehicle yielding doses of 0, 1, 2.5 or 7.5 mg of dust per rat.

### Collection of bronchoalveolar lavage fluid

To study biomarkers of toxicity in the bronchoalveolar lavage fluid (BALF), rats were deeply anesthetized with an overdose of Sleepaway (26% sodium pentobarbital, 7.8% isopropyl alcohol and 20.7% propylene glycol) (Fort Dodge Animal Health, Fort Dodge, IA) at 1 or 4 weeks after the dust instillation. Blood samples were collected by cardiac puncture; then the vena cava was cut to exsanguinate the rat. The lung was lavaged with 6 ml of phosphate-buffered saline (PBS) and then was further washed five times with 8 ml of PBS, for a total subsequent lavage fluid volume of 40 ml. The first lavage sample was centrifuged, and its supernatant was used for measuring the acellular BALF biomarkers. The cell pellets of the first and subsequent lavages were combined and suspended in 1 ml of HEPES-buffered solution for assessment of cell numbers and differentials.

### Assessment of biochemical components of the BALF

Albumin in BALF was determined according to a Sigma Diagnostics method utilizing the reaction of albumin with bromocresol green. The reaction product was then measured with a spectrophotometer at 628 nm and quantified against known concentrations of bovine serum albumin. Lactate

dehydrogenase (LDH) activity was determined by the oxidation of lactate coupled to the reduction of  $\text{NAD}^+$  at 340 nm over time. Measurements were performed with a Cobas c111 analyzer (Roche Diagnostics, Indianapolis, IN). Cytokine protein concentrations were determined with enzyme-linked immunosorbent assay (ELISA) kits that were specifically used to identify MPC-1, TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10 (Biosource International, Camarillo, CA). The results of this colorimetric assay were obtained with a Spectramax 250-plate spectrophotometer using *Softmax Pro 2.6* software (Molecular Devices Corp., Sunnyvale, CA). The samples were analyzed using a Beckman/Olympus AU480<sup>®</sup> chemistry analyzer (Marietta, GA). All BALF assays followed NIOSH standard protocols.

### Assessment of BAL cells

The total numbers of bronchoalveolar lavage cells were counted using a Coulter Counter equipped with a Channelizer (model Z<sub>b</sub>, Coulter Electronics, Hialeah, FL). Cell differentials were performed by visually counting 300 cells after making Cytospin preparations of microscope slides (Shandon Cytospin II, Shandon Inc., Pittsburgh, PA) and performing Wright-Giemsa staining (Hema-Tec 2000, Bayer Corp., Elkhart, IN). The number of macrophages and neutrophils was obtained by multiplying the total number of cells by the percentage of macrophages or neutrophils, respectively.

### Zymosan-stimulated chemiluminescence produced by alveolar macrophages

The chemiluminescence of alveolar macrophages (AMs) was assayed on AMs isolated from BALF of the dust-treated rats. The assay utilizes an un-opsonized zymosan solution (*Saccharomyces cerevisiae* cell wall preparation [Sigma-Aldrich<sup>™</sup>, St. Louis, MO]) to stimulate degranulation and release of reactive oxygen and reactive nitrogen species (predominantly nitric oxide) selectively from the macrophages. Thus zymosan A was suspended in HEPES buffered medium in a concentration of 20 mg/ml and the suspension was heated in boiling water bath for 5 min. It was then centrifuged at 1500 rpm for 5 min to remove debris. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione [Sigma-Aldrich<sup>™</sup>, St. Louis, MO]) was dissolved in dimethylsulfoxide (ATCC, Manassas, VA) to produce a solution of 10 mg/ml. The concentrated luminol solution was diluted with HEPES buffer to yield a working luminol solution of 0.4  $\mu\text{g}/\text{ml}$ . And, 0.5 ml of aliquot of AM suspension containing about  $1.0 \times 10^6$  cells was incubated at 37 °C for 10 min for measuring the resting stage of the cells (blank). Luminol solution was added to achieve a final concentration of 0.08 mg/ml with a total volume of 2 ml. The resultant chemiluminescence was determined with an automated luminometer (Berthold AutoLumat Plus LB 953, Gaithersburg, MD) set to collect light from 390 to 620 nm for 15 min. The integral of counts per minute (cpm) per cell versus time was calculated, and net signal was determined by subtracting cpm for blank.

### Statistical analysis and BMD modeling of biomarkers

The dose–response data obtained from each biomarker at 1 and 4 weeks were inspected to identify the biomarkers that

were sensitive to the 50-fold difference in the PELs for the standard respirable-size dusts,  $\text{TiO}_2$  and quartz. The PELs for the respirable fractions of these dusts are 5 and 0.1  $\text{mg}/\text{m}^3$ , respectively (U.S. Department of Labor, 2012). Dose–response profiles suitable for modeling were required to be monotonically increasing. If the dose–response curves for the standard dusts appeared suitable, data were screened to remove any outlier values according to two procedures. In one, we used commercial software (Stata, 2012) for a “robust regression” fit to the data, not including the control data. Control data were not included because the dispersion of biomarker data from controls was typically much less than the dispersion in data from animals receiving dust. We iterated upon an exclusion value of  $<0.25$ . In the second procedure, values greater than 2 SD from the mean value of the six data points were excluded. Of the 162 data points that were available for useful comparisons, the 2-SD exclusion rejected four points, all associated with one rat 4 weeks after exposure to  $\text{TiO}_2$ , whereas the robust regression exclusion rejected many more points, and was used only once to derive a plausible SEE (4 week macrophage data). Further elaboration of the statistical analysis is shown in Figure 1.

Targeting only the sensitive biomarkers, we attempted to model the data using BMD software from the Environmental Protection Agency (EPA, Version 2.1.2). Five models for continuous-type data were available in the software package. Variables modeled were those for which there were significant dose-related responses. If the variance was found to be non-homogeneous then dose-dependent variance was applied by the model for curve fitting. The best model was chosen for each dust on the basis of assessments of goodness of fit, values of scaled residuals of interest, and Akaike information criterion or the model with the lowest BMDL (when the range of BMDL estimates was greater than a factor of 3, some model dependence is assumed). The software predicted the weight of dust (mg) that would result in a 1-SD increase in biomarker response over the control mean, which is the default approach in the EPA software. A biomarker was deemed acceptable if  $[\log \text{BMD}_{1\text{SD}}(\text{TiO}_2) - \log \text{BMD}_{1\text{SD}}(\text{Quartz})]$  was greater than 1.0.

### Example calculation of SEE

The first step in the calculation was to establish the line between the  $\log \text{BMD}_{1\text{SD}}(\text{TiO}_2)$  and  $\log \text{BMD}_{1\text{SD}}(\text{Qz})$  using  $\log_{\text{PEL}}\text{TiO}_2$  and  $\log_{\text{PEL}}\text{Qz}$ , respectively, for a given toxic endpoint. Here, we will show a sample calculation for total white cells (Figure 2). Once the line is established on a log–log basis, the responses to each of the lunar dusts in terms of total white cell count can be used to calculate the  $\log_{\text{PEL}}$  for each dust. In this example, the  $\log_{\text{PEL}}$ s were as follows:  $\text{LD}_{\text{jm}} = 0.12$ ,  $\text{LD}_{\text{ug}} = 0.07$  and  $\text{LD}_{\text{bm}} = -0.08$  (last column of table in Figure 3). Taking the anti-log of these gives SEEs of 1.32, 1.17 and 0.83, respectively, in the same units as the units of the known PELs, which is  $\text{mg}/\text{m}^3$ .

### Results

The best-fit BMD profiles, as given by the EPA software, are shown (Figures 3–11) for each of the five dusts for sensitive biomarkers. The table with each figure shows the comparison

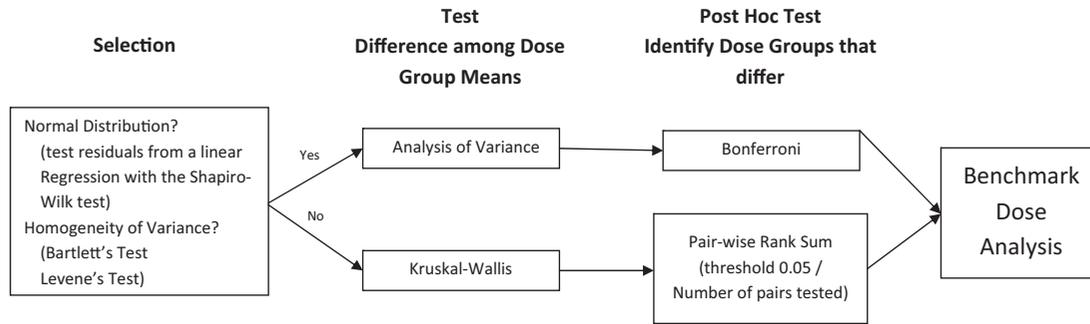
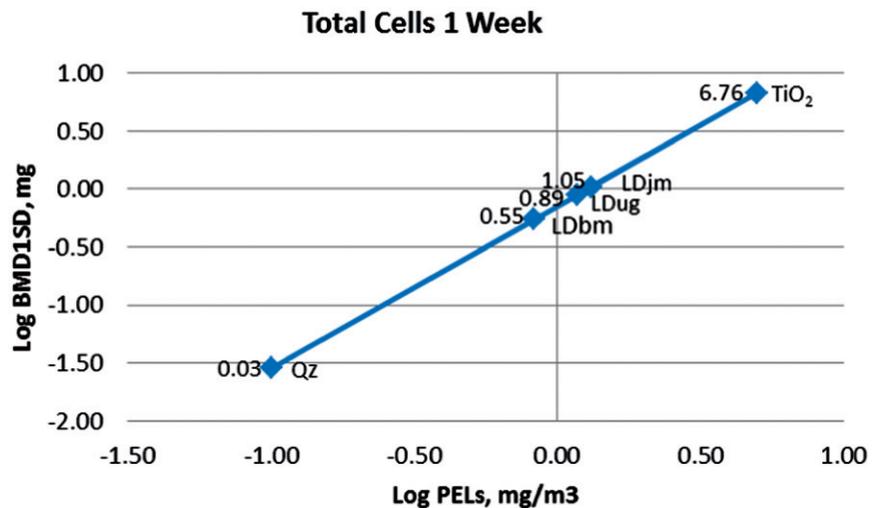


Figure 1. Statistical analyses were performed using Stata (version 12) (StataCorp, College Station, TX). In order to determine if conditions necessary for the application of analysis of variance were met, normality of distribution was assessed by testing residuals from a linear regression with the Shapiro–Wilk test (Swilk), and homogeneity of variance was assessed both with Bartlett's test and with a more robust method (robvar) based upon Levene's test. If conditions were appropriate, then testing of treatment means was performed with one way analysis of variance and *post-hoc* testing was accomplished by the method of Bonferroni. If either of the conditions for parametric testing were not satisfied then the nonparametric method of Kruskal–Wallis was utilized to test for difference among treatment groups and *post-hoc* testing was accomplished by pair-wise rank sum test and the threshold for significance was taken as  $p < 0.5/\text{number of pairs tested}$ .

Figure 2. The relationship between the responses for total white cells 1 week after dosing is given along a log–log line defined by  $\text{TiO}_2$  and Qz responses. Responses to the lunar dusts are shown on this line according to the total-white-cell count they elicited. The  $\text{BMD}_{1\text{SD}}$  for each dust is shown next to the dust label as follows: LDjm (jet-milled dust), LDug (unground dust) and LDbm (ball-milled dust). The equation of the log–log line is as follows: slope = 1.396393 and intercept = -0.14609.



on a linearized logarithmical scale in order to estimate the SEEs for the three lunar dusts from the known PELs of the reference dusts. Data in Table 1 show a statistical difference between jet-milled and unground dust, but the difference in SEEs, 1.3 versus 0.9  $\text{mg}/\text{m}^3$ , respectively, is not considered toxicologically significant.

## Discussion

This is a powerful new approach to estimate safe exposure levels for humans who could be exposed to mineral dust. It is “anchored” on either end by two PELs based on large amounts of data and considerable expert opinion, on what the safe human exposure levels should be. Quartz is among the most toxic of mineral dusts, and  $\text{TiO}_2$  is among the least toxic. Their respective PELs have been applied for years as a safety measure to control exposures of Earth-based workers. By anchoring our approach to these human exposure values, we have avoided having to apply large uncertainty factors to an arbitrary point of departure. Furthermore, we anticipate that astronaut exposures will be much like those of

ground-based workers. Astronauts may be exposed to dust for a few hours when they return to their habitat on “weekdays” until the air revitalization system purifies the air. This is similar to industrial workers’ exposure. Astronauts, like industrial workers, will have weekends off, and this will mean no scheduled work outside the habitat and no new dust in the habitat.

This approach also achieves efficacy by making use of a wide array of quantitative biomarkers of dust-induced lung cell injury. Among this large array of biomarkers, we expected to find several that were exquisitely sensitive to the differences in toxicity of the two standard dusts. In fact, five biomarkers met our criterion for acceptable sensitivity  $\{[\log \text{BMD}_{1\text{SD}}(\text{TiO}_2) - \log \text{BMD}_{1\text{SD}}(\text{Qz})] \text{ was greater than } 1.0\}$  at 1 week, 4 weeks or both times after instillation. Several biomarkers failed because  $\text{TiO}_2$  elicited no convincing dose response. Other biomarker profiles failed because the only dust that elicited a biomarker response was quartz.

Our approach also gained power because it depended on the entire dose–response curve instead of extrapolation below a single-value NOAEL, which has commonly been used as a

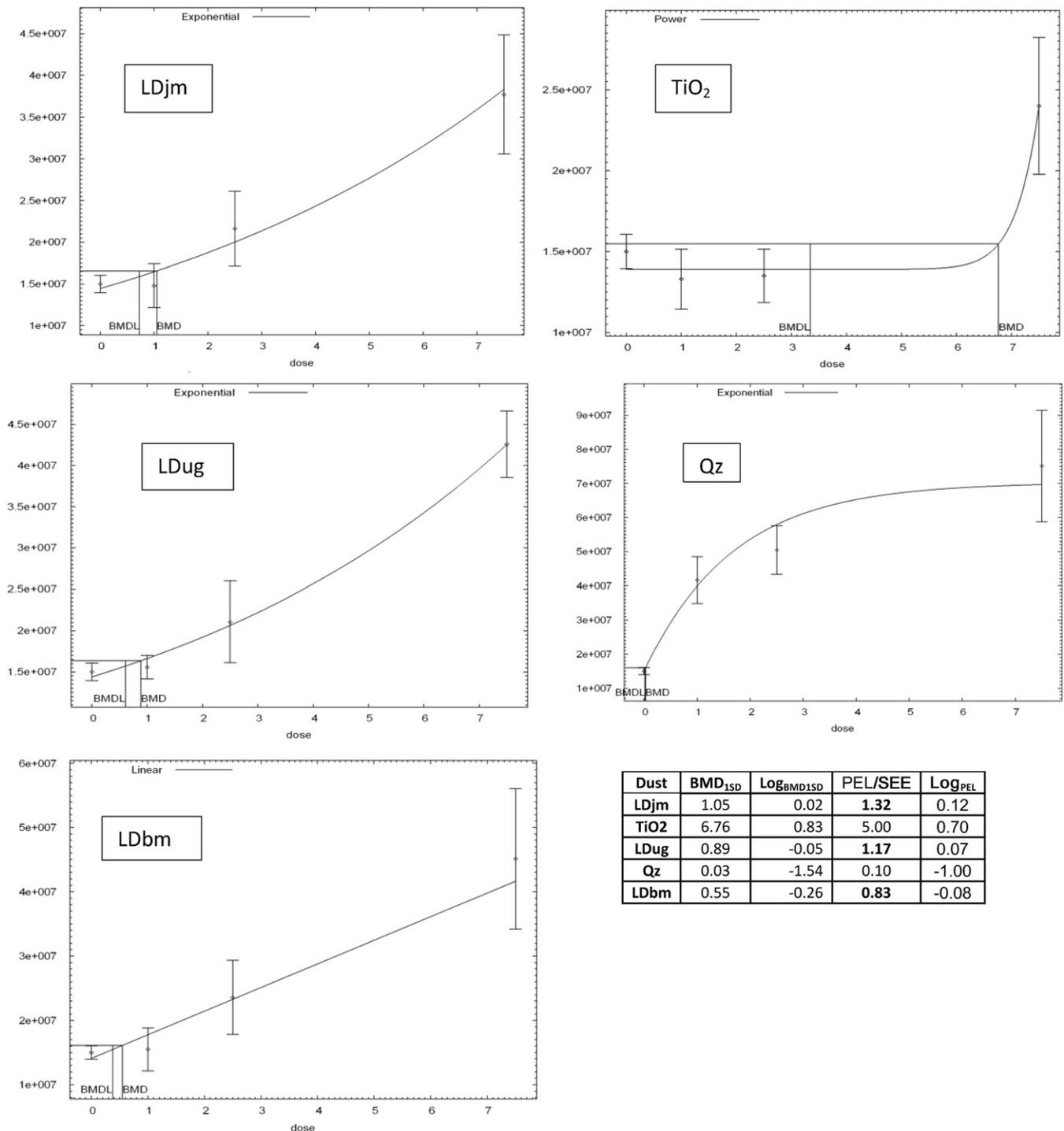


Figure 3. Total white cells 1 week after dust instillation and table showing calculations to estimate SEEs. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for total white cells ( $10^6$ /rat) 1 week after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The “maximum likelihood” BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (Figure 2). The SEEs in  $\text{mg}/\text{m}^3$  for the lunar dusts are shown in bold in the table.

point of departure on which various uncertainty factors are applied. NOAELs are very sensitive to the number of test subjects, the choice of dose intervals, and the choice of dose ranges. By using comparative BMD<sub>1SD</sub> values, we avoided limitations associated with using the NOAELs as a point of departure. BMD modeling of dose–response data has been developed and refined by the EPA over many years, but

toxicity data in the literature have typically not been obtained with BMD analysis in mind as a tool to be used in directly setting a safe exposure level. For example, of 19 volatile compounds reviewed for setting safe astronaut exposures, data suitable for benchmark–dose analysis were available for only three (National Research Council, 2008). Our experiments were designed *a priori* to facilitate BMD analyses.

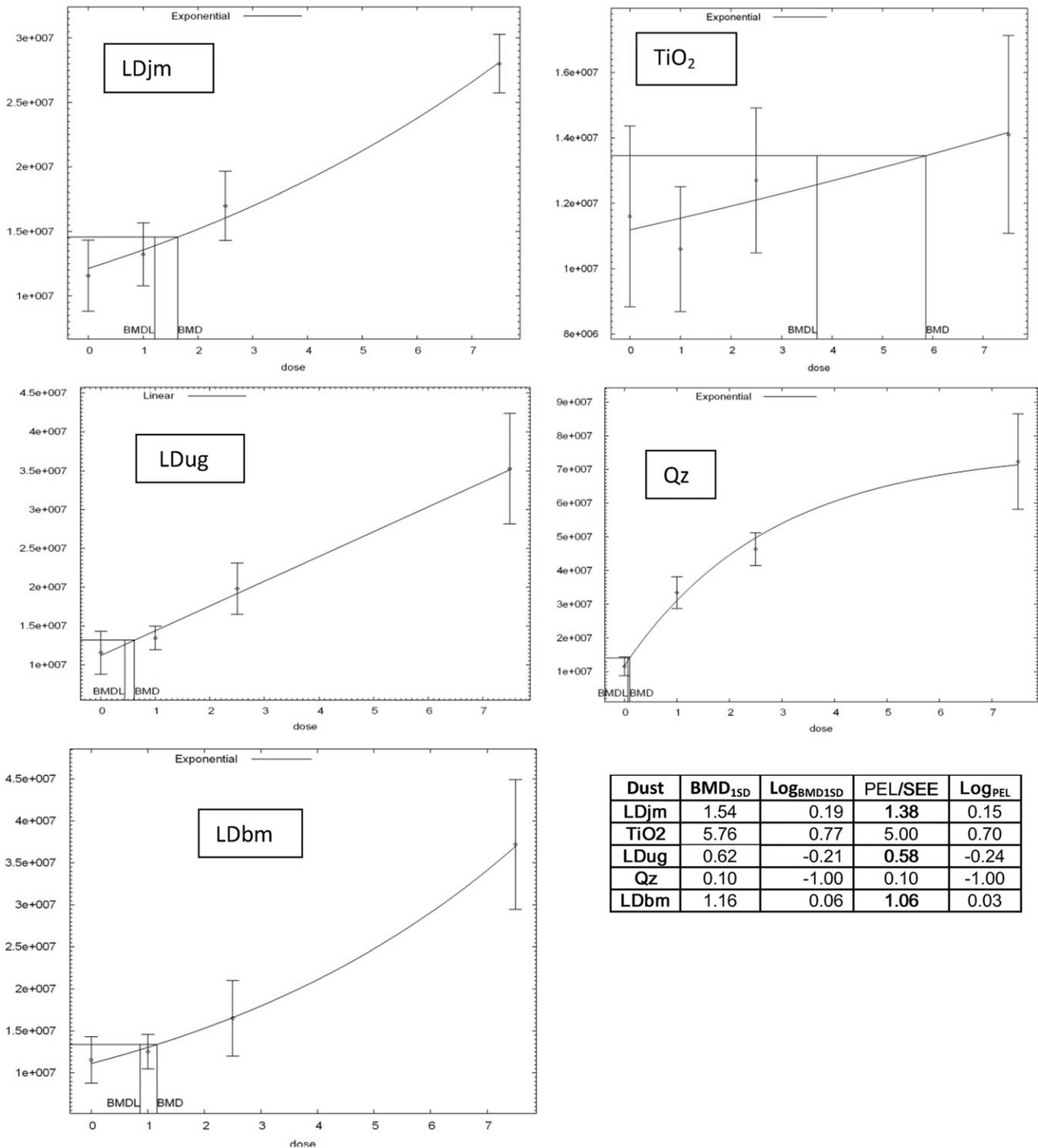


Figure 4. Total white cells 4 weeks after instillation of dust. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for total white cells ( $10^6$ /rat) 4 weeks after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The ‘‘maximum likelihood’’ BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

Use of a common set of control data and a common set of experimental parameters also adds strength to the analyses. When comparing the toxicities of dusts, one typically faces a collection of data from various laboratories, on various species, with various endpoints, and with various exposure regimens. All of these potentially confounding factors are

removed by using data from a single study obtained simultaneously and using the same set of control data for each biomarker. Our data on the selected biomarkers support the conclusion that the three lunar dusts are toxicologically indistinguishable (Table 1). The lowest SEEs were just above 0.5 mg/m<sup>3</sup>, and the averages were near 1 mg/m<sup>3</sup>; therefore, a

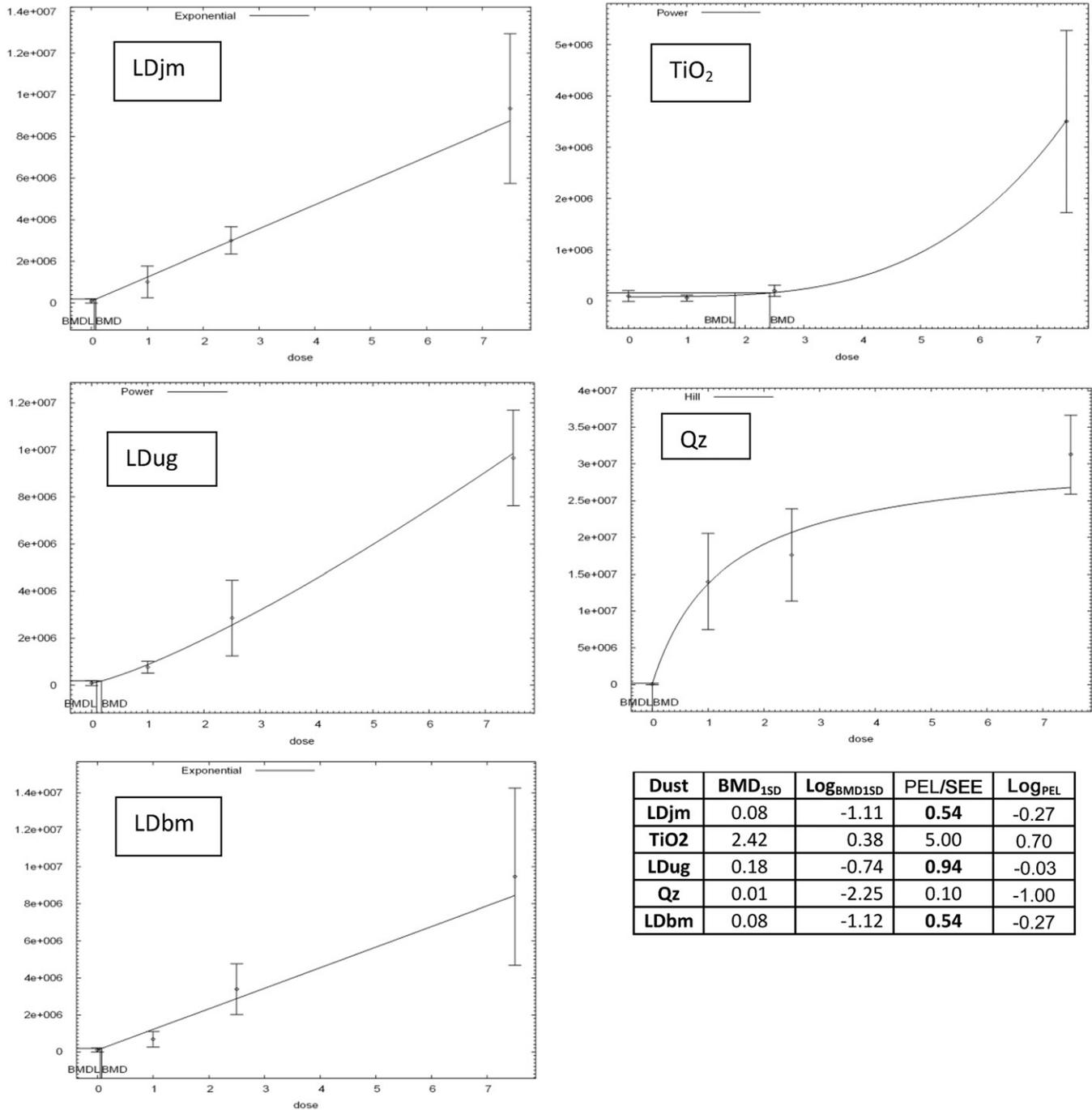


Figure 5. Total neutrophils 1 week after instillation of dust. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for total neutrophils ( $10^6$ /rat) 1 week after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The ‘‘maximum likelihood’’ BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

SEE of 0.5 to 1 mg/m<sup>3</sup> is reasonable for the episodic exposures we would expect inside a lunar habitat during a prolonged mission on the lunar surface.

Using multiple endpoints, our results show that the toxicities of ground and unground lunar dust are about the same, suggesting that any surface activation caused by grinding is not an important factor in the pulmonary toxicity of this dust. This observation is further supported by the finding that dry LDjm kept in upN did not elicit a significant

reaction when placed directly on the surface of a bed of cultured corneal cells (Meyers et al., 2012). In any case, grinding of any sort has a limited ability to simulate conditions on the lunar surface that contribute to surface activation of respirable size dust; however, we note that the vast majority of respirable lunar dust from our Apollo 14 sample consists of glassy beads (David McKay, unpublished data). These have undergone melting and in the process would have lost any surface activation up to the time of

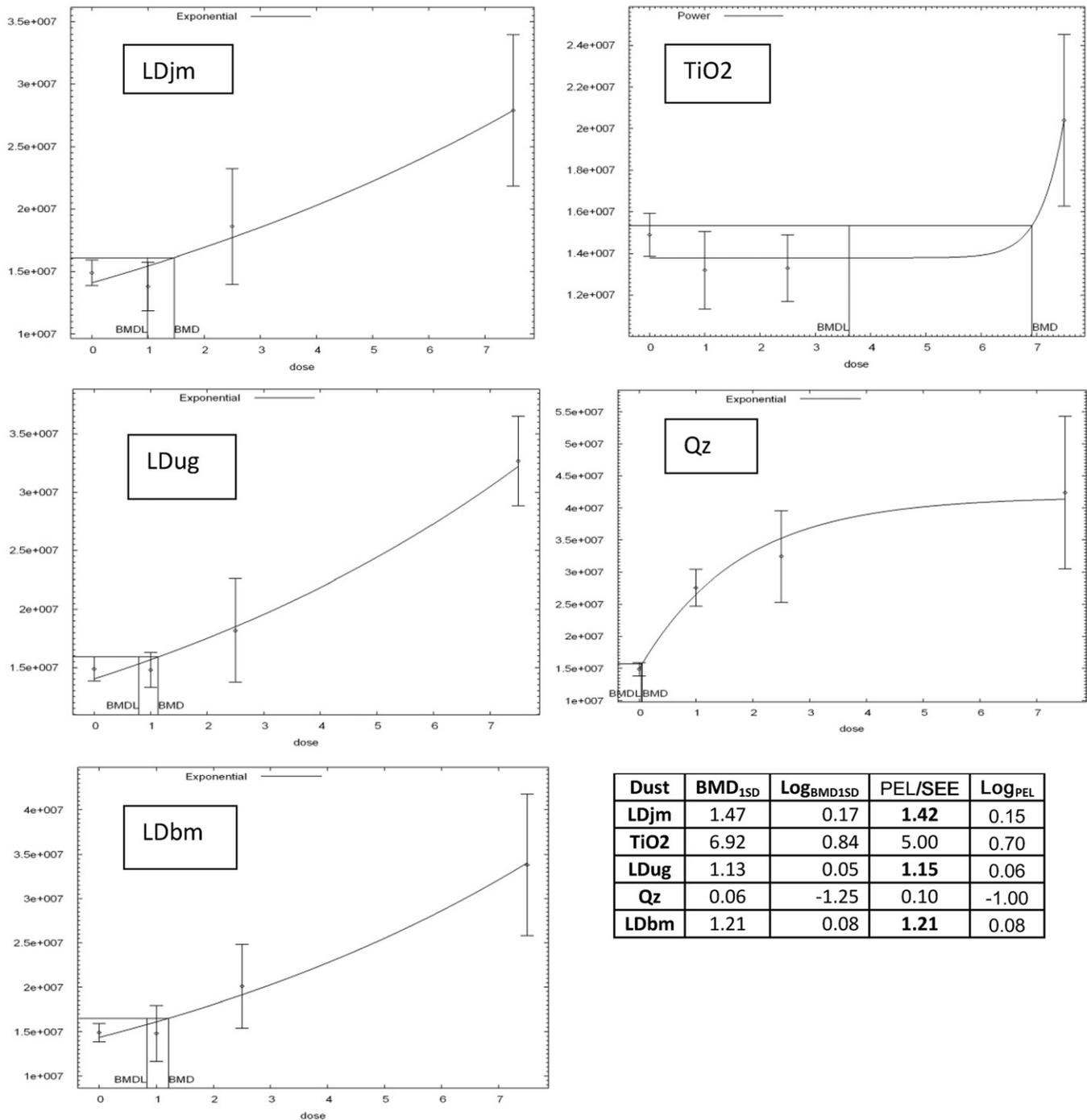


Figure 6. AMs 1 week after instillation of dust. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for AMs ( $10^6$ /rat) 1 week after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The ‘maximum likelihood’ BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

the melt. The question of how important surface activation might be has by no means been completely settled, but the weight of evidence favors no more than a small secondary effect from such activation, which is likely to be rapidly dissipated when the dust encounters a humid, oxygen-rich environment in which astronauts will be living on the lunar surface.

The estimate presented here is based on initial data obtained on a limited amount of lunar dust from a specific

location on the lunar surface. The present estimate should be applied with caution to lunar dust from other locations on the moon and is subject to refinement as more data become available. The finest dust at the lunar surface is continuously, but very slowly redistributed so that there is some mixing of the dust at the surface; however, dust below the surface or in exotic locations, such as permanently dark crater floors, may be much different in physical and chemical properties and have different toxicological properties.

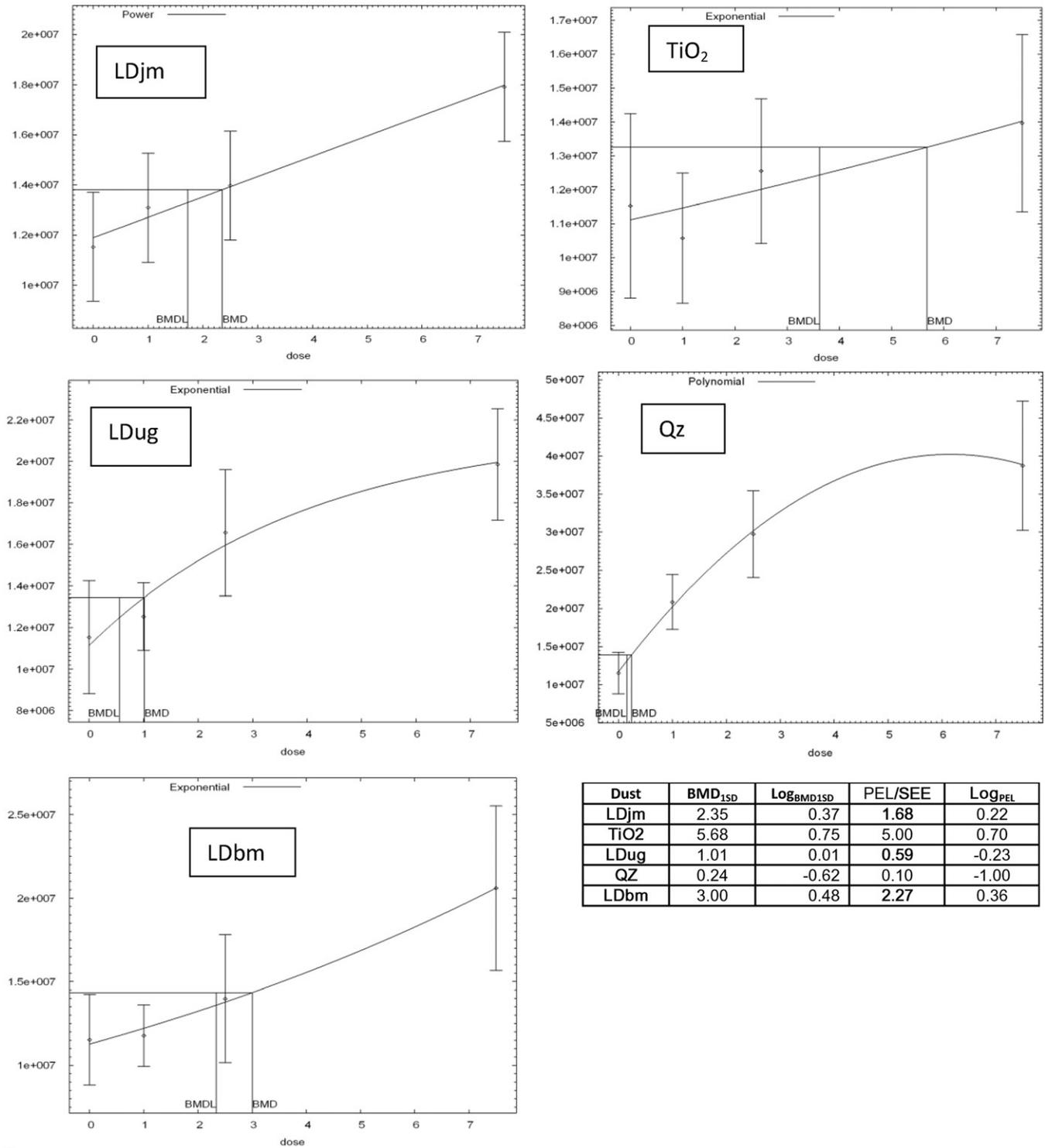


Figure 7. AMs 4 weeks after instillation. Data from robust regression analyses. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for AMs ( $10^6$ /rat) 4 weeks after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The “maximum likelihood” BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in  $\text{mg}/\text{m}^3$  for the lunar dusts are shown in bold in the table.

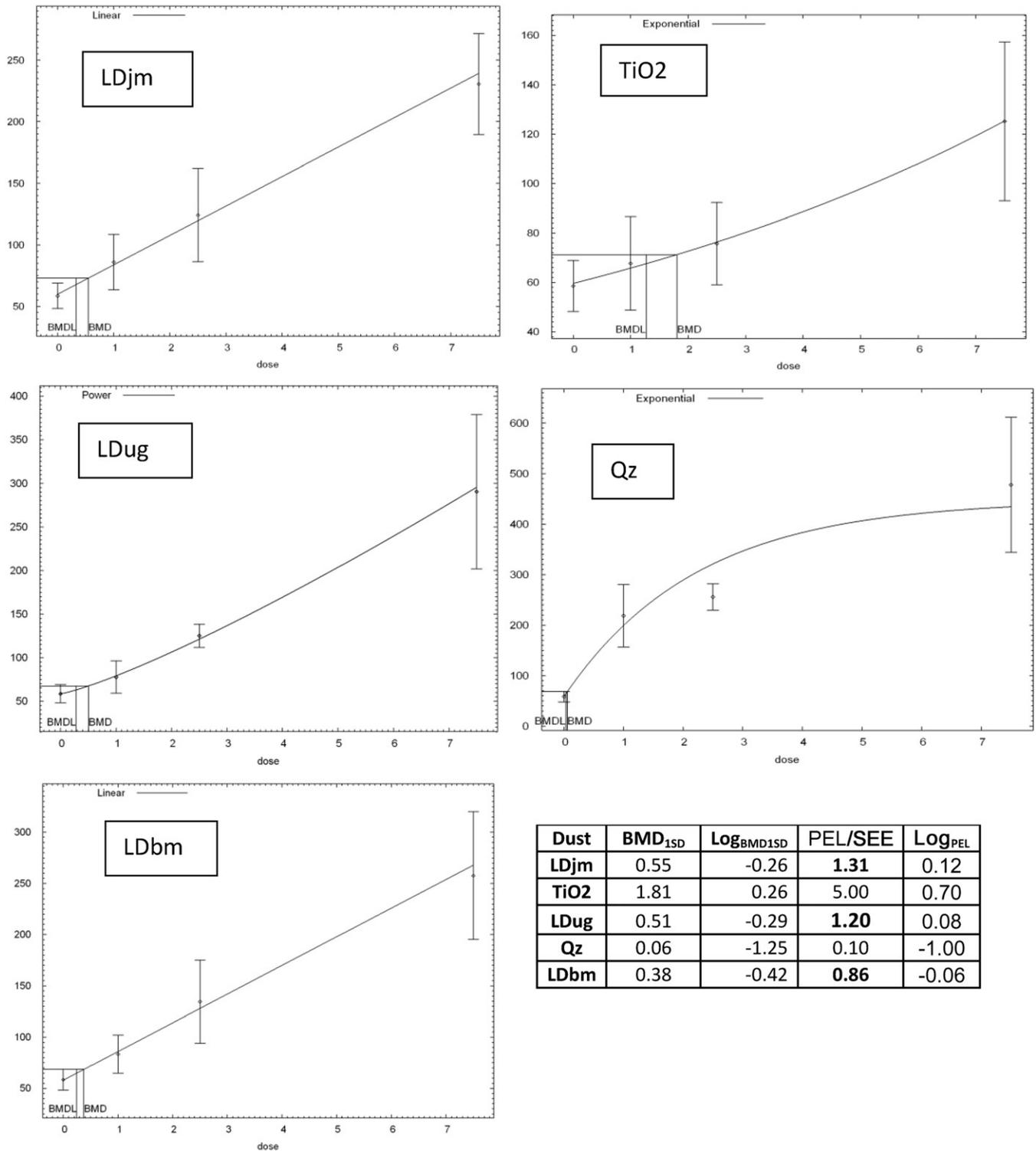


Figure 8. LDH activity 1 week after instillation of dusts. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for LDH (U/L) 1 week after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The ‘‘maximum likelihood’’ BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

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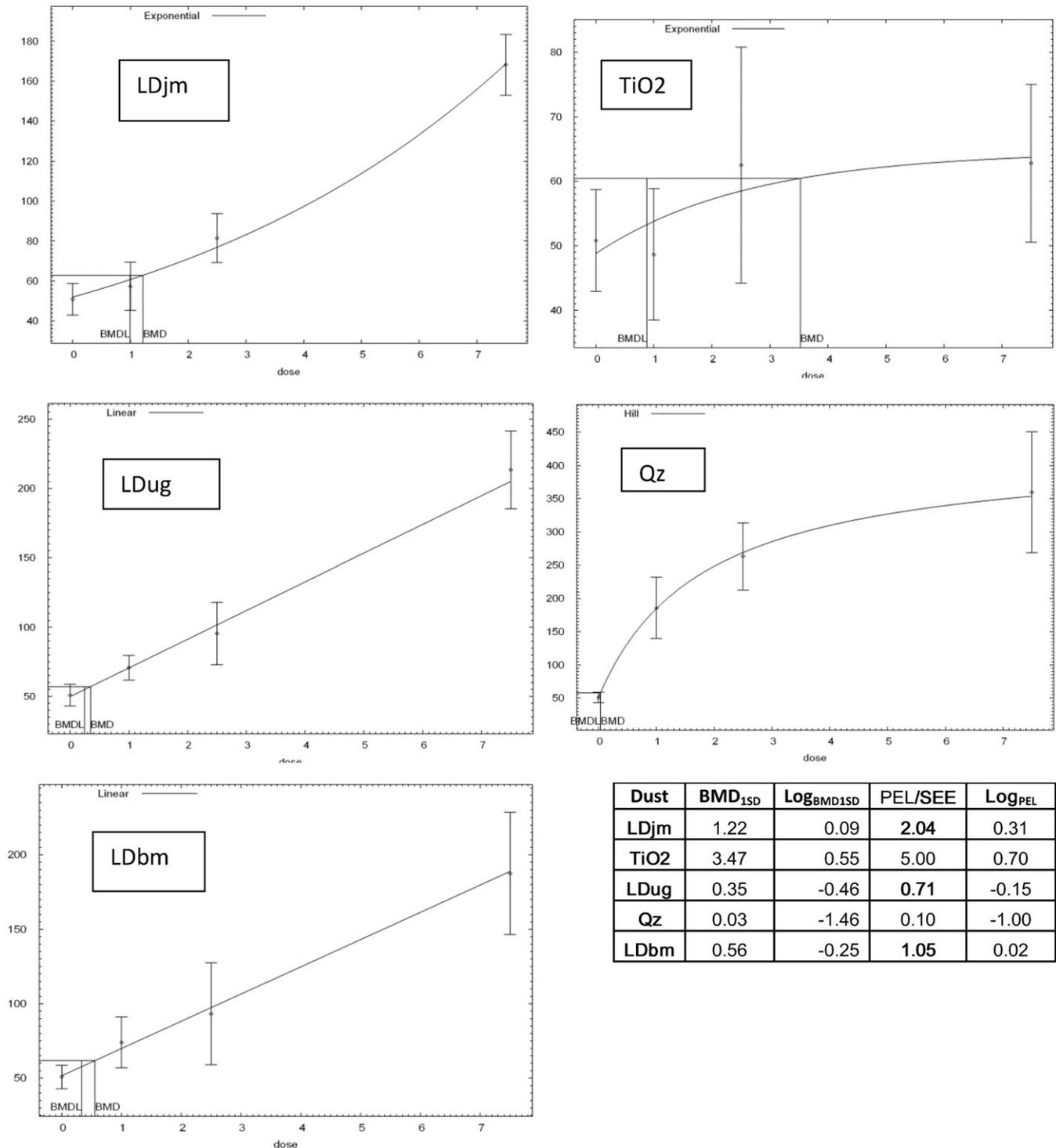


Figure 9. LDH activity 4 weeks after instillation of dusts. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for LDH (U/L) 4 weeks after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The ‘maximum likelihood’ BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the tables.

A relatively small number of animals per group were used to respect the legal mandate to involve the minimal number of animals expected to yield satisfactory scientific results. In hindsight, our choice of six animals per group was appropriate. However, any results like ours, which are based on statistical analyses, could always be improved by using a

larger group of test animals. The maximal amount of confidence in the results must be tempered with the reality of maintaining minimal use of animals. Furthermore, hindsight shows that dose selection could have been improved to generate better sets of comparative dose–response curves. For example, the amount of quartz instilled could have been

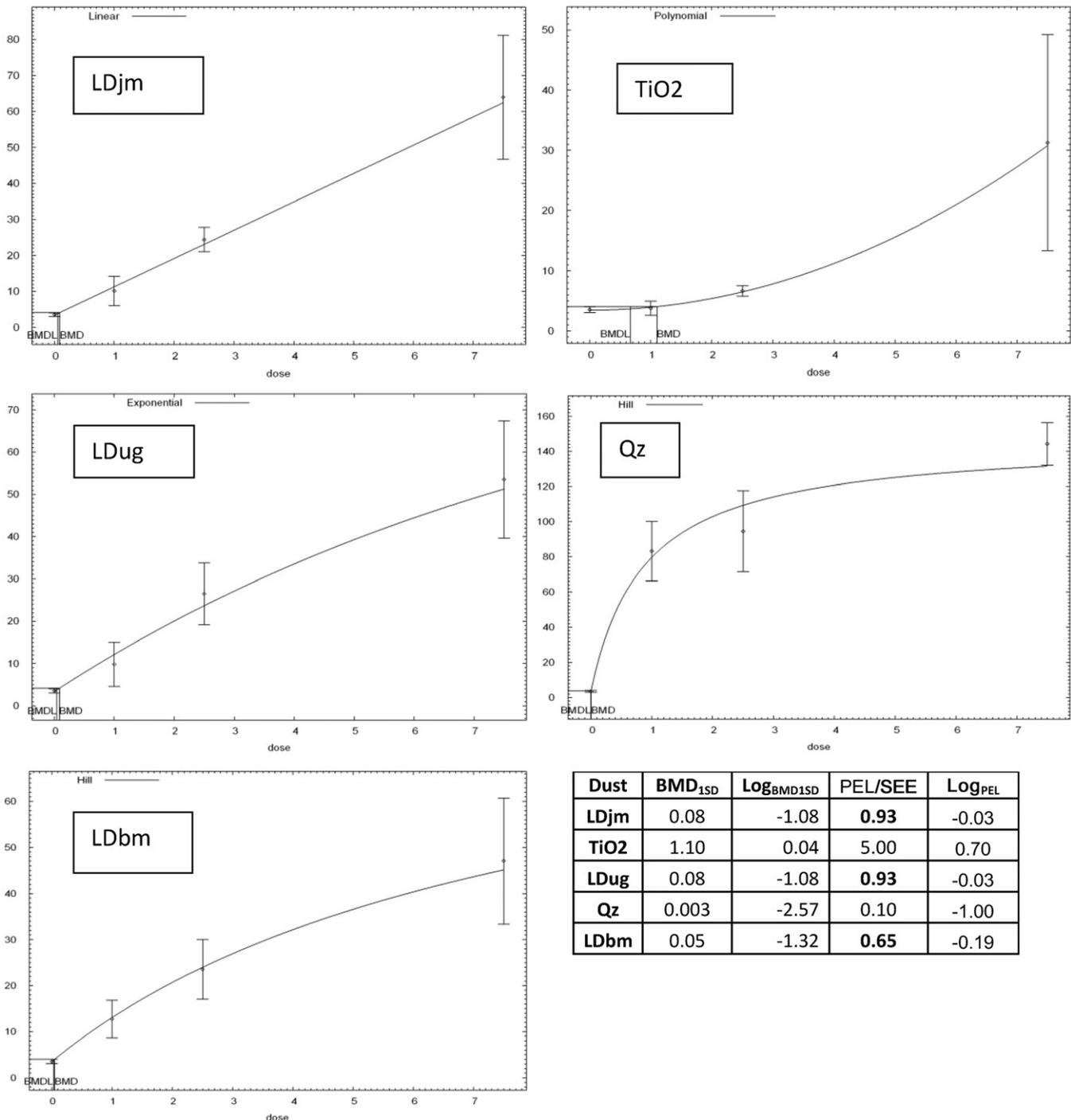


Figure 10. Macrophage stimulation 1 week after dust instillation. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for macrophage stimulation (cpm) 1 week after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The “maximum likelihood” BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

three-fold lower and the amount of TiO<sub>2</sub> three-fold higher to improve BMD modeling.

A single bolus dose of dust delivered into a rat in a vehicle has a limited ability to reveal long-term toxicity of that dust if it were inhaled for months or years by humans. However, we have selected biomarkers that are known to be associated with lung injury in rats and show high sensitivity to the known

differences in long-term toxicity of TiO<sub>2</sub> and Quartz in humans. Furthermore, we have followed these markers for a month after instillation into rats to determine the persistence of biomarker changes. The markers are reflective of cell damage (LDH) or inflammation (cell counts), which are harbingers of pathological changes, but are not of themselves pathological changes.

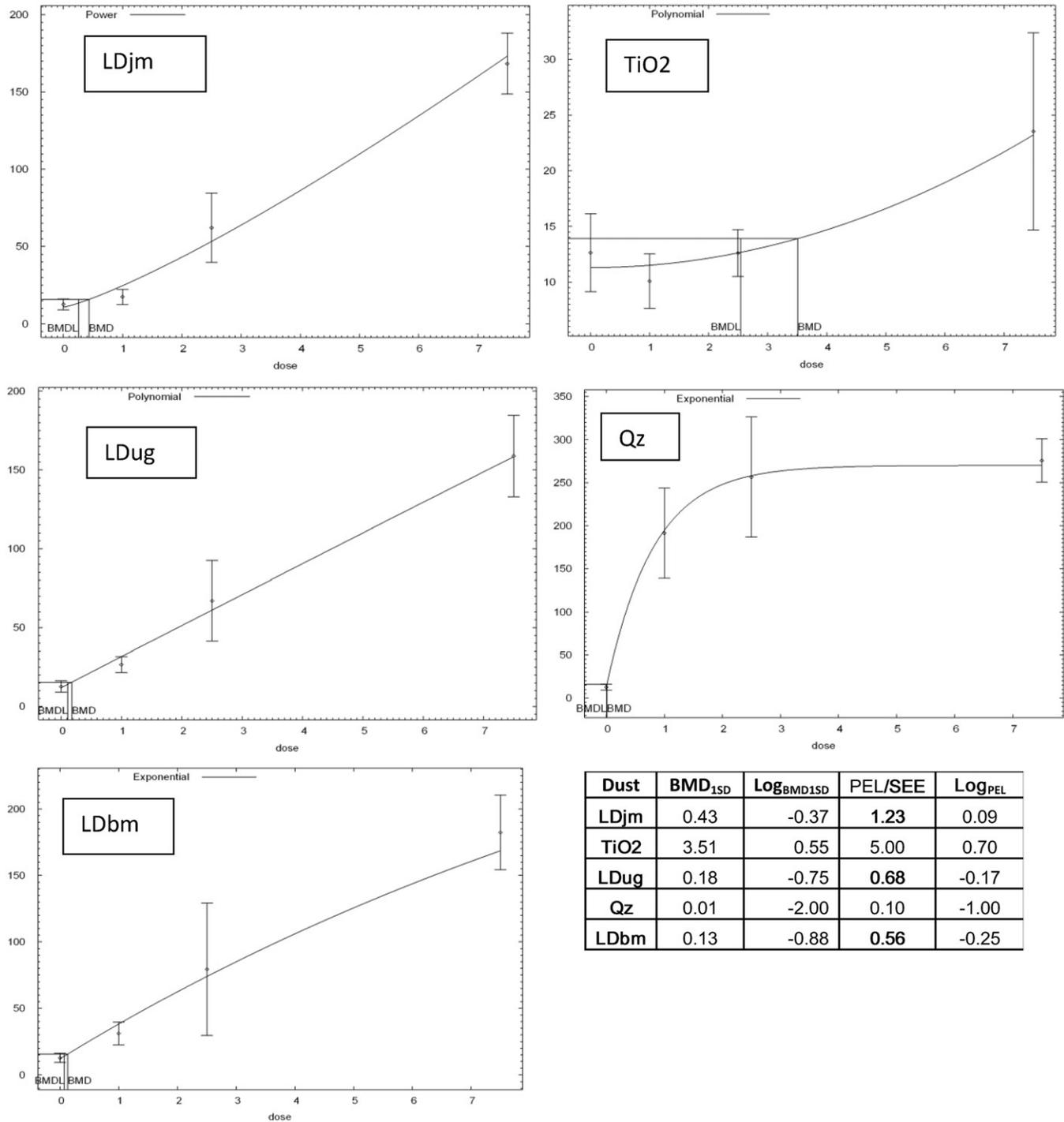


Figure 11. Macrophage stimulation 4 weeks after dust instillation. The best BMD fits to the dose–response data from lung lavage fluid parameters are shown for macrophage stimulation (cpm) 4 weeks after instillation of the dusts, which are identified as follows: LDjm (jet-milled lunar dust), TiO<sub>2</sub>, LDug (unground lunar dust), Qz (quartz) and LDbm (ball-milled lunar dust). The instilled dose of dust was 0, 1, 2.5 or 7.5 mg/rat. The “maximum likelihood” BMD is shown as well as the lower 95% confidence interval on the BMD, which is labeled BMDL. The accompanying table reflects the analysis and transformation for the log–log plot (example in Figure 2). The SEEs in mg/m<sup>3</sup> for the lunar dusts are shown in bold in the table.

## Conclusions

We have demonstrated that comparative BMD modeling facilitates estimation of safe exposure levels for lunar dust while using a minimal quantity of dust. Our study offers the first evidence-based estimate of safe exposure levels to lunar dust during long stays on the lunar surface. To achieve this,

we used a new method, which we call “comparative BMD modeling”, on multiple indices of toxicity derived from lung lavage fluid. Although our study was conducted in rats, by anchoring the indices to human PELs, we have shown direct relevance to protection of human explorers as they live and work on the lunar surface for decades to come.

Table 1. SEE (mg/m<sup>3</sup>) for three types of lunar dust from biomarkers that were sensitive to the differences in toxicity of TiO<sub>2</sub> and quartz.

Lavage fluid biomarker	Jet-milled dust*	Unground (original) dust*	Ball-milled dust
Total white cells, 1 week	1.32	1.17	0.83
Total white cells, 4 week	1.38	0.58	1.06
Neutrophils, 1 week**	0.54	0.94	0.54
Macrophages, 1 week	1.42	1.15	1.21
Macrophages, 4 week	1.68	0.59	2.27
LDH, 1 week	1.31	1.20	0.86
LDH, 4 week	2.04	0.71	1.05
Macrophage stimulation, 1 week	0.93	0.93	0.65
Macrophage stimulation, 4 week	1.23	0.68	0.56
AVERAGE SEE (mg/m <sup>3</sup> )	1.32 ± 0.42	0.88 ± 0.25	1.00 ± 0.53

\*Mann–Whitney *U* test gave  $p = 0.01$  in comparing jet-milled and unground dust SEEs. Other pair-wise comparisons were  $p > 0.05$ .

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

## Acknowledgements

We thank David McKay and Bonnie Cooper of Johnson Space Center (JSC) for preparation of the lunar dusts, Alan H. Feiveson of JSC for statistical consulting, Jon Rask of Ames Research Center for excellent technical assistance, Vincent Castranova for welcoming us to work in his laboratory at the NIOSH and Patti C. Erdely, Terrence G. Meighan, Mark Barger and Shih-Houng Young of the NIOSH for technical expertise. We thank Jane Krauhs for editorial assistance and Cynthia Bush for graphics assistance.

## Declaration of interest

This work was supported by the Human Research Program of the NASA. The estimates provided herein should not be construed as official NASA standards. The conclusions are those of the authors alone, and we report no conflicts of interest.

## References

Cooper BL, McKay DS, Taylor LA, et al. (2010). Extracting respirable particles from lunar regolith for toxicology studies. In: Song G, Malla RB (eds.) Proceedings of the 12th international conference on engineering, science, construction, and operations in challenging environments, Reston: American Society of Civil Engineers, 66–73.

Driscoll KE, Costa DL, Hatch G, et al. (2000). Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol Sci* 55:24–35.

Henderson RF, Driscoll KE, Harkema JR, et al. (1995). A comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. *Fundam Appl Toxicol* 24:183–97.

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

Horwell CJ, Baxter PJ. (2006). The respiratory health hazards of volcanic ash: a review for volcanic risk mitigation. *Bull Volcanol* 69: 1–24.

McKay DS, Heiken G, Basu A, et al. (1991). The lunar regolith. In: Heiken G, Vaniman D, French BM (eds.) Lunar sourcebook: a user's guide to the moon. Houston, TX: Lunar Planetary Institute, 285–356.

Meyers VE, Garcia HD, Monds K, et al. (2012). Ocular toxicity of authentic lunar dust. *BMC Ophthalmol* 12:26–32.

National Research Council. (2008). Spacecraft maximum allowable concentrations for selected airborne contaminants (vol. 5). Washington, DC: National Academies Press.

Papike JJ, Taylor LA, Simon S. (1991). Lunar minerals. In: Heiken G, Vaniman D, French BM (eds.) Lunar sourcebook: a user's guide to the moon. Houston, TX: Lunar Planetary Institute, 121–81.

Sand S, von Rosen D, Victorin K, Filipsson AF. (2006). Identification of a critical dose level for risk assessment: developments in benchmark dose analysis of continuous endpoints. *Toxicol Sci* 90:241–51.

Stata. Available from: <http://www.stata.com/> [last accessed: 6 Dec 2012].

U.S. Department of Labor. Available from: [http://www.osha.gov/dts/chemicalsampling/data/CH\\_272100.html](http://www.osha.gov/dts/chemicalsampling/data/CH_272100.html); [http://www.osha.gov/pls/oshaweb/owadis.show\\_document?p\\_table=standards&p\\_id=9994](http://www.osha.gov/pls/oshaweb/owadis.show_document?p_table=standards&p_id=9994) [last accessed: 6 Dec 2012].