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Toxicology 177 (2002) 285–297

TOXICOLOGY

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Metal working fluids: sub-chronic effects on pulmonary functions in B6C3F1 mice given vitamin E deficient and sufficient diets

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Received 20 September 2001; accepted 11 April 2002

Abstract

Metal working fluids (MWFs) have been widely known to cause asthma and neoplasia of the larynx, pancreas, rectum, skin and urinary bladder (Textbook of Clinical Occupational and Environmental Medicine (1994) 814; Am. J. Ind. Med. 32 (1997) 240; Am. J. Ind. Med. 33 (1997) 282; Am. J. Ind. Med. 22 (1994) 185). Other non-neoplastic respiratory effects in industrial workers attributed to MWFs include increased rates of cough, phlegm production, wheeze, chronic bronchitis and chest tightness (Eur. J. Resir. Dis. 63(118) (1982), 79; J. Occup. Med. 24 (1982) 473; Am. J. Ind. Med. 32 (1997) 450). The epidemic and endemic nature of immune mediated lung morbidity commonly known as hypersensitivity pneumonitis in workers from several different industries using MWFs has been well documented (J. Allergy clin. Immunol. 91 (1993) 311; Chest 108 (1995) 636; MMWR45 (1996) 606; Am. J. Ind. Med. 32 (1997) 423). We studied morphological/functional and antioxidant outcomes in lungs after inhalation exposure of vitamin E deficient mice to MWF (27 mg m^{-3} 17 weeks, 5 days a week, 6 h a day). Mice were given vitamin E deficient (< 10 IU kg^{-1} vitamin E) or basal diets (50 IU kg^{-1} vitamin E) for 35 weeks. Inhalation exposure to MWF started after 18 weeks on diet. Microscopic observation of lungs from mice given vitamin E deficient or sufficient diets revealed no inflammation or morphological alteration after exposure to MWF. Mice given vitamin E deficient diet exhibited a significant decrease ($P < 0.05$) in breathing rate, peak inspiratory/expiratory flow, minute ventilation, and tidal volume compared with sufficient controls. However, no differences were found after exposure to MWF in pulmonary function, with the exception of tidal volume which also significantly decreased ($P < 0.05$). Exposure to MWF reduced vitamin E, protein thiol and ascorbate level in lungs. Exposure to MWF in combination with a vitamin E deficient diet resulted in significantly enhanced accumulation of peroxidative products compared with vitamin E deficient controls. This is the first report that describes the increase of oxidative stress in the lungs after MWF exposure.

Keywords: Metal working fluid; Oxidative stress; GSH; Vitamin E; Lung

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0300-483X/02/$ - see front matter
PII: S0300-483X(02)00188-9

1. Introduction

Metal working fluids (MWFs) are widely used in a variety of industrial operations to cool, flush, and lubricate tools and working surfaces. The National Institute of Occupational Safety and Health (NIOSH, 1998) estimates that 1.2 million workers in the United States are exposed to MWFs, while over 6 million are exposed to specific components. Soluble oil and semi-synthetic MWFs prevail in automotive manufacturing and present mixtures of lubricant oils, emulsifiers, and other performance additives. Synthetic MWFs contain no petroleum oil and consist of water dispersible and soluble ingredients. Because of the physical conditions that exist during the use of MWFs in these industries, large amounts of aerosol of the (used or in-use) oil mist and other notable contaminants like bacteria, bacterial metabolic products, fungi, and metal chips or shavings are generated.

Metal working fluids have been widely known to cause skin diseases like irritant and contact dermatitis, oil folliculitis and skin cancer (Sprince et al., 1994), In addition, previous studies in US automobile workers have also reported increased rates of laryngeal and certain digestive tract cancers in relation to MWF exposures (Bardin et al., 1997; Eisen et al., 1992, 1994; Tolbert et al., 1992; Calvert et al., 1997). Occupational exposures to semi- and synthetic MWFs have been associated with adverse pulmonary outcomes, e.g. respiratory irritation with symptoms of cough, phlegm, wheezing and chronic bronchitis, and hypersensitivity pneumonitis (Zacharisen et al., 1998; Greaves et al., 1997; Fox et al., 1999). Clinical and epidemiological findings suggested that MWFs were the second most common cause of work-related asthma in the state of Michigan from 1988 to 1994 (Rosenman et al., 1997; Kennedy et al., 1999; Robins et al., 1997).

NIOSH (1998) recommends 0.4 mg m^{-3} exposure level for aerosol MWF; however, some occupational setting have a higher level of exposure. This recommended low level of exposure is for used MWF containing many moieties not present in the unused MWF. Our preliminary studies yielded data that 27 mg m^{-3} concentration of unused MWF does not cause adverse effects in mice after long-term exposure; therefore, this was the level used to determine if biological outcomes are changed after long-term exposure.

Among pulmonary diseases, oxidative stress is associated with adult respiratory distress syndrome (Baldwin et al., 1986), silicosis (Castranova et al., 1998; Castranova and Vallyathan, 2000) and pulmonary fibrosis (Jack et al., 1996). Oxidative stress has been implicated as an important pathological factor in pulmonary airway obstruction (Schunemann et al., 1997). Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants and/or a depletion of antioxidants. Vitamin E is an important component of lung's defense against oxidant injury (Richard et al., 1990; Schunemann et al., 2001). The chronic effects of inhalation exposure to unused semi-synthetic MWF were studied in B6C3F1 mice given vitamin E deficient or sufficient diets to evaluate respiratory function, pulmonary inflammation and oxidant/antioxidant balance in lung tissue following 85 days of exposure (27 mg m^{-3} MWF for 17 weeks, 5 days a week, 6 h a day).

2. Materials and methods

Fatty acid-free human serum albumin (hSA), luminol, sodium dodecyl sulfate (SDS), L-ascorbic acid, and glutathione were purchased from Sigma Chemicals Co. (St. Louis, MO). Methanol, ethanol, hexane (HPLC grade), and water (HPLC grade) were purchased from Aldrich Chemical Co. (Milwaukee, WI). ThioGlo-1™ was obtained from Covalent Inc. (Wobum, MA). Metaphosphoric acid was purchased from Fisher Scientific Co. (Pittsburgh, PA), Unused semi-synthetic metal working fluid was purchased from Milacron Inc. (Cincinnati, OH). Basal and Vitamin E deficient mouse diets were purchased from Purina Mill (Richmond, IN).

2.1. Animals

Twenty four male B6C3F1 mice (3–4 weeks old) weighing 16–18 g were obtained from Hilltop (Scottsdale, PA). The animals were single housed,

given food and water ad libitum and were maintained at an ambient temperature of 20 ± 10 °C with a 12-h dark:12-h light cycle.

2.2. Supplementation with basal or vitamin E deficient diets

The basal diet (Basal Diet™ 5755, Test Diet, Purina Mill, Richmond, IN) is a purified, synthetic diet that provides all the essential nutrients to support maintenance, growth, gestation and lactation in laboratory mice and rats (Table 1). Vitamin E deficient diet (Purina Mill, Richmond, IN) is based on the Basal Diet™ 5755 from which α-tocopherol was removed. Basal and vitamin E deficient diets contained respectively 50 IU kg^{-1} and less than 10 IU kg^{-1} of α-tocopherol. Animals were daily supplied with fresh diets refrigerated at 4 °C.

Following 2 weeks of acclimation, mice were randomly divided into four groups. Groups 1 and 2 were placed on vitamin E deficient diet and groups 3 and 4 were given basal diet. The mice were kept on their respective diets for a total of 35 weeks.

2.3. Inhalation exposure protocol

After 18 weeks, mice (average weight of 28.0 g) were exposed to either semi-synthetic unused MWF (groups 1 and 4) or were left housed in their holding cages during exposure periods (control groups 2 and 3). A whole body inhalation exposure system with individual cages was used to expose the mice (Fig. 1). Animals were exposed for 6 h a day from Monday through Friday 17 weeks, which corresponded to 85 days of exposure.

A syringe pump (KD Scientific, model # 200, New Hope, PA) delivered a constant flow (3 ml h^{-1}) of MWF to an atomizer (TSI, model # 3076, St. Paul, MN). The atomizer used conditioned input air regulated at a pressure of 35 PSI to produce the MWF aerosol. Any large droplets that were formed during atomization fell out of the air stream and were deposited into a collection chamber by gravitational forces. The smaller respirable suspended droplets were mixed with diluent air. The mixture was introduced to animals in

Table 1
Composition of diets

Typical analysis	
Protein (%)	19.3
Fat (%)	10.0
Fiber[1] (%)	4.3
Carbohydrate (%)	60.6
Ingredients	
Casein-vitamin free (%)	21.00
Sucrose (%)	5.00
Non-nutritive fiber (Solka-floc) (%)	3.00
Corn oil (%)	5.00
Lard (%)	5.00
Dextrin (%)	43.65
DL-methionine (%)	0.15
RP vitamin mixture[2] (%)	2.00
Choline Chloride (%)	0.20
RP mineral mixture # 10[1] (%)	5.00
Total (%)	100
Chemical composition[1]	
Nutrients[2]	
Protein (%)	19.3
Fat (%)	10.0
Fiber (Crude) (%)	4.3
Carbohydrate (%)	60.6
Gross Energy (kcal g^{-1})	4.1
Minerals	
Calcium (%)	0.6
Phosphorus	0.4
Potassium (%)	0.4
Magnesium (%)	0.065
Sodium (%)	0.2
Chlorine (%)	0.2
Fluorine (ppm)	5.0
Iron (ppm)	60.0
Zinc (ppm)	20.0
Manganese (ppm)	65.0
Copper (ppm)	15.0
Cobalt (ppm)	3.2
Iodine (ppm)	0.6
Chromium (ppm)	3.0
Molybdenum (ppm)	0.8
Selenium (ppm)	0.2
Vitamins	
Thiamin hydrochloride (ppm)	20.0
Riboflavin (ppm)	20.0
Nicotinic acid (ppm)	90.0
Pyridoxine hydrochloride (ppm)	20.0
d-Calcium pantothenate (ppm)	60.0
Folic acid (ppm)	4.0
Biotin (ppm)	0.4
i-Inositol (ppm)	200.0
Vitamin, B_{12} (mcg kg^{-1})	20.0
Menadione dimethylpyrimidmol bisulfite (ppm)	20.0
Vitamin A acetate, (IU g^{-1})	22.0
Vitamin D (IU g^{-1})	2.2
dl-Alpha tocopheryl acetate (IU kg^{-1}) (sufficient diet)	50.0
dl-Alpha tocopheryl acetate (IU kg^{-1}) (deficient diet)	<10.0

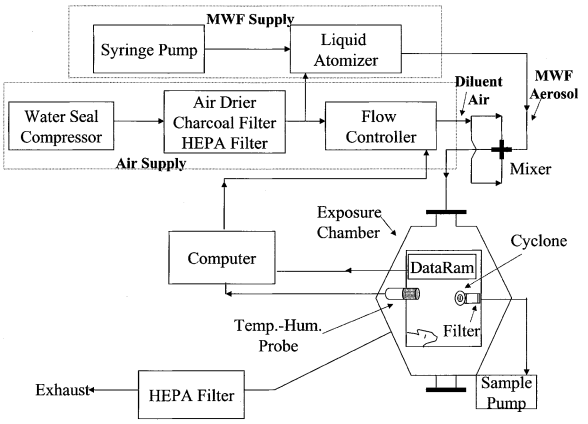

Fig. 1. Block diagram of the metal working fluids mice inhalation exposure system.

the exposure chamber. Any remaining aerosol was filtered and passed into the exposure facility exhaust system. A DataRam (MIE, model # PDR-1000 AN, Bedford, MA), placed directly above the animal cages, was used to estimate the total mass particulate concentration of aerosol in the exposure chamber. These measurements were based on the light scattering characteristics of the MWF aerosol. This DataRAM provided a real-time analog signal proportional to the aerosol concentration. The signal was acquired and digitized by a computer. A custom software proportional-derivative controller was designed and implemented to determine the amount of feedback to apply to achieve the desired concentration based on the DataRam output. A computer controlled stepper motor attached to a needle valve governed the diluent air flow. When the concentration exceeded the desired concentration, based on a control law, diluent flow was increased, and vice-versa. In this manner the level of MWF in the exposure chamber held within set limits. Chamber temperature and relative humidity were also continuously measured (Vaisala, model # HMP233, Boston, MA) and acquired by the computer to confirm acceptable levels (22–24 °C and 25–75%, respectively). Gravimetric samples were collected from the exposure chamber at 2 h intervals by continuously drawing the aerosol through a thoracic sampler, and a Gelman 37 mm PVC filter at a flow rate of 1.6 l m^{-1} according to NIOSH Method 0500. The entire system was housed in a safety hood maintained at negative pressure to ensure safe working conditions.

In order to calibrate the DataRam with respect to the gravimetric samples, test runs were performed. Average DataRam values were compared with gravimetric samples during the same time periods and a calibration factor of 2.21 was attained. The desired concentration for the exposures was 60 DataRam Units which corresponded to a MWF concentration of 27 mg m^{-3}. The actual average and standard error (S.E.) over the 85 inhalation exposures was 26.8 ± 0.87 mg m^{-3}. Particle size measurements were also performed to verify that a respirable fraction of aerosol was delivered to the mice. Results from a custom cyclone and an aerodynamic particle sizer (TSI, model # 3020, St. Paul, MN), indicated that the major peak of the mass concentration centered around 2 μm.

2.4. Pulmonary function monitoring

Tidal volume and breathing rate were measured in a double chamber plethysmograph (Fig. 2). The plethysmograph consists of a chamber that holds the mouse, a headpiece containing a nose-out seal, a plunger, and a screen for measuring flow produced by movement of the animal's thorax. A mouse was placed in the plethysmograph head forward. The headpiece was inserted into the main tube. The plunger was used to position the animal such that the animal's nose is placed into the seal, separating the flow produced by the thorax and the flow

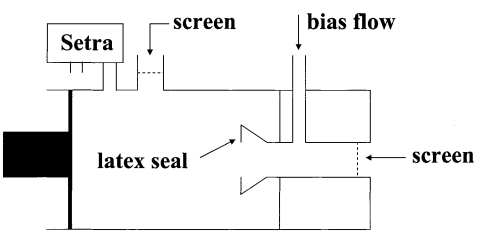

Fig. 2. Diagram of a double chamber (Plethysmograph) for measurements of tidal volume and breathing rate.

produced at the nares. A differential pressure transducer (Model 239E, Setra Systems Inc., Boxborough, MA) was used to measure thorax chamber pressure. This pressure was directly proportional to rate of change of volume of the thorax (thoracic flow). Flow calibration was performed as follows: the main body chamber was sealed; a known volume of air was injected into each chamber; the signal from the pressure transducer was acquired and integrated to obtain flow volume. A calibration constant was chosen so that the integral of this signal matched the injected gas volume. All signals were acquired with a National Instruments data acquisition card (DAQCARD 700, Austin, TX) controlled using custom LABVIEW software on a laptop computer (Dell Inspiron 7000, PII 333 MHz, Austin, TX), Three sets of data were collected for each mouse at approximately 1 min intervals. Each set consisted of 6 s of data acquired at a rate of 1000 samples per second.

Ventilatory parameters were calculated using programs written in the MATLAB computing environment. For each mouse, the data sets were split into individual inspiration/expiration flow pairs using a zero flow crossing based method. These flows were then integrated to yield inspiratory and expiratory tidal volumes. All the tidal volumes for an individual data set were then averaged yielding a tidal volume measurement for each file (three tidal volume measurements per mouse). Breathing rate was calculated by dividing the number of whole breaths in a data set by the time span over which they occurred. This measurement was cross-checked with a spectral based method that calculates the fundamental frequency of the signal. Minute ventilation was calculated by multiplying tidal volume by the breathing frequency. Expiratory time, inspiratory time, peak expiratory flow, and peak inspiratory flow were found directly by taking the average of these values calculated from the inspiratory/expiratory flow signals.

2.5. Necropsy

After 17 weeks of exposure, the mice were euthanized by inhalation using an excess quantity of carbon dioxide. The lungs were excised, and equal pieces were taken with care that the same area of mouse lung was collected for histology and biochemical assays from each mouse.

2.6. Tissue processing

The lung was processed after fixation in 10% neutral buffered formalin, following the standard operating procedures of our laboratory. Lung sections were placed on slides and stained with hematoxylin and eosin to detect inflammatory cells and determine lung histology. Using a light microscope (Olympus B × 40) with a high dry objective (40 ×), 10 random fields were examined for inflammation.

2.7. Homogenate preparation

The lungs were excised promptly after the mice were sacrificed, washed with phosphate buffered saline and samples for biochemical analysis were frozen at −80 °C until processed. The lung homogenates were prepared from frozen tissues with phosphate buffered saline (PBS, pH 7.4) using a tissue tearer (model 985-370, Biospec Products Inc., Racine, WI). Homogenates were stored at −80 °C until processed further.

2.8. HPLC assay of α-tocopherol in the lung homogenates

α-Tocopherol from the above lung homogenates was extracted using the procedure described by Lang et al. (1986). A Waters HPLC system with an HP ODS Hypersil column (5 μm, 200 × 4.6 mm) was employed to measure α-tocopherol (Waters Associates, Milford, MA). A Waters HPLC system with a 717 autosampler, Waters 600 controller pump and an 474 fluorescence detector was used. The wavelengths employed in the assay were 292 nm (excitation) and 324 nm (emission). Eluent was methyl alcohol (CH_3OH) and the flow rate was 1 ml min^{-1}. Under these conditions, the retention time for α-tocopherol was 8.5 min. The data acquired were exported from the Waters 474 de-

tector using MILLENNIUM 2000 software for further analysis (Waters Associates, Milford, MA).

2.9. Fluorescence assay of GSH and protein sulfhydryls

Total protein sylfhydryl concentration in homogenates of lung was determined using ThioGlo-1™, a maleimide reagent which produces a highly fluorescent product upon reaction with SH-groups (Shvedova et al., 2000). A standard curve was established by addition of GSH (0.04–4.0 mM) to 0.1 M phosphate buffer (pH 7.4) containing 10 μM ThioGlo-1™. GSH content was estimated from the immediate fluorescence response registered upon addition of ThioGlo-1™ to a tissue homogenate. Total protein sulfhydryls were determined from the augmentation of the fluorescence response after addition of SDS (4 mM) to the same homogenate. A spectrofluorophotometer (Shimadzu RF-5000 U, Kyoto, Japan) was employed in the assay (excitation $\lambda = 388$ nm and emission $\lambda = 500$ nm).

2.10. Colorimetric determination of ascorbate

Ascorbate concentration in lung homogenates was determined using a L-ascorbic acid determination kit (Cat. Number 409 677, Boehringer Mannheim, Germany). In brief, the sample and sample blank were each prepared by mixing 50 μl of lung homogenate with 1.0 ml of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide. An ascorbate oxidase was added to the sample blank. The samples were incubated at 37 °C for 6 min and absorbances were measured at 578 nm using spectrophotometer (Beckman DU640, Fullerton, CA). After recording this spectra, 5-methylphenazinium methosulfate (100 μl, pH 3.5) was added to the samples and the mixture was incubated for 15 min at 37 °C. The absorbances were immediately measured at $\lambda = 578$ nm. Concentration of ascorbate in samples was calculated as differences between the sample and sample blank.

A standard curve was prepared using 1.5% meta-phosphoric acid (pH 4.0) (Fisher Scientific) and L-ascorbic acid (Sigma).

2.11. Measurements of peroxidative products

Peroxidative products were determined using the procedure described by Buege and Aust (1978). The formation of thiobarbituric acid-reactive substances (TBARS) was measured in lung homogenates. Tissue homogenates (0.5 mg of protein) were mixed with 1 ml 0.67% thiobarbituric acid: 30% trichloroacetic acid (1:1). The samples were heated for 20 min at 100 °C and centrifuged for 15 min at $5000 \times g$. The absorbency of the supernatant was determined at $\lambda = 535$ nm using a spectrophotometer (Beckman DU640, Fullerton CA). A molar extinction coefficient of $\epsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ was used for calculations.

2.12. Protein assay

Measurements of protein in lung homogenates of mice were conducted using a Bio-Rad protein assay kit (cat. # 500-0006, Richmond, CA).

2.13. Statistics

Data were expressed as the mean $\pm$ S.E. of the mean for each group. One-way ANOVA was employed to compare the responses between treatments. Statistical significance was set at $P < 0.05$.

3. Results

The body weight gains for mice given basal and vitamin E deficient diets for 35 weeks were not significantly different (Table 2). After mice were on the respective diets for 18 week, mice were exposed to MWF (27 mg m^{-3}) or allowed to breathe filtered air for 17 weeks. Mice given a basal or vitamin E deficient diet and exposed to MWF gained about 8 or 12% less weight than the respective air controls (Table 2). However, this decrease in body weight with MWF was significant only in the vitamin E deficient group.

Microscopic observation of lungs from mice given vitamin E deficient or sufficient diets revealed no morphological alteration after exposure to MWF (Fig. 3). The morphological appearance

Table 2
Weights of B6C3F1 mice given basal or vitamin E deficient diet after inhalation exposure to MWF

Basal diet		Vitamin E deficient diet	
Control	MWF	Control	MWF
34.25 ± 1.35	31.27 ± 1.58	32.32 ± 0.97	28.38 ± 0.66[a]

[a]Significant from the air/vitamin E deficient group ($P<0.05$).

in the figures A, B, C, and D is that of normal lung architecture containing branch(es) of the bronchial tree, blood vessels and numerous alveoli. None of these components of the lung showed any abnormalities that were related to the vitamin E deficiency or inhalation exposure to the metal working fluid aerosol.

Mice given a vitamin E deficient diet exhibited decreases in breathing rate of 13.8%, in peak inspiratory flow of 36.2%, in peak expiratory flow of 30%, in minute ventilation of 37%, and in tidal volume of 27.5% compared with sufficient controls (Figs. 4, 5, 7 and 8).

No differences were found after exposure to MWF in breathing rate, peak inspiratory/expiratory flow, inspiratory/expiratory time, and minute ventilation in lungs of mice given vitamin E deficient or sufficient diets (Figs. 4–7). After exposure to MWF, tidal volume was decreased by 16 or 21% compared with mice breathing air and given a basal or vitamin E deficient diet, respectively (Fig. 8).

After 35 weeks of vitamin E deprivation, the level of vitamin E in the lungs was reduced by 73.5% compared with controls (Fig. 9). After MWF exposure, the level of vitamin E in the lungs of vitamin E sufficient mice was reduced by 82.2% compared with control; in mice maintained on vitamin E deficient diet, the vitamin E was at the detectable limit (<0.1 pmol mg^{-1} protein) after MWF exposure.

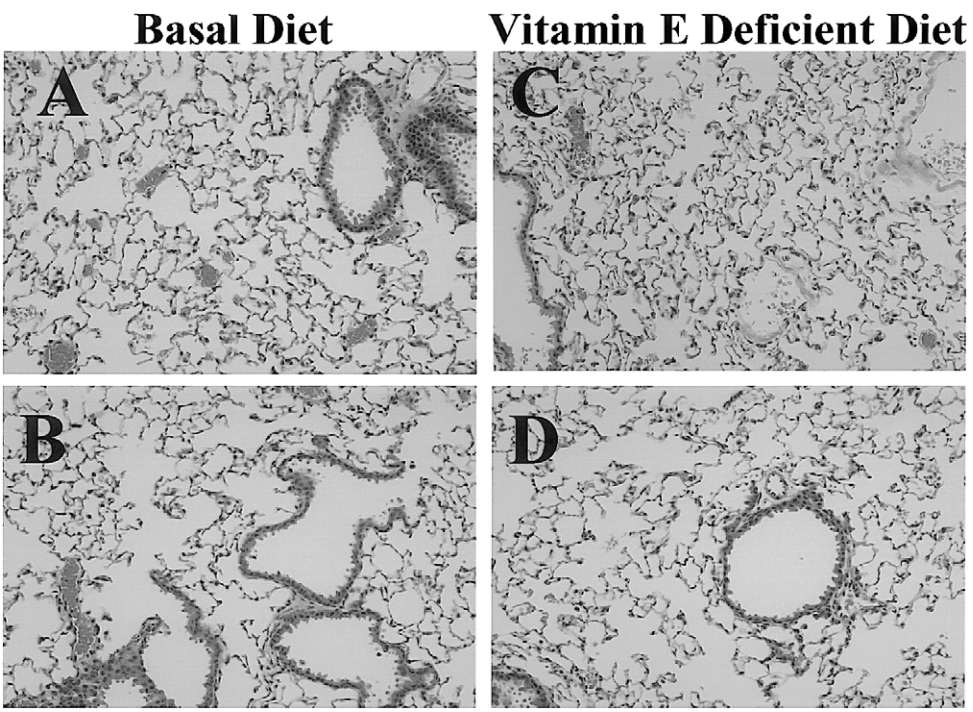

Fig. 3. Microscopic observation of lungs from mice given vitamin E deficient or sufficient diets, breathing air or after inhalation exposure to MWF. A and C—lung histology of mice given basal or vitamin E deficient diets, air controls. B and D—lung histology of mice given basal or vitamin E deficient diets after exposure to MWF.

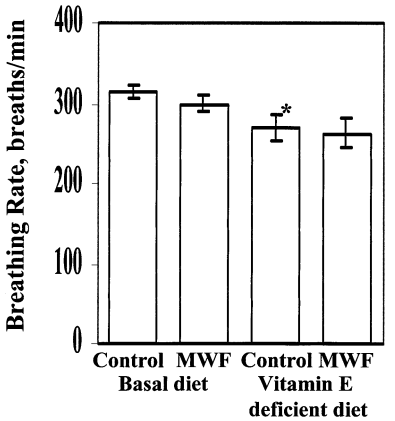

Fig. 4. Breathing rates of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or and after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal air controls.

The L-ascorbic acid and other reducing substances present in lung homogenates are able to reduce 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide in the presence of the electron carrier 5-methylphenazinium (pH 3.5). To detect ascorbate content, absorbency of a blank sample containing ascorbate oxidase was measured and data were subtracted from the absorbencies (λ_{578}) of the total reduced substances in lung homogenates. The concentration of L-ascorbic was calculated as the difference in absorbency readings between the samples and the sample blanks. The lung ascorbate level of mice maintained on vitamin E deficient diet was reduced by 57.5% compared with sufficient controls (Fig. 10). After exposure to MWF the levels of ascorbate in lungs of vitamin E sufficient or deficient mice were decreased by 68.5 or 52.2% compared with respective controls.

Addition of ThioGlo-1™ to lung homogenates produced an instantaneous increase in fluorescence due to the formation of the GSH–ThioGlo-1™ reaction products (Fig. 11). The intensity of the response did not change further unless SDS was added to the incubation system to unfold protein SH-groups at which point a slow increase of fluorescence was observed which leveled-off after approximately 45–60 min. This latter fluorescence response was due to the interaction of protein SH-groups with ThioGlo-1™ (Shvedova et al., 2000). As shown in Fig. 11, depletion of GSH and protein thiols in lungs of mice given vitamin E deficient diet was 20.5 and 20.8%, respectively, compared with sufficient controls. The exposure of mice to MWF did not alter the lung GSH levels from the respective controls. However, protein thiol levels were decreased in mice exposed to MWF and on a basal diet by 23% compared with control.

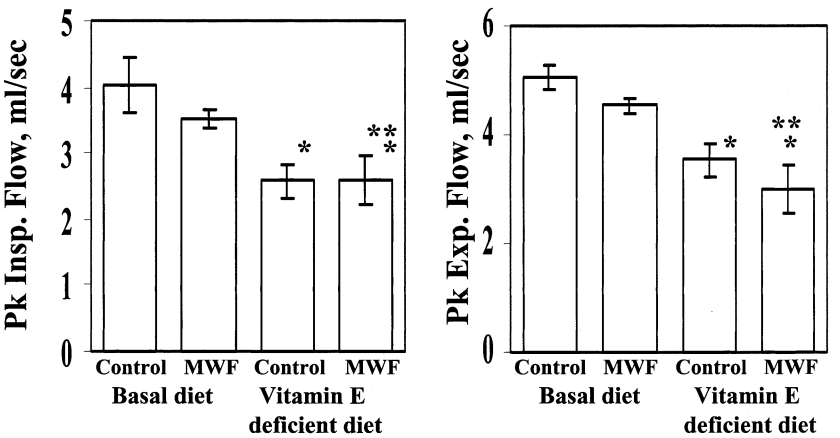

Fig. 5. Peak inspiratory/expiratory flows B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal air controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF.

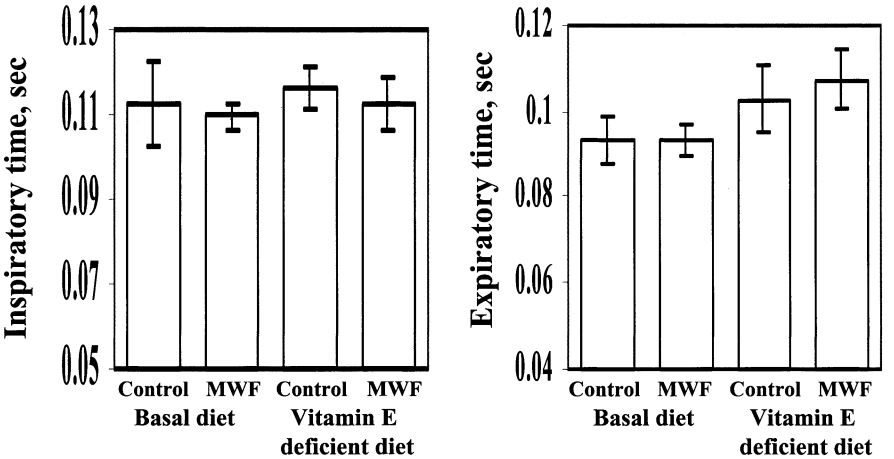

Fig. 6. Inspiratory/expiratory times of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M.

Vitamin E deficiency increased the level of oxidative TBARS products in control mice by 51%. Exposure of vitamin E deficient mice to MWF cause a further increase (21%) in lung oxidation compared with vitamin E deficient controls (Fig. 12). No accumulation of TBARS products in lung homogenates of mice given vitamin E sufficient diet was detected after MWF exposure.

4. Discussion

The results of this study indicate that subchronic inhalation exposure to MWF resulted in a substantial reduction (82%) in pulmonary levels of vitamin E in mice given a basal diet. Depletion of vitamin E levels in the lungs of mice given vitamin E deficient diet and exposed to MWF aerosol was so marked that it was at the minimum detection level of our methods. These results affirm our earlier results that MWF induced oxidative stress in the target organs and organs distant from the site of exposure (Al-Humadi et al., 2000aAl-Humadi et al., 2000b). The body weight gain of mice maintained on basal diet with or without exposure to MWFs did not vary much. However, the body weight gain of mice given vitamin E deficient diet and exposed to MWFs by inhalation was about 12% lower than vitamin E deficient mice breathing air. None of the mice showed any clinical symptoms of ill health.

Oxidant stress caused by inhalation of MWF is also supported by a decrease in lung ascorbate levels of 68.5 or 52.2% after MWF exposure in normal or vitamin E deficient mice compared with their respective air controls. In contrast, GSH levels of mice after exposure to MWFs were not

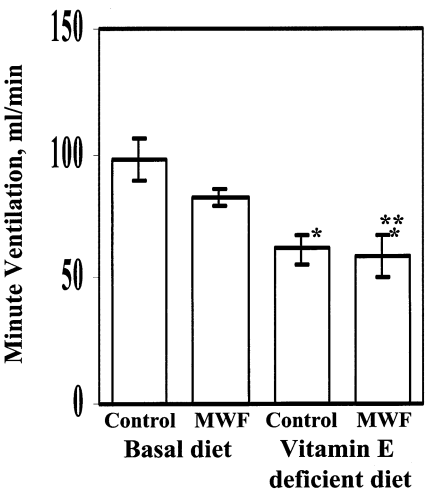

Fig. 7. Levels of minute ventilation of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal air controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF.

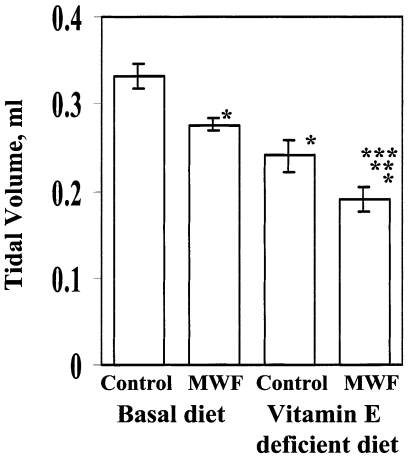

Fig. 8. Tidal volume data of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal air controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF.

lower than their respective controls. However, MWF exposure did significantly lower total thiol levels in mice on a basal diet by 23%. The metabolic products of oxidative stress expressed as TBARS did not show any differences between the groups of mice given basal diet alone and those with basal diet and exposed to MWFs. This anomaly is hard to

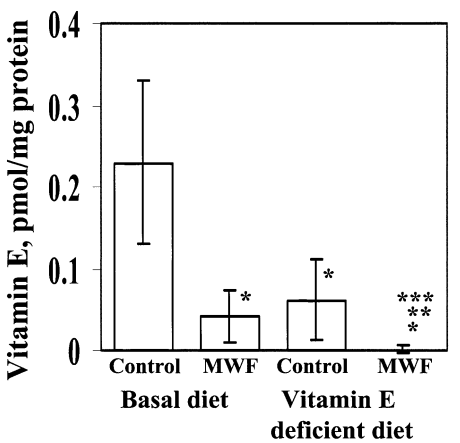

Fig. 9. Levels of vitamin E in the lungs of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M., * $P < 0.05$, vs. basal controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF, *** $P < 0.05$, vs. vitamin E deficient controls.

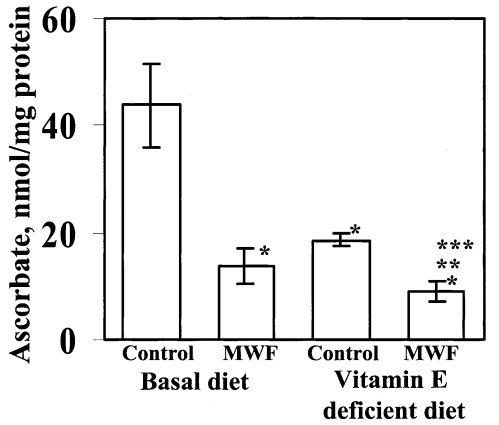

Fig. 10. Levels of ascorbate in the lungs of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$ vs. basal controls, ** $P < 0.05$, vs. mice given a basal diet, and exposed to MWF, *** $P < 0.05$, vs. vitamin E deficient controls.

explain in the light of lower lung vitamin E, ascorbate and protein thiol levels in these MWF-exposed mice. However, lungs of mice breathing air but maintained on vitamin E deficient diet showed significantly higher TBARS levels compared with basal diet controls. TBARS levels were increased further in vitamin E deficient mice exposed to MWF confirming that vitamin E deficiency induced oxidative stress in the lung and the exposure of these mice to MWFs further exaggerated the preexisting oxidative stress.

The lack of changes in the respiratory parameters such as the peak inspiratory and expiratory flows and breathing rates suggest that MWF exposure levels used in this protocol did not induce sensory or pulmonary irritation. In contrast, Detwiler-Okabayashi and Schaper (1996), Schaper and Detwiler (1991), Schaper and Detwiler-Okabayashi (1995) reported short-term inhalation exposure to MWF induced sensory and pulmonary irritation. The Detwiler and Schaper studies were designed to investigate short-term rather than sub-chronic exposures using a 'head only' exposure system. Reason for such a discrepancy in results might be the differences in conditions, levels of or duration of exposure to MWF. Indeed, it is possible that the mice became desensitized to the irritant effects of MWF after sub-chronic exposure for 85 days.

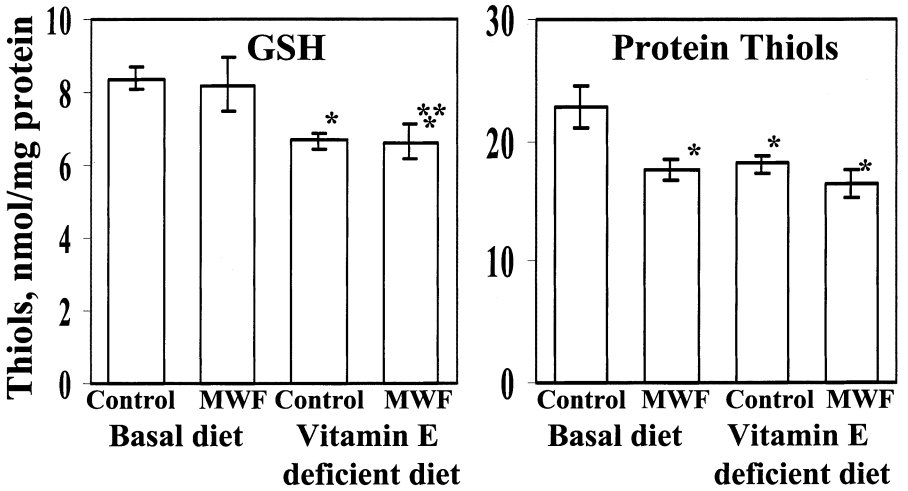

Fig. 11. Levels of GSH and protein thiols in the lungs of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF.

Finding of decrease in tidal volume in our study may suggest that the exposure to MWFs may induce restrictive effects on the respiratory system. Earlier inhalation studies (Gordon and Galdanes, 1999; Thorne and DcKoster, 1996) with MWFs using guinea pigs reported lung inflammatory changes. The mice in our study did not show any inflammatory or any other structural changes in the lung. These differences probably may be due to the fact that we used unused semisynthetic MWF, a shorter duration of exposure, a lower lung burden of MWF, and a different species of animal model. Interestingly, the vitamin E deficiency did not play any role in the induction of morphologic changes in the mice exposed to MWF in our study. Similarly, Uejima et al. (1995) also observed no changes in morphological appearance of lungs of weaning rats given a vitamin E deficient diet for 5 weeks.

One should note that the results presented here were obtained using unused MWF. In reality, the workers are exposed to aerosols of a highly complex mixture of MWF that may have been further contaminated with oils from other operations and numerous biological agents and their degradative products including sensitizing agents and endotoxins (Laitinen et al., 1999), Considering reports of hypersensitivity pneumonitis epidemics and endemics in industrial workers using metal working fluids, detailed systematic inhalation studies of 'in use' (used) MWFs is warranted.

In summary, exposure of mice to 27 mg m^{-3} MWF for 85 days did not cause substantial changes in pulmonary function, inflammation, or lung structure. However, MWF exposure did result in oxidative stress as evidenced by a decrease

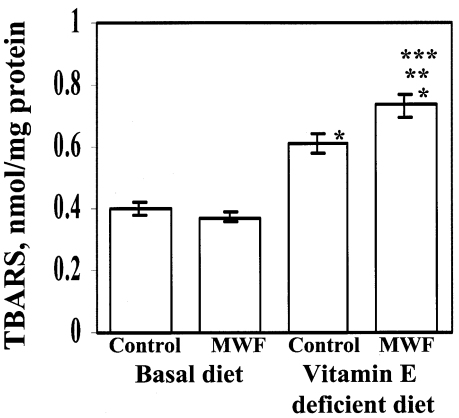

Fig. 12. Levels of TBARS in the lungs of B6C3F1 mice given vitamin E deficient or basal diets, breathing air or after inhalation exposure to MWF. Values are means ± S.E.M. * $P < 0.05$, vs. basal controls, ** $P < 0.05$, vs. mice given a basal diet and exposed to MWF, *** $P < 0.05$, vs. vitamin E deficient controls.

in lung vitamin E, ascorbate, and total thiol levels. This MWF-induced reduction in antioxidants did not result in enhanced lipid peroxidation in lungs of MWF-exposed mice unless they were also on a vitamin E deficient diet.

Acknowledgements

We are extremely grateful to Charlotte Smith for her assistance with some sample measurements. We would also like to thank Dean Newcomer and Patsy Willard for their processing of histology slides.

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