Chapter 11

EVALUATION OF IMMUNOTOXICITY FOLLOWING A 3-DAY EXPOSURE TO DUST SAMPLES COLLECTED FROM NELLIS DUNES RECREATION AREA

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1. Introduction

Epidemiologic studies have demonstrated an association between particulate matter (PM) and daily mortality (Dockery et al., 1994), increased emergency room visits and hospital admissions (Schwartz et al., 1993), or decreased pulmonary function (Boezen et al., 1998). In mouse models, repeated airway exposure of dust induces lung inflammation in the presence or the absence of allergen (Ichinose et al., 2005; Hiyoshi et al, 2005; Ichinose et al., 2006). Many of these studies have focused on urban sources of dust, where fine particles (aerodynamic diameters equal to or less than 2.5 μm; PM2.5) are the major type of dust exposure consisting of acid condensates, sulfate, and nitrate particles (Yang et al., 2005). However, few studies have examined the specific health risks associated with dust generated in non-urban areas, and even less is known about potential health risks to dust exposure while recreating in the desert.

Recent epidemiologic studies have shown that dust events are associated with an increase in daily mortality in Seoul, Korea, and Taipei, Taiwan (Kwon et al., 2002), and that these dust particles cause cardiovascular and respiratory dysfunction in Taipei (Chan, 2002). Furthermore, the PM2.5 and PM10 fractions of dust contains various metals (i.e., Pb, Cd, Zn, As, Mn, etc.). One study identified that ambient concentrations of PM2.5 and PM10 were not significantly associated with changes in peak expiratory lung flow rates, but that

most of the metal concentrations bound to the particulates were significantly associated with decreases in pulmonary function (Hong et al., 2010). This study also reported similar potency in reduction of pulmonary function regardless of whether the source of metals on the dust particles was anthropogenic or natural occurring (Hong et al., 2010).

Understanding the chemical composition of PM exposure is key in determining health risks. In many cases, much is known about single metal toxicity, but little is known regarding exposure to complex metal mixtures. Although each metal exhibits unique toxicity, there are common toxic pathways to include mimicry, oxidative damage, and adduct formation with DNA or protein. Nonessential metals may mimic essential metals causing a disruption in cellular and enzymatic mechanisms. Examples include the replacement of essential zinc by cadmium, replacement of potassium by thallium, replacement of phosphates by arsenate, and mimicry of manganese in place of iron. Further, the generation of reactive oxidative species is often induced by metals in their ionic form, resulting in oxidative modification of DNA or proteins, including aberrant gene expression and carcinogenesis (Ballatori, 2002; Basalt, 2004).

Dust released from the Nellis Dunes Recreation Area (NDRA) consists of many metals at the parts per million level, adsorbed to PM10 or smaller particles (see Chapter 9). Therefore, an initial study was undertaken to examine the toxicological and histopathological effects following exposure to dust samples from NDRA.

2. Procedures

2.1 Dust collection and characterization

Samples were collected from 3 different surface units in the NDRA: unit 2.2 (silt and clay deposits with gravel), unit 3.1 (desert pavement with a silty Av horizon underneath), and unit 3.2 (rock-covered silt deposits). All dust was extracted from samples taken from the uppermost cm of the topsoil using a Soil Fine Particle Extractor (Goossens, 2011). This instrument enables one to select the finest fractions of the soil for analysis. Median diameter of the dust used in the tests was 4.2 μm (unit 2.2), 2.4 μm (unit 3.1), and 3.1 μm (unit 3.2).

Dust samples were acid-digested in the Environmental Soil Analytical Laboratory (UNLV) in accordance with EPA Method 3052 prior to total elemental analysis using inductively coupled plasma mass spectroscopy (ICP-MS) analysis. ICP-MS analyses were performed for the following elements: arsenic (As), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), cadmium (Cd), nickel (Ni), lead (Pb), strontium (Sr), uranium (U), vanadium (V), thorium (Th), boron (B), molybdenum (Mo), selenium (Se), manganese (Mn), zinc (Zn), barium (Ba), titanium (Ti), iron (Fe), and aluminum (Al).

Concentrations of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were determined using atomic absorption spectroscopy (AAS). Phosphorus (P) concentrations were determined colorimetrically using molybdate-ascorbic acid method (Kuo 1996). To ensure quality control for the ICP-MS and AAS analyses, Buffalo River Sediment Reference Material 8704 was obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA. Samples of this material were digested in accordance with EPA Method 3052 and analyzed along with the NDRA samples. Satisfactory recoveries were found for the trace elements analyzed with the exception of Cs, Ba, Fe, and Al. Based on comparison with the Buffalo River standard, the concentrations of these elements were underestimated. It is likely that these results were underestimated because of interferences caused by high soluble salt concentrations in the samples and some insoluble mineral fluorides may have been formed during digestion (Kingston and Haswell, 1997). The analytical results are shown in Table 1.

2.2 Exposure route and dose

To learn about potential toxicological effects caused by acute exposure to NDRA dust, a standardized rodent exposure model and assays were utilized. B6C3F1 mice were exposed for 3 consecutive days to the dust extracted at 0, 0.1, 1.0, 100, or 1000 mg/kg/day. Intratracheal aspiration was the route of exposure used as it has several advantages over inhalation exposure (Driscoll et al, 2000). The small particle size, 4.3 micrometer in diameter or less (Table 1), is appropriate for the smaller size of the rodent while also relevant to human health concerns regarding exposure to small dust particles (i.e., PM2.5). Limitations of this type of exposure include that a bolus amount of dust is delivered as compared to smaller amounts over a period of hours or day.

As the level of dust exposure in humans is not known at NDRA, the exposures applied in this study ranged from a 0.1 to 1000 mg/kg/day to capture dose-responsive effects applicable to lower, reasonable levels that might be anticipated in human exposures at NDRA. This range was derived based on previous studies in the literature that have examined exposure to metal dust during welding activities (Anderson et al., 2007).

3. Results and discussion

3.1 Immunotoxicology

The plaque forming cell (PFC) assay and flow cytometric evaluation of lymphocytic subpopulations were assessed in this study. These two assays are recommended by the US Environmental Protection Agency (USEPA) when assessing risk of immunotoxicity

Table 1: Metal concentrations and median diameters for the dust extracts used in the mouse exposure study

	NDRA Samples			Buffalo River Sample (BRS)	
	Unit 2.2	Unit 3.1	Unit 3.2	BRS Value (µg/g)	BRS Reference Material 8704 (Provided value/range for SRM) (µg/g)
Median Diameter (µm)	4.30	2.40	3.10		
Metal Concentration (µg/g)					
As	142.48	24.89	23.19	16.64	17.000
Co	9.12	12.76	10.79	13.82	13.14 - 14.00
Cr	9.26	19.50	17.96	92.61	118.1 - 125.7
Cs	3.05	0.46	0.38	0.86	5.71 - 5.95
Cu	71.43	36.07	43.45	79.90	NP
Cd	0.34	0.46	0.65	3.90	2.65 - 3.23
Ni	17.97	29.21	27.09	40.92	39.2 - 46.6
Pb	23.52	23.04	18.26	159.47	133 - 167
Sr	182.76	125.60	106.39	51.74	NP
U	8.27	3.88	4.15	2.24	2.96 - 3.22
V	105.06	78.35	72.75	99.63	90.6 - 98.6
Th	2.18	3.15	0.49	3.83	8.91 - 9.23
B	41.19	51.50	50.04	73.19	NP
Mo	6.10	3.10	2.93	4.47	NP
Se	ND	ND	ND	ND	NP
Mn	274.52	428.12	419.21	482.70	523 - 565
Zn	68.20	92.13	86.18	323.74	393 - 423
Ba	641.75	296.86	72.34	152.69	400 -426
Ti	2411.07	3127.00	2647.99	4575.10	4370 - 4770
Fe	4949.43	9223.15	8865.79	26696.80	38700 - 40700
Al	8452.57	9745.57	6660.24	13538.97	59200 - 62800
Ca	40900.00	36020.00	100541.00	26400.00	25580 - 27240
Mg	40370.00	29620.00	45190.00	14450.00	11820 - 12180
K	22230.00	32000.00	28090.00	17730.00	19600 - 20420
Na	660.00	1130.00	1960.00	5880.00	5380 -5680
P	420.00	1280.00	1030.00	900.00	NP

Notes:
1. ND= Not Done
2. NP= Not Provided
3. SRM= Standard Reference Material

to humans. These two assays are known to be predictive of alterations in immune function (Luster et al., 1992, 1993). The standardized plaque forming cell (PFC) assay measures the ability to mount an IgM immune response to a foreign antigen, in this case, sheep red blood cells. The PFC response was dose-responsively suppressed beginning at 0.1 mg/kg/d exposure to map unit 2.2 and 1.0 mg/kg/d to exposure to map units 3.1 and

3.2 (Fig. 1). Flow cytometric studies identified dose-responsive decreases in splenic lymphocytic populations beginning at 0.1 mg/kg/d for map unit 2.2 and 1.0 mg/kg/d for map unit 3.1 (Fig. 2). No significant changes were detected with map unit 3.2 (Fig. 2). In these studies, the lowest observed adverse effect level (LOAEL) was determined to be 0.1 mg/kg/d for map unit 2.2 and 1.0 mg/kg/d for map units 3.1 and 3.2.

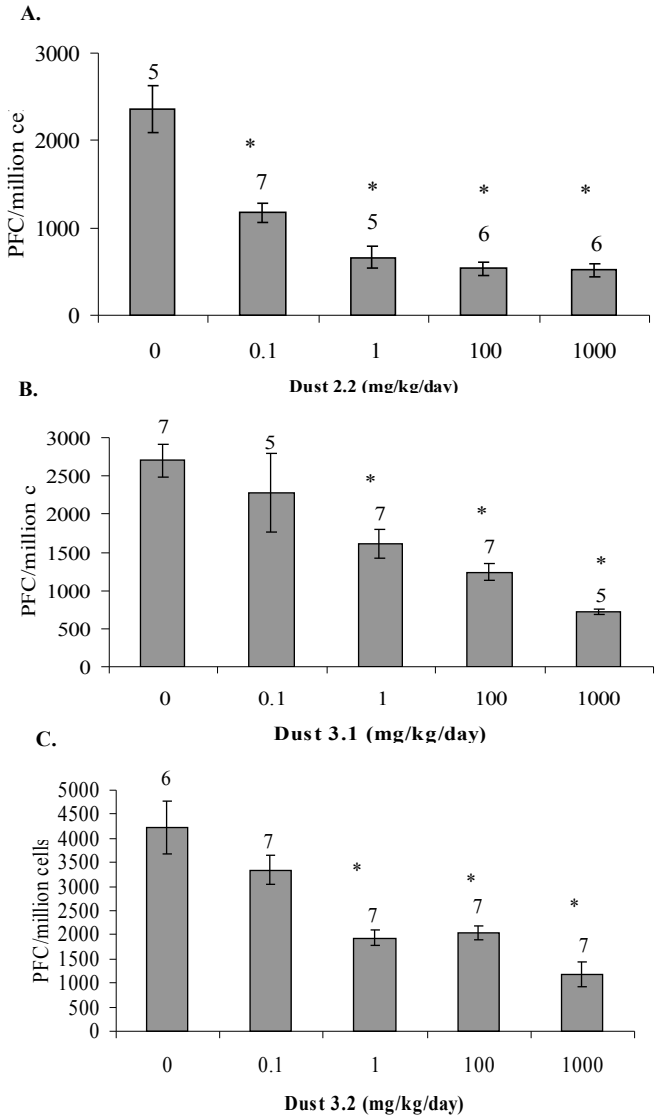

Fig. 1: Sheep red blood cell-specific-IgM antibody production in adult female B6C3F1 mice following intratracheal aspiration exposure to dust 2.2 (A), 3.1 (B), or 3.2 (C). Data are presented as mean ± SEM. Numbers above SEM bars indicate sample size. (*)Indicates significantly different from respective control ($p < 0.05$). Dust 2.2 = dust samples collected from unit 2.2. Dust 3.1 = dust samples collected from unit 3.1. Dust 3.2 = dust samples collected from unit 3.2.

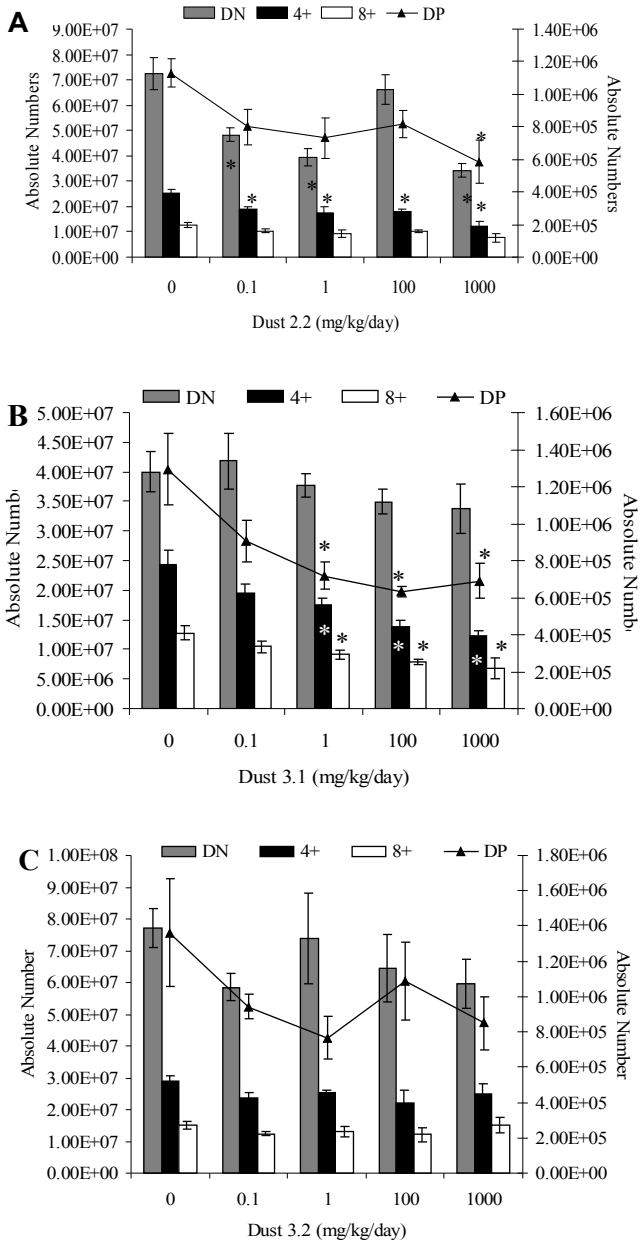

Fig. 2: Splenic T-cell populations (CD4CD8) in adult female B6C3F1 mice following intratracheal aspiration exposure to dust 2.2 (A), 3.1 (B), or 3.2 (C). Data are presented as mean ± SEM. Sample sizes for 0, 0.1, 1, 100, and 1000 mg/kg/day for dust 2.2 are 5, 7, 5, 6 and 6, respectively. Sample sizes for 0, 0.1, 1, 100, and 1000 mg/kg/day for dust 3.1 are 7, 5, 7, 7 and 5, respectively. Sample sizes for 0, 0.1, 1, 100, and 1000 mg/kg/day for dust 3.2 are 6, 7, 7, 7, and 7, respectively. (*)Indicates significantly different from respective control ($p < 0.05$). Double positive (DP; CD4+DC8+) cells are one the second axis. DN= Double negative cells (CD4-CD8-), 4+ = CD4+CD8-, 8+ =CD4-CD8+. Dust 2.2= dust samples collected from unit 2.2. Dust 3.1= dust samples collected from unit 3.1. Dust 3.2= dust samples collected from unit 3.2.

3.2 General toxicology

The most notable changes in body weight occurred at exposure levels of 100 or 1000 mg/kg/d (Fig. 3). Significant decreases in body weight occurred at the 1000 mg/kg/d exposure level for dust map units 2.2 and 3.1 (Fig. 3), while 100 and 1000 mg/kg/d exposure significantly decreased body weight following exposure to dust map unit 3.2. Significant changes in body weight suggest overt toxicity has occurred. It is important that overt toxicity is not present when defining a LOAEL. In this study, overt toxicity was not present at the LOAELs of 0.1 and 1.0 mg/kg/d established from the immunotoxicity data.

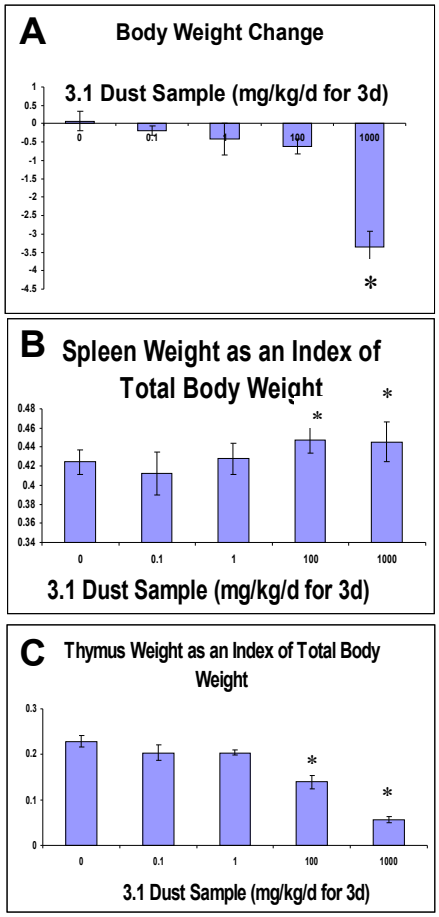

Fig. 3: Body (A), spleen (B) and thymus (C) weight in adult female B6C3F1 mice following intratracheal aspiration exposure to dust map unit 3.1 at levels of 0, 0.1, 1.0, 100 and 1000 mg/kg/d. Data are presented as mean ± SEM. (*)Indicates significantly different from respective control ($p < 0.05$). Spleen and thymus weight are represented an index to body weight (organ weight/body weight).

Thymus weight is often a sensitive target of environmental agents and in the current study, significant decreases in thymus weight were observed at 100 and 1000 mg/kg/d for dust map units 2.2 and 3.1 (Fig. 3), but only 1000 mg/kg/d for dust map unit 3.2 (data not shown). Thymus cellularity was consistent with changes in this organ weight in that the total cell counts were decreased at 100 and 1000 mg/kg/d for all map units tested.

Significant changes in liver weight (hepatic) were different for each of the map units examined in this study. For map unit 2.2, 100 mg/kg/d increased liver weight, while 1000 mg/kg/d decreased liver weight. For map unit 3.1, 1000 mg/kg/d exposure decreased liver weight (Fig. 4). Lastly, exposure to map unit 3.2 caused decreases in liver weight at 100 and 1000 mg/kg/d.

Kidney weight was unaffected following exposure to dust map units 2.2 and 3.2. However, dust exposure to map unit 3.1 caused significant increases in kidney weight at 100 and 1000 mg/kg/d (Fig. 4).

Overall, these data indicate that changes in body and organ weight occurred only in the 100 and 1000 mg/kg/d exposure groups. These changes were different for each map unit. As each map unit is a complex mixture with varying metal content, it is not surprising that alterations in organ weight also vary.

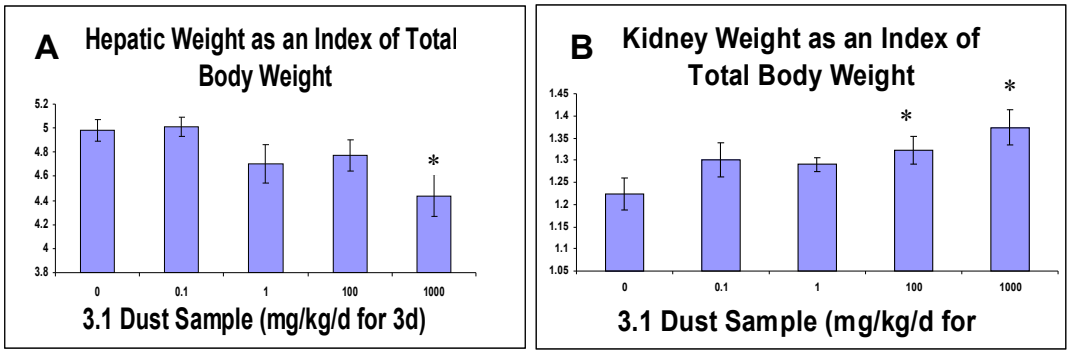

Fig. 4: Hepatic (A) and liver (B) weight in adult female B6C3F1 mice following intratracheal aspiration exposure to dust map unit 3.1 at levels of 0, 0.1, 1.0, 100 and 1000 mg/kg/d. Data are presented as mean ± SEM. (*)Indicates significantly different from respective control ($p < 0.05$). Organ weights are represented an index to body weight (organ weight/body weight).

3.3 Hematology

Peripheral white blood cell differentials were examined in the mice exposed to NDRA dust. No changes in distribution of cells were evident following exposure to dust map unit

3.2. Following exposure to map unit 2.2 and 3.1 (Fig. 5), changes were only evident at the highest exposure level, 1000 mg/kg/d. Due to minimal amounts of blood available for analysis, total white blood cell counts were not done.

3.4 Histopathology of lungs

Pulmonary histopathology was evident in mice that received dust from surface units 2.2, 3.1 or 3.2, but was restricted to the high dose groups (100 and 1000 mg/kg/d) from each site. The character of the lung lesions was similar among the different dust samples with greatest severity in the highest dose group. The principal morphologic lesion was a multifocal, centriacinar brochiolitis characterized by marked accumulation of dust-filled macrophages associated with interstitial fibrosis and a mixed inflammatory cell infiltrate of neutrophils, lymphocytes, and lesser numbers of eosinophils. Alveolar bronchiolarization and bronchiolitis obliterans were also common features of these lung lesions characteristic of pneumoconiosis.

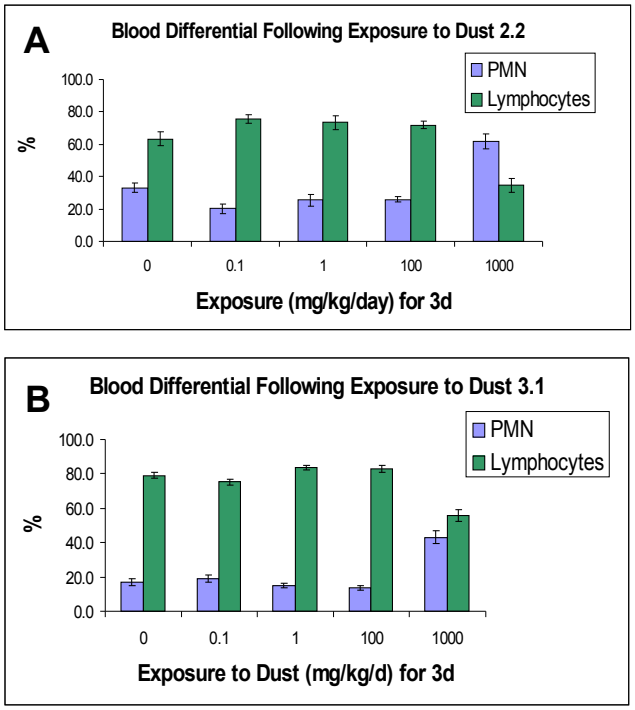

Fig. 5: Peripheral white blood cell differentials were performed on map units 2.2 (A), 3.1 (B) and 3.2 (data not shown). Significant changes were evident at the 1000 mg/kg/d exposure group only. Neutrophils (PMN) and lymphocytes are represented as these are the two primary cell population in the peripheral blood. No changes were observed following exposure to map unit 3.2 (data not shown).

4. Summary and conclusions

The level of human exposure to dust generated at the NDRA is not known. Therefore, a large dose-response range was utilized in these toxicology studies. Changes in immune function and suppression of humoral immunity were the most sensitive parameters affected by the surface units tested in this study. Immunotoxicity occurred at test exposures where no overt toxicity was indicated. The immunological parameters affected in this study are known to be predictive of increased disease susceptibility (Luster et al., 1992, 1993) and, therefore, are key to the maintenance of good health and disease resistance. The *LOAEL based on immunotoxicology parameters are 0.1 mg/kg/d for map unit 2.2 and 1.0 mg/kg/d for map units 3.1 and 3.2.* The present data indicate the need for further studies to characterize the potential risks to human health for exposure to dust from NDRA map units 2.2, 3.1, and 3.2.

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References

Anderson, S., Meade, J., Butterworth, L.F., Munson, A., 2007. The humoral immune response of mice exposed to manual metal arc stainless steel-welding fumes. Journal of Immunotoxicology 4, 15-23.

Ballatori, N., 2002. Transport of toxic metals by molecular mimicry. Environmental Health Perspectives110, Supplement 5, 689-694.

Baselt, R.C., 2004. Disposition of Toxic Drugs and Chemicals in Man, 7th ed. Biomedical Publications, Foster City.

Boezen, M., Schouten, J., Rijcken, B., Vonk, J., Gerritsen, J., van der Zee, S., et al., 1998. Peak expiratory flow variability, bronchial responsiveness, and susceptibility to ambient air pollution in adults. American Journal of Respiratory and Critical Care Medicine 158, 1848-54.

Chan, C.C., 2002. Adverse health effects of China sandstorms in Taipei, Taiwan. Environmental Protection Administration, EPA-91-FA11-03-D036.

Dockery, D.W., Pope, C.A., 1994. Acute respiratory effects of particulate air pollution. Annual Review of Public Health 15, 107-132.

Driscoll, K.E., Costa, D.L., Hatch, G., Henderson, R., Oberdorster, G., Salem, H., Schlesinger, R.B., 2000. Intratracheal instillation as an exposure technique for the

evaluation of respiratory tract toxicity: Uses and limitations. Toxicological Sciences 55, 24-35.

Goossens, D., 2011. A method for dry extracting large volumes of fine particulate matter from bulk soil samples. Air Quality Atmosphere and Health, in press.

Hiyoshi, K., Ichinose, T., Sadakane, K., Takano, H., Nishikawa, M., Mori, I., Yanagisawa, R., Yoshida, S., Kumagai, Y., Tomura, S., Shibamoto, T., 2005. Asian sand dust enhances ovalbumin-induced eosinophil recruitment in the alveoli and airway of mice. Environmental Research 99, 361-368.

Hong, Y.C., Pan, X.C., Kim, S.Y., Park, K., Park, E.J., Jin, X, Yi, S.M., Kim, Y.H., Park, C.H., Song, S., Kim, H., 2010. Asian dust storm and pulmonary function of school children in Seoul. Science of the Total Environment 408, 754-759.

Ichinose, T., Nishikawa, M., Takano, H., Sera, N., Sadakane, K., Mori, I., Yanagisawa, R., Oda, T., Tamura, H., Hiyoshi, K., Quan, H., Tomura, S., Shibamoto, T., 2005. Pulmonary toxicity induced by intratracheal instillation of Asian yellow dust (Kosa) in mice. Environmental Toxicology and Pharmacology 20, 48-56.

Ichinose, T., Sadakane, K., Takano, H., Yanagisawa, R., Nishikawa, M., Mori, I., Kawazato, H., Yasuda, A., Hiyoshi, K., Shibamoto, T., 2006. Enhancement of mite allergen-induced eosinophil infiltration in the murine airway and local cytokine/chemokine expression by Asian sand dust. Journal of Toxicology and Environmental Health A 69, 1571-1585.

Kingston, H.M., (Skip), Haswell, S.J. (eds.), 1997. Microwave Enhanced Chemistry. Chapter 3: Environmental microwave sample preparation: fundamentals, methods and applications.American Chemical Society, Washington DC., 772 pp.

Kuo, S., 1996. In: Sparks, D.L. et al. (eds.). Methods of soil analysis. Part 3: Chemical Methods. SSSA Book Series 5, Soil Science Society of America, Inc. and America Society of Agronomy, Inc., Madison, 1390 pp.

Kwon, H.J., Cho, S.H., Chun, Y., Lagarde, .F, Pershagen, G., 2002. Effects of the Asian dust events on daily mortality in Seoul, Korea. Environmental Research 90, 1-5.

Luster, M.I., Portier, C., Pait, D.G., White, K.L., Gennings, C., Munson, A.E., Rosenthal, G.J., 1992. Risk assessment in immunotoxicology. I: Sensitivity and predictability of immune tests. Fundamental and Applied Toxicology 18, 200-210.

Luster, M.I., Munson, A.E., Thomas, P.T., Holsapple, M.P., Fenters, J.D., White, K.L. Jr, Lauer, L.D., Germolec, D.R., Rosenthal, G.J., Dean, J.H., 1988. Development of a testing battery to assess chemical induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. Fundamental and Applied Toxicology 10, 2-19.

Luster, M.I., Portier, C., Pait, D.G., Rosenthal, G.J., Germolec, D.R., Corsini, E., Blaylock, B.L., Pollock, P., Kouchi, Y., Craig, W., et al., 1993. Risk assessment in immunotoxicology. II:. Relationships between immune and host resistance tests. Fundamental and Applied Toxicology 21, 71-82.

Schwartz, J., Slater, D., Larson, T.V., Pierson, W.E., Koenig, J.Q., 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. American Review of Respiratory Disease 147, 826-31.

Schwartz, J., Norris, G., Larson, T., Sheppard, L., Claiborne, C., Koenig, J., 1999. Episodes of high coarse particle concentrations are not associated with increased mortality. Environmental Health Perspectives 107, 339-342.

Yang, C.Y., Chen, Y.S., Chiu, H.F., Goggins, W.B., 2005. Effects of Asian dust storm events on daily stroke admissions in Taipei, Taiwan. Environmental Research 99, 79-84.