

Oxidative stress risk analysis for exposure to diesel exhaust particle-induced reactive oxygen species

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Abstract

We constructed a probabilistic risk-based framework to assess the human oxidative stress (OS) risk from diesel exhaust particle (DEP)-induced reactive oxygen species. A human respiratory tract model was used to estimate DEP concentration and cumulative doses in lung regions for three occupational groupings (*driver*, *homeworker* and *student*) in northern, central, and southern Taiwan. Dose–response profiles were reconstructed in terms of the specific ratio of the reduced and oxidized forms of glutathione (GSH/GSSG ratio) for the human macrophage cell (THP-1) and human bronchial epithelial cell (BEAS-2B). The highest estimated median daily cumulative dose of DEP with 95% CI was for *driver* in northern Taiwan (DEP_{2.5}: 0.716 (0.443–1.197) mg and DEP_{0.18}: 0.584 (0.417–0.822) mg), significantly higher than that of the other settings. The *driver* in northern Taiwan setting had the highest cumulative dose–response calculated over a 2-year exposure period: 0.57 (0.41–0.76) and 0.70 (0.53–0.87) for DEP_{2.5} and 0.40 (0.25–0.70) and 0.47 (0.34–0.80) for DEP_{0.18}, respectively, in THP-1 and BEAS-2B cells. Our results implicate that potential risks of OS from above-critical exposure to DEP_{2.5} in all settings are found, whereas from DEP_{0.18} in the *driver* in northern Taiwan setting is also alarming.

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1. Introduction

Epidemiological studies show a strong correlation between ambient particulate matter (PM) concentrations and adverse health (Donaldson et al., 1998; Cho et al., 2005; Nel, 2005; Reliene et al., 2005; Chen et al., 2006; Tovalin et al., 2006). Chio et al. (2004) identified that the vehicle emissions as the largest source of airborne PM in

Taiwan, and evidence also shows that diesel vehicle emissions contribute more to the ambient PM load than gasoline vehicle emissions (Gertler, 2005). Furthermore, diesel vehicle emissions are the major source of ultrafine particulate matter (PM of diameter <0.1 μm) (Lin et al., 2005), which is more readily respirable than larger diameter particulate. Knaapen et al. (2004) found that inhalation of diesel exhaust particle (DEP) can induce epithelial cells in the lung to generate cell-damaging intracellular reactive oxygen species (ROS), and the USEPA (2002) has listed DEP as an atmospheric toxin due to the cancer and other disease effects associated with whole diesel exhaust exposure.

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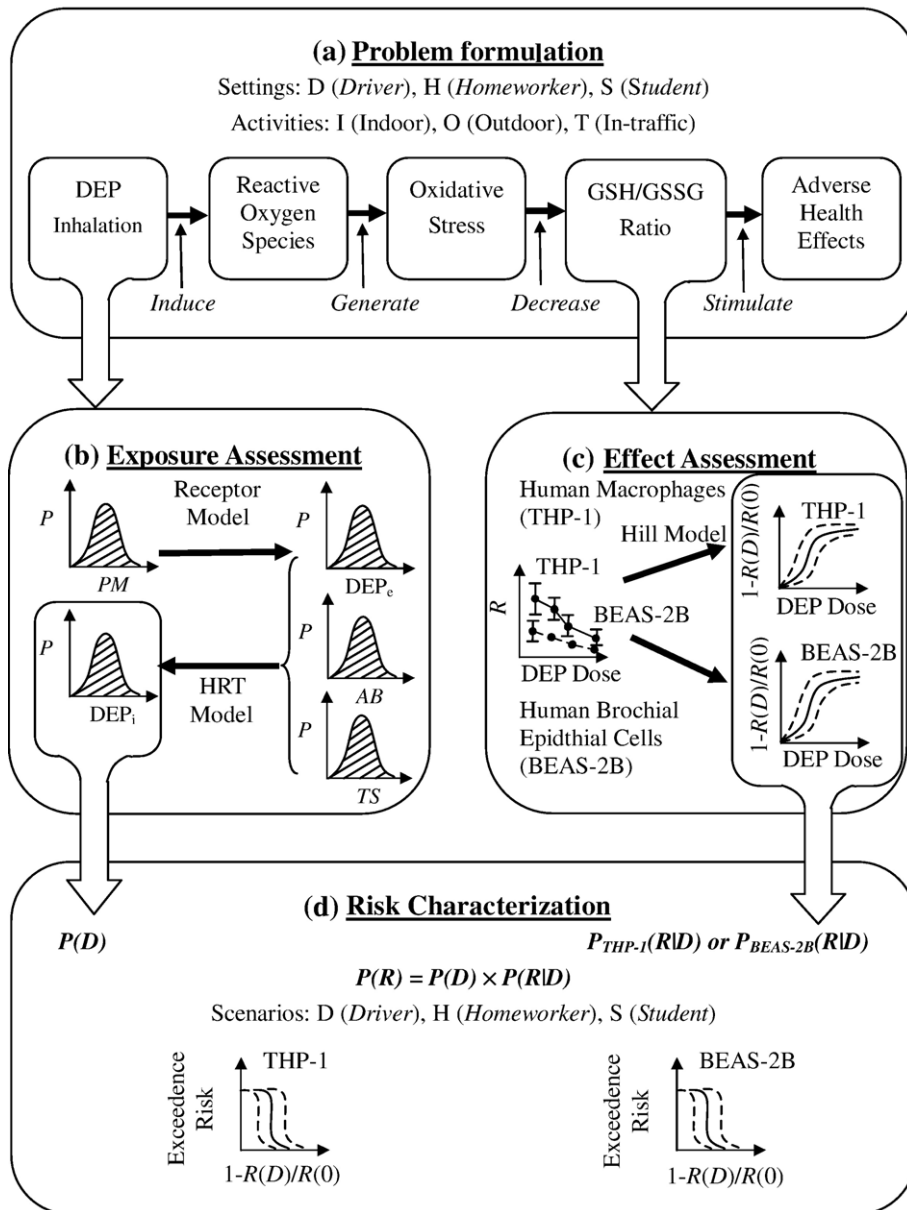


Fig. 1. Schematic diagram showing the proposed probabilistic assessment framework for oxidative stress risk for human exposed to diesel exhaust particle-induced reactive oxygen species.

ROS are broadly defined as oxygen-containing chemical species with reactive chemical properties, and include free radicals, superoxide ($O_2^{\bullet-}$), and hydroxyl radicals (HO^{\bullet}) (Pelicano et al., 2004). Hung and Wang (2001) measured H_2O_2 concentration as a surrogate for ROS concentration in Taipei and found it ranged from 0.016 to 0.146 and 0.026 to 0.592 $nM m^{-3}$ in fine ($PM_{2.5}$, PM of diameter $<2.5 \mu m$) and ultrafine PM, respectively. Kao and Wang (2002) also indicated that the mean ROS concentrations in PM_1 (PM of diameter

$<1.0 \mu m$) and $PM_{2.5}$ from incense smoke were 15.6 ± 1.0 and $13.5 \pm 1.3 \text{ nmol } H_2O_2 \text{ mg}^{-1}$ of particle, respectively. ROS are also constantly generated in the cell via a variety of pathways, including enzyme-catalyzed and non-enzyme-catalyzed reactions (Pelicano et al., 2004), and can damage cellular proteins, lipid, membranes, and DNA (Iwai et al., 2000; Nel, 2005; Reliene et al., 2005; Tovalin et al., 2006). Ordinarily ROS are cleared from the cell by the action of antioxidants such as glutathione (GSH). However, Nel (2005) reported that the generation

Table 1
Published data for PM_{2.5} and PM_{0.18} in Taiwan

Activity/regions	Measured PM _{0.18} ($\mu\text{g m}^{-3}$)	Estimated PM _{0.18} ($\mu\text{g m}^{-3}$) ^a	Measured PM _{2.5} ($\mu\text{g m}^{-3}$)	References
Indoors ^b	NA ^c	6±3	39±20	Li and Lin (2003)
Northern Taiwan				
Taipei, underpass ^d (n=5)	117±14	NA	223±31	Hung and Wang (2001)
Taipei, urban ^c	NA	9±2	51±8	Li and Lin (2002)
Central Taiwan				
Taichung, roadside ^d (n=9)	42±25	NA	93±53	Chang et al. (2001)
Taichung, urban ^c	6±3	NA	65±40	Chio (2005)
Southern Taiwan				
Tainan, urban ^c (n=59)	NA	NA	73±38	Tsai and Kuo (2005)
Kaohsiung, urban ^c (n=20)	NA	10±4	NA	Tsai et al. (2005)
Pingtung, roadside ^d	31±19	NA	140±41	Lin et al. (2005)
Pingtung, urban ^c (n=21)	NA	NA	72±21	Chen et al. (2004)

^a Estimated PM_{0.18} concentrations are calculated by Max (0.20×PM₁, 0.15×PM_{2.5}). This empirical equation is adopted from Chio et al. (2006).

^b Assumed that PM_{2.5} and PM_{0.18} measured indoors are representative of northern, central and southern Taiwan.

^c NA: Data not available.

^d PM measured at underpass and roadside is classified as in-traffic.

^e PM measured in urban area is classified as outdoor.

of ROS consequent to inhalation of PM particularly that derived from combustion of fossil fuel was a likely cause of inflammation in the lung, while Hiura et al. (1999) have demonstrated that DEP-induced ROS can cause cell apoptosis. Finkel and Holbrook (2000) indicated that the balance between ROS production and antioxidant defenses determines the degree of oxidative stress (OS), and Kelly (2000) reported that OS is instrumental in the aetiology of numerous human diseases (e.g. coronary heart disease, myocardial ischemia, cystic fibrosis, adult respiratory disease syndrome, and asthma).

Gilmour et al. (2004) studied that the inflammatory and cytokine responses in plasma caused from the PM-induced ROS determining by Trolox equivalent antioxidant capacity (TEAC) assay. Chen et al. (2006) described a positive correlation between PM₁₀ concentrations and the cumene-hydroperoxide equivalent concentration (CEC) in that the CEC represented the ROS level generated by cumene-hydroperoxide. Furthermore, Kuo et al. (1998) indicated that ROS caused

the inhibition of gap junctional intercellular communication (GJIC) and intercellular glutathione (GSH) content, the major small molecular weight cellular and epithelial surface thiol antioxidant. Nel et al. (2006) posited that the ratio of GSH to its oxidized form, glutathione disulphide (GSSG), should be a suitable indicator to describe several tiers of OS level including normal response, antioxidant defense, inflammation, and cytotoxicity. In support, Yeh et al. (2005) observed that there were significant decreases in the levels of GSH, GSSG, and the ratio of GSH/GSSG in the blood of patients with breast cancer compared to those of a control group ($p < 0.05$), yet a non-significant decrease in the vitamin E level. Chang et al. (2005) also reported an increase in OS and ROS production and a decrease in GSH/GSSG ratio in BEAS-2B cells on exposure to cooking oil fumes. OS is evident in the cell in increased conversion of GSH to GSSG (Donaldson et al., 1998), and hence the GSH/GSSG ratio in the cell is a good indicator of the level of OS (Halliwell and Gutteridge, 1999; Schafer and Buettner, 2001). Li et al. (2002) employed this ratio in determining level of injury suffered by human macrophage (THP-1) and human bronchial epithelial (BEAS-2B) cells exposed to DEP.

DEP cannot be measured directly in the atmospheric environment because it is only one of many sources in ambient aerosol. A receptor model, however, allows the source apportionment of an ambient mixture of pollutants to the contributing pollution sources, and can be used in this instance to quantify the DEP concentration. Receptor models include the Bayesian model (Chan et al., 1996), the chemical mass balance (CMB) model (Watson et al., 1991), the positive matrix factorization (PMF) model (Paatero and Tapper, 1994; Paatero, 1997), and other statistical methods (Henry, 1991). In this research we also employed the human respiratory tract (HRT) model (ICRP, 1994) to estimate the internal DEP concentration or dose in human lung regions. Chen et al. (2005) reported that the haematological markers of increased cardiovascular disease risks were associated with long driving time (10 h/day and 26 days/month) for taxi drivers in Taipei. In their opinion described in the discussion section, they indicated that the drivers exposed to vehicle exhaust may be one of the reasons to support their results. Therefore, we considered that the *driver* setting was the noteworthy subgroup in Taiwan for our study, and chose the *homeworker* and *student* settings as compared subgroups (lesser exposure time in traffic activity) in the same time.

We reanalyzed the published data for indoor, outdoor and in-traffic particulate matter from different regions in Taiwan, incorporated the HRT model into dose–

Table 2

The estimated volume of air breathed for *driver*, *homeworker*, and *student* at indoor, outdoor, and in-traffic activities

Activity	AB ₁₇₊ ^a	AB _{5–15} ^a	Daily time-spent-specific air breathed (m ³ d ⁻¹)		
	(m ³ h ⁻¹)	(m ³ h ⁻¹)	driver ^b	homeworker ^c	student ^d
Sleep	0.385 (0.092) ^e	0.312 (0.073)	7.37 (0.96)	13.80 (1.71)	10.03 (2.36)
Indoor	1.070 (0.156)	0.753 (0.229)			
Outdoor	1.105 (0.148)	0.937 (0.483)	2.21 (0.30)	4.42 (0.59)	3.74 (1.96)
In-traffic	1.105 (0.148)	0.937 (0.483)	11.06 (1.48)	2.21 (0.30)	1.87 (0.96)
Total air breathed			20.64 (1.79)	20.43 (1.83)	15.64 (3.21)

^a Data adapted from Table B.16A in ICRP66 report (ICRP, 1994).

^b The *driver* setting spent 12 h indoor (8 h in bed, 4 h at home), 10 h in-traffic, and 2 h outdoor (walking and other activity). Chen et al. (2005) indicated that the driving time of taxi and lorry drivers in Taipei was about 10 h per day by questionnaire survey.

^c The *homeworker* setting spent 18 h indoor (8 h in bed, 10 h at home), 2 h in-traffic, and 4 h outdoor (shopping and other activity). The *homeworker* mainly referred to housewife, but could equally apply to any home-based worker.

^d The *student* setting spent 18 h indoor (8 h in bed, 6 h in classroom, and 4 h at home), 2 h in-traffic, and 4 h outdoor (sports and other activity).

^e Mean (SD).

response profiles to assess the risk estimates, and performed a Monte Carlo (MC) simulation to evaluate the uncertainties resulting from the assessments of exposure and dose–response in the risk estimates. The purposes of this study were (1) to quantify the effective or internal DEP exposure in actual atmospheric environments and (2) to assess probabilistically the DEP-induced OS risk in selected occupational settings.

2. Materials and methods

Our probabilistic risk assessment (PRA) framework is divided into four parts (Fig. 1) and will be described in the subsequent sections.

2.1. Problem formulation: data reanalysis

Studied designs of our study include the northern, central, and southern Taiwan regions, three selected settings (*driver*, *homeworker* and *student*) and three activities (indoor, outdoor and in-traffic) are taken into account for exposure assessment (Fig. 1a). The excellent available published data for PM_{2.5} and PM_{0.18} in Taiwan (Chang et al., 2001; Hung and Wang, 2001; Li and Lin, 2002, 2003; Chen et al., 2004; Chio, 2005; Lin et al., 2005; Tsai and Kuo, 2005; Tsai et al., 2005) was divided into several subgroups representing (1) different regions in Taiwan (northern, central, and southern) and (2) different activities (indoor, outdoor and in-traffic) within those regions (see Table 1 for complete data sources).

2.2. Exposure assessment

In order to quantify the DEP dose, it is necessary to determine the percentage of DEP in vehicle-emitted

particulate and the vehicle contribution to the ambient PM_{2.5} prior to estimating DEP_{2.5} concentration from atmospheric environment (Fig. 1b). Chio (2005) estimated the percentage of vehicle contribution to ambient PM_{2.5} as 34.1–84.8% using a CMB model, while the percentage of DEP in vehicle-emitted particulate has been estimated at between 26 and 69%, based on calculations using Bayesian (Chio, 2005), CMB (Gertler, 2005) and PMF (Zhao and Hopke, 2004) models. Furthermore, Lin et al. (2005) found that the major fraction in DEP was ultrafine, and ranged from 41.5 to 45.9% of the total. We are able to estimate the contribution of DEP_{0.18} to PM_{0.18} based on that finding. DEP contributory ratios were estimated in this study using the source apportionment technologies (Bayesian, CMB, and PMF), and we employed Crystal Ball® (Version 2000.2, Decisioneering, Inc., Denver, Colorado, USA) to perform the MC simulation, with simulation frequencies of 10,000 iterations to ensure the variability of results. A lognormal probability distribution was used to define the possible variables.

We apply the HRT model (ICRP, 1994) to estimate the internal exposure doses in different lung regions (Fig. 1b). The model divides the respiratory tract into five major compartments: (i) the nasal passage (ET₁), comprising the anterior nose and posterior nasal passages; (ii) the pharynx (ET₂), comprising larynx and mouth; (iii) the bronchial region (BB), comprising the airway from the trachea, main bronchi, and intrapulmonary bronchi; (iv) the bronchiolar region (bb), comprising bronchioles and terminal bronchioles; and (v) the alveolar-interstitial region (AI), comprising the airway from respiratory bronchioli through alveolar sacs (ICRP, 1994; Liao et al., 2003, 2006). Based on the principle of mass balance, the HRT model, varying with

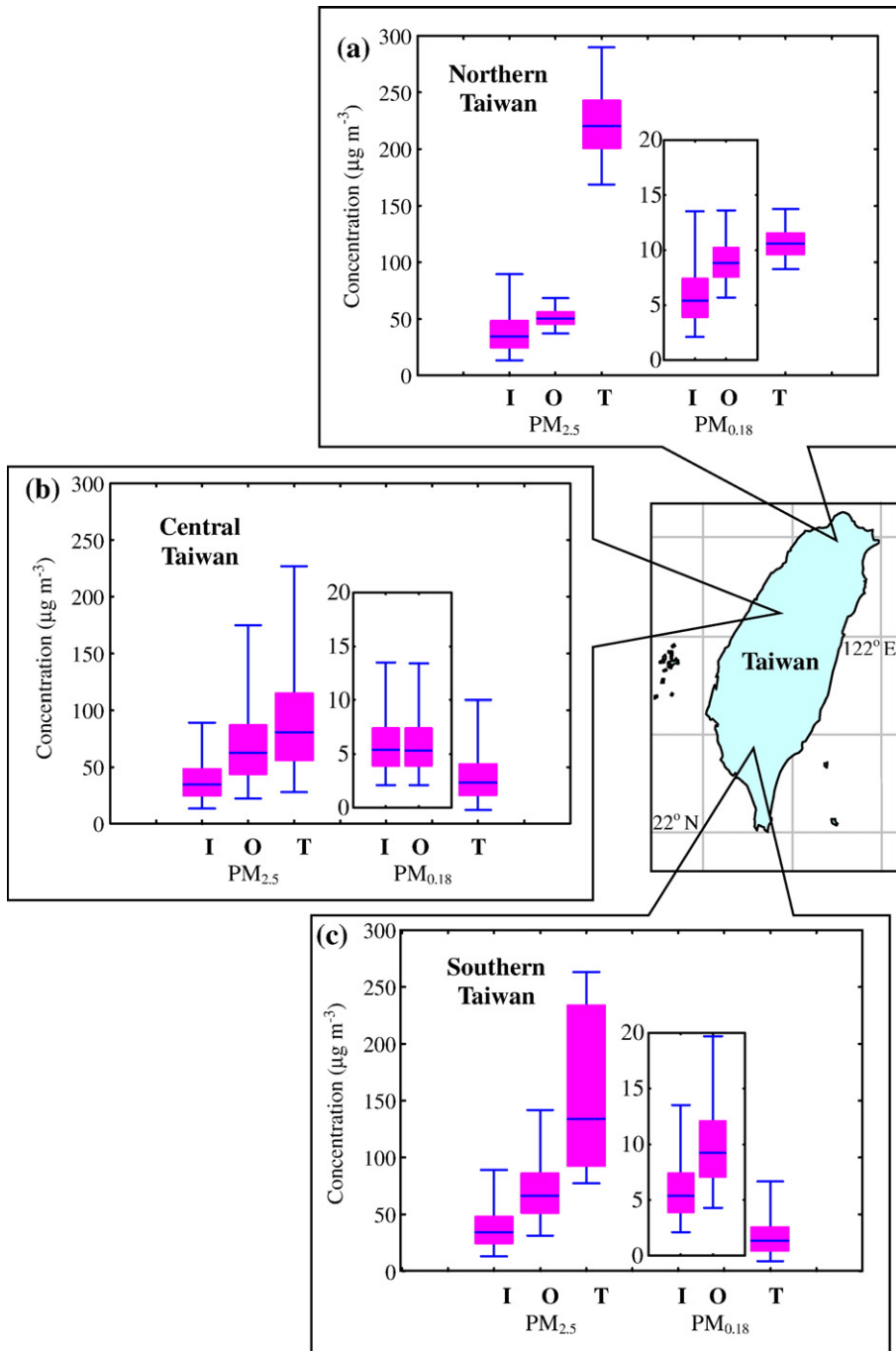


Fig. 2. Distribution of $\text{PM}_{2.5}$ and $\text{PM}_{0.18}$ mass concentration estimated during indoor (I), outdoor (O), and in-traffic (T) activities in (a) northern, (b) central, and (c) southern Taiwan using 10,000 Monte Carlo simulations.

particle size ranges of $\text{PM}_{2.5}$ and $\text{PM}_{0.18}$ and equilibrium time to each regional compartment and represented by a linear dynamic equation (Liao et al., 2003, 2006), was used to estimate DEP concentration in lung regions. We solved the linear dynamic equation to obtain the mass

lung/indoor (L/I) ratio based on an equilibrium state in each compartment, and the specific fractional deposition (d_F) for $\text{PM}_{2.5}$ and $\text{PM}_{0.18}$ in each compartment was then estimated, with a focus on the lung tissue of the lower respiratory tract (BB, bb and AI).

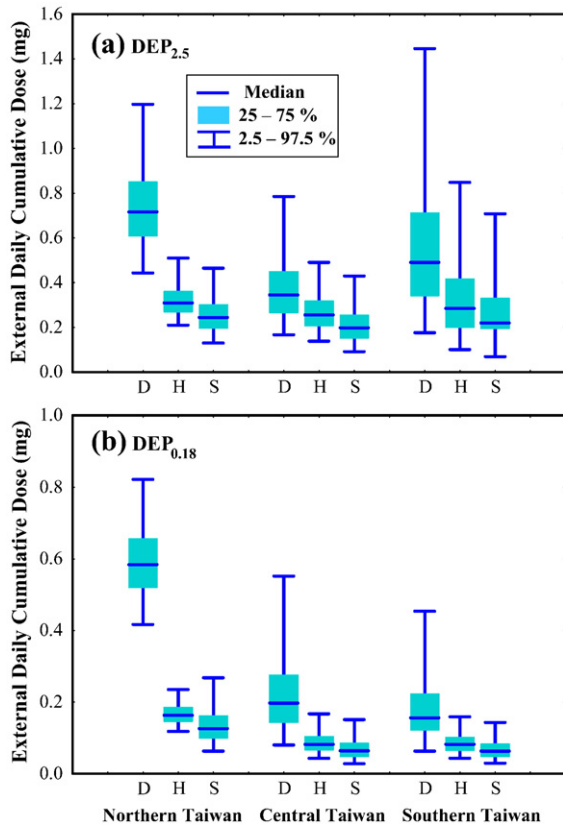


Fig. 3. Daily cumulative dose exposure to (a) DEP_{2.5} and (b) DEP_{0.18} for driver (D), homemaker (H), and student (S) in different Taiwan regions.

We converted the data into a daily cumulative dose, the product of exposure to DEP concentration (C_{DEP}), volume of air breathed (AB), and time spent (TS) of each activity.

We assume that the *driver* setting will spent half time indoors in one day, and while the *homeworker* and *student* settings spent 75% of the time indoors (see Table 2 for complete activity breakdown). The activity profiles placed *driver* indoors 50% of the time and allowance was made for an interchange ratio (IR , ratio of alveolar ventilation to blood flow rate: $230/290=0.7931$) between air breathed and blood interfaces (Ramsey and Anderson, 1984) and for the clearance effect in the lung tissue while DEP deposited on BB, bb and AI regions. A 2-year duration was adopted as an appropriate exposure time based on the half-time of particulate removed from AI to ET_2 (ICRP, 1994). The total cumulative dose in lung tissue was calculated as the sum of the doses in individual compartments multiplied by the exposure time (T). The following equation could describe clearly the algorithm of the total internal cumulative DEP dose (D):

$$D = C_{DEP} \times AB \times TS \times d_F \times IR \times T. \tag{1}$$

2.3. Effect assessment

We constructed a dose–response profile by fitting an empirical three parameters Hill equation model to the published data (Li et al., 2002) on DEP dose–GSH/GSSG ratio in human macrophages (THP-1) and human bronchial epithelial cells (BEAS-2B) (Fig. 1c):

$$R = 1 - \frac{R(D)}{R(0)} = \frac{R_{max} \times D^n}{K_{0.5}^n \times D^n}, \tag{2}$$

where R is the measured response (%), $R(D)$ is the GSH/GSSG ratio response for a specific DEP dose, R

Table 3
Fractional deposition in respiratory tract regions for DEP_{0.18} and DEP_{2.5}

	$\lambda_{d_i}^a$ (s ⁻¹)	$\lambda_{s_i}^a$ (s ⁻¹)	$\lambda_{im_i}^a$ (s ⁻¹)	ϵ_i^a (-)	Q^b (m ³ d ⁻¹)	V_i^a (cm ³)	L/I ratio ^c (-)	$d_{F_i}^d$ (-)
DEP _{0.18}								
BB	1.40E-4	1.80E-4	4.90E-3	4.21E-9	2.04E+1	9.46E+1	5.98E-1	1.25E-3
bb	1.68E-3	7.42E-3	5.89E-2	3.61E-6	2.04E+1	5.10E+2	3.98E-1	5.85E-2
AI	6.34E-3	1.48E-2	8.24E-2	2.43E-4	2.04E+1	1.58E+3	2.40E-1	1.66E-1
							Total d_{F_i} =	2.26E-1
DEP _{2.5}								
BB	7.99E-5	1.04E-2	2.45E-2	4.22E-9	2.04E+1	9.46E+1	4.53E-1	6.35E-3
bb	9.59E-4	1.24E-1	2.94E-1	3.62E-6	2.04E+1	5.10E+2	1.86E-1	1.68E-1
AI	2.01E-3	2.49E-1	3.53E-1	2.45E-4	2.04E+1	1.58E+3	3.70E-2	1.50E-1
							Total d_{F_i} =	3.24E-1

^aAdapted from Liao et al. (2006) in that λ_{d_i} , λ_{s_i} , and λ_{im_i} represent the turbulent diffusive deposition rate, gravitational settling rate, and inertial impaction rate, respectively, and ϵ_i is the interception deposition efficiency in compartment i .

^b Q is the daily volume of air breathed (AB).

^cL/I ratio ($=C_i/C_l$) is estimated from HRT model.

^d $d_{F_i} = \frac{C_i}{C_l} \left[(\lambda_{d_i} + \lambda_{s_i} + \lambda_{im_i}) \frac{V_i}{Q} + \epsilon_i \right]$ (Liao et al., 2006).

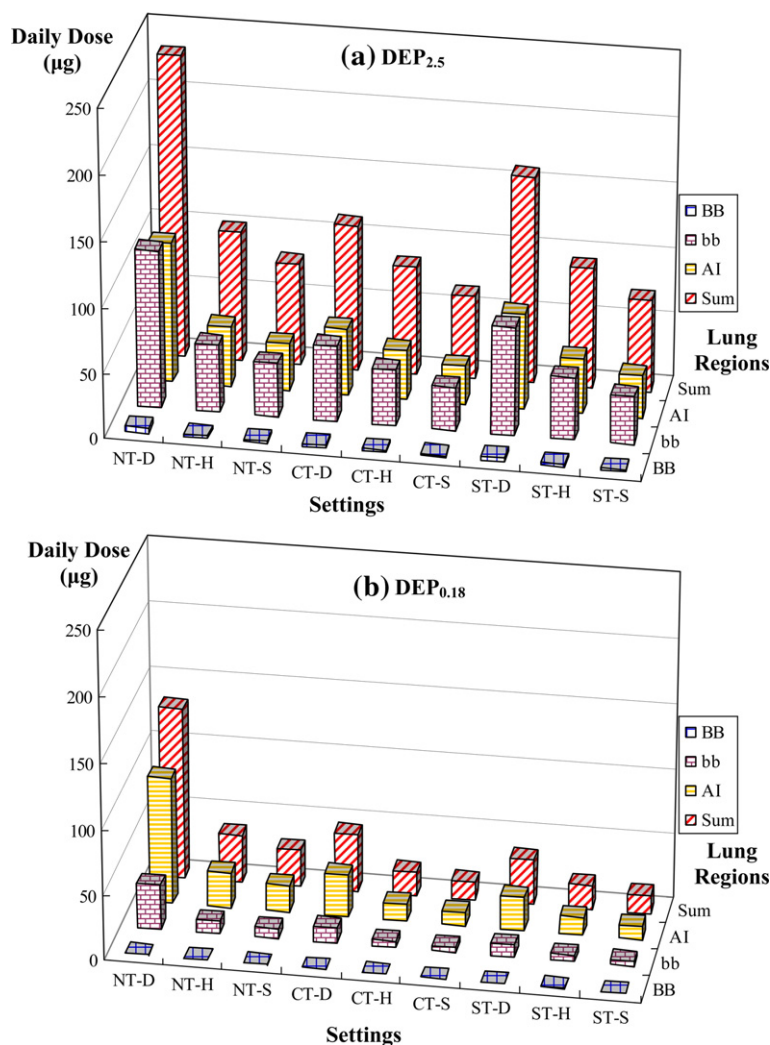


Fig. 4. Daily (a) DEP_{2.5} and (b) DEP_{0.18} dose (µg) distributed in different lung regions at steady-state condition (BB: the bronchial region, bb: the bronchiolar region, AI: alveolar-interstitial region, Sum: BB+bb+AI).

(0) is the GSH/GSSG ratio response when the dose equal to zero, $K_{0.5}$ is the DEP cumulative dose yielding half the maximal response of R_{\max} (%), D is the DEP cumulative dose in the lung (mg), and the exponent n is a fitted average value, the Hill coefficient, a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity. We used TableCurve 2D (Version 5, AISN Software Inc., Mapleton, OR, USA) to optimize the dose–response profile with significance at $p < 0.05$. We converted the DEP concentration in $\mu\text{g ml}^{-1}$ to the cumulative dose in mg under the assumptions that DEP is carried in the blood and that the total volume of blood is nearly 5.5 L (Fox, 2004).

We treated the $K_{0.5}$ value in Eq. (2) probabilistically. The cumulative distribution function (CDF) of the

predicted response function for a given DEP dose is expressed as a conditional CDF:

$$P(R|D) = \Phi\left(\frac{R_{\max} \times D^n}{K_{0.5}^n \times D^n}\right), \quad (3)$$

where $\Phi(\bullet)$ is the cumulative standard normal distribution.

Li et al. (2002) also reported that the induction of cellular toxicity in THP-1 cells occurred at a DEP dose $> 25 \mu\text{g ml}^{-1}$, whereas the onset of cell death in BEAS-2B cells was nearly linear at DEP doses $\geq 10 \mu\text{g ml}^{-1}$. We considered that these two concentration-based doses as the critical doses for their responses, and the corresponding values can be found in the model for DEP dose–

response profiles. Nel et al. (2006) constructed a hierarchical OS model to describe the relationships among GSH/GSSG ratio (as the level of OS), genetic responses and obvious outcomes through response and signaling pathways. We employed these concepts to predict the DEP-induced GSH/GSSG ratio decreasing situations when people exposed to different scenarios.

2.4. Risk characterization

Risk characterization provides an estimate of risk for the specific subpopulation under study (Fig. 1d). The risk at a specific DEP dose of OS can be calculated as the proportion of human lung expected to be subjected to that DEP dose multiplied by the conditional probability of OS. A joint probability function (JPF) or exceedence profile describes the probability of exceeding the cumulative dose associated with the related response and can be expressed mathematically as

$$P(R) = P(R|D) \times P(D), \quad (4)$$

where $P(R)$ is the probabilistic risk for a certain cumulative dose D , $P(R|D)$ is the CDF of having DEP cumulative dose D in human lung tissue, and $P(D)$ is the probability of DEP cumulative dose D in human lung tissue.

3. Results and discussion

3.1. Exposure analysis

3.1.1. Data treatment

From published data (Table 1) we produced estimations of the $PM_{2.5}$ and $PM_{0.18}$ mass concentration in indoor, outdoor and in-traffic activities in northern, central, and southern Taiwan (Fig. 2). Mass concentrations in traffic were generally higher than in indoor and outdoor activities. Uncertainties associated with in-traffic $PM_{2.5}$ levels in central and southern Taiwan (28.1–227 and 77.3–234 $\mu\text{g m}^{-3}$, respectively) were higher than in northern Taiwan (168.8–243.6 $\mu\text{g m}^{-3}$). While $PM_{2.5}$ concentration in indoor and outdoor activities tended to be lower in northern than that in central and southern Taiwan, no similar phenomenon was apparent for $PM_{0.18}$ (Fig. 2).

3.1.2. DEP concentration conversion

We incorporated the ratio of the vehicle contribution to the ambient $PM_{2.5}$ and the ratio of $DEP_{2.5}$ to the total vehicle exhaust particle to estimate the $DEP_{2.5}$ concentration. The former is best fitted as a lognormal distribu-

tion ($GM=0.507$ and $GSD=1.4$) and the latter estimated as a lognormal distribution ($GM=50.7$ and $GSD=1.4$). The transformation factor of $PM_{0.18}$ to $DEP_{0.18}$ was then obtained ($GM=0.436$ and $GSD=1.1$). The $DEP_{2.5}$ and $DEP_{0.18}$ daily cumulative doses were calculated using the average $DEP_{2.5}$ and $DEP_{0.18}$ concentrations multiplied by the specific daily air breathed (AB) rates, based on ICRP66 (ICRP, 1994) and shown in Table 2 as 20.64, 20.43, and 15.64 $\text{m}^3 \text{d}^{-1}$ for *driver*, *homeworker*, and *student*, respectively. The median daily cumulative dose of external $DEP_{2.5}$ with 95% confidence intervals (CIs) for *driver* was 0.716 (0.443–1.197), 0.345 (0.167–0.785), and 0.491 (0.176–1.446) mg in northern, central, and southern Taiwan, respectively. It was lower in all three regions for *homeworker* and *student*, and ranged from 0.198 to 0.309 mg. The median daily cumulative dose data for $DEP_{0.18}$ show even more forcefully that *driver* in northern Taiwan is the highest dose category, experiencing a dose of 0.584 (0.417–0.822) mg of $DEP_{0.18}$ compared with a range of 0.083 to 0.197 mg for other settings (Fig. 3).

3.1.3. Human lung deposition

By applying the HRT model to estimate the internal DEP dose, the results indicate that the fractional deposition value for $DEP_{2.5}$ in bb (0.168) region is higher than that in BB (0.006) and AI (0.150) regions, whereas it was highest for $DEP_{0.18}$ in AI (0.166) region. The total fractional deposition in BB, bb, and AI regions for $DEP_{2.5}$ and $DEP_{0.18}$ was 0.324 and 0.226, respectively (Table 3). We combined the external DEP doses from three different regions for three settings (Fig. 3) and the size-specific fractional deposition values (Table 3), we could calculate the medians and uncertainties of internal DEP doses for nine selected subgroups. We performed the median internal $DEP_{2.5}$ and $DEP_{0.18}$ in Fig. 4. We assumed that the DEP dose deposited on low respiratory tract (BB, bb, and AI regions) could cause the macrophages to swallow those particles. In light of this concept, the *driver* in northern Taiwan had the highest internal $DEP_{2.5}$ and $DEP_{0.18}$ dose (232.2 and 132.1 μg) compared with those in southern (159.2 and 14.2 μg) and central (111.9 and 44.5 μg) Taiwan (Fig. 4a, b).

The median $DEP_{2.5}$ daily cumulative dose for *driver*, *homeworker*, and *student* in northern Taiwan was 0.716, 0.309, and 0.244 mg, respectively (Fig. 3a), leading to an estimated median $DEP_{2.5}$ daily cumulative dose in BB, bb, and AI regions for those settings of 232.2, 100.2, and 79.1 μg (Fig. 4a). We applied the ratio of the vehicle contribution to the ambient PM ($GM=0.507$, $GSD=1.4$) and the ratio of DEP to the vehicle exhaust particle ($GM=0.507$, $GSD=1.4$) in northern Taiwan to estimate

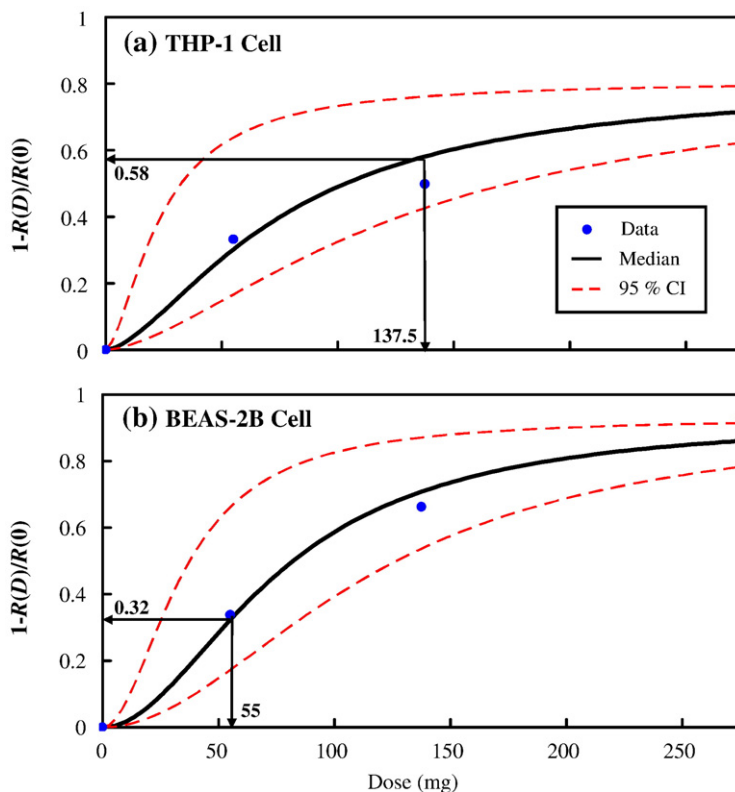


Fig. 5. Dose–response profiles optimally fitted by a three parameters Hill equation model with 95% confidence interval in (a) THP-1 and (b) BEAS-2B cells for DEP cumulative dose.

the $PM_{2.5}$ dose in deep lung tissue, producing a median daily $PM_{2.5}$ dose in lung tissue for *driver*, *homeworker*, and *student* of 631, 272, and 215 μg , respectively. The median daily $PM_{2.5}$ dose for *driver* (can be seen as male adult), *homeworker* (can be seen as female adult), and *student* (can be seen as youth) estimated by the PEARLS (Particulate Exposure from Ambient to Region Lung by Subgroup) model is 24.6, 20.8, and 22.4 μg based on an average $PM_{2.5}$ concentration of 15 $\mu\text{g m}^{-3}$ (Gower and McColl, 2005).

In our study the estimations are based on $PM_{2.5}$ concentration of 34.7–220.9 $\mu\text{g m}^{-3}$ (Fig. 2a). The conversion to a concentration of 15 $\mu\text{g m}^{-3}$ produces a median daily $PM_{2.5}$ dose for *driver*, *homeworker* and *student* of 184.6, 89.5, and 82.7 μg , respectively, resulting in our estimated doses in lung tissue nearly 2.5–3.4 times higher than the values estimated via PEARLS model.

We also apply the multiple-path particle dosimetry (MPPD) model version 2 developed by CIIT Centers for Health Research (2006) to validate our d_F values by using HRT model. The d_F values of $PM_{0.18}$ and $PM_{2.5}$ by using MPPD model are 0.204 and 0.277, respectively, whereas the values conducted from HRT model are 0.226 and

0.324, indicating that the d_F values of $PM_{0.18}$ and $PM_{2.5}$ from MPPD and HRT models are insignificantly different.

3.2. Effect analysis

3.2.1. Dose–response profiles construction

The dose–response profiles in THP-1 and BEAS-2B cells indicate that the 95% CIs for $K_{0.5}$ are estimated by the corresponding value of maximal residual multiplied by t statistical value ($t_{(0.025,3)}=3.182$) (Fig. 5). The maximum response R_{max} in THP-1 and BEAS-2B cells was estimated at 0.8088 and 0.9297, respectively. The Hill coefficient (n) in THP-1 cells was estimated at 1.59, and that in BEAS-2B at 1.96, with a $r^2 > 0.95$. The specific DEP cumulative dose yielding half of maximal response $K_{0.5}$ for THP-1 and BEAS-2B cells with 95% CIs was estimated at 76.29 (24.01–128.57) mg and 76.03 (34.77–117.29) mg, respectively.

3.2.2. Hierarchical OS model and critical levels

The hierarchical OS model could be used to describe the relationships among GSH/GSSG ratio (i.e. level of OS), cell response pathways, signaling pathways, genetic

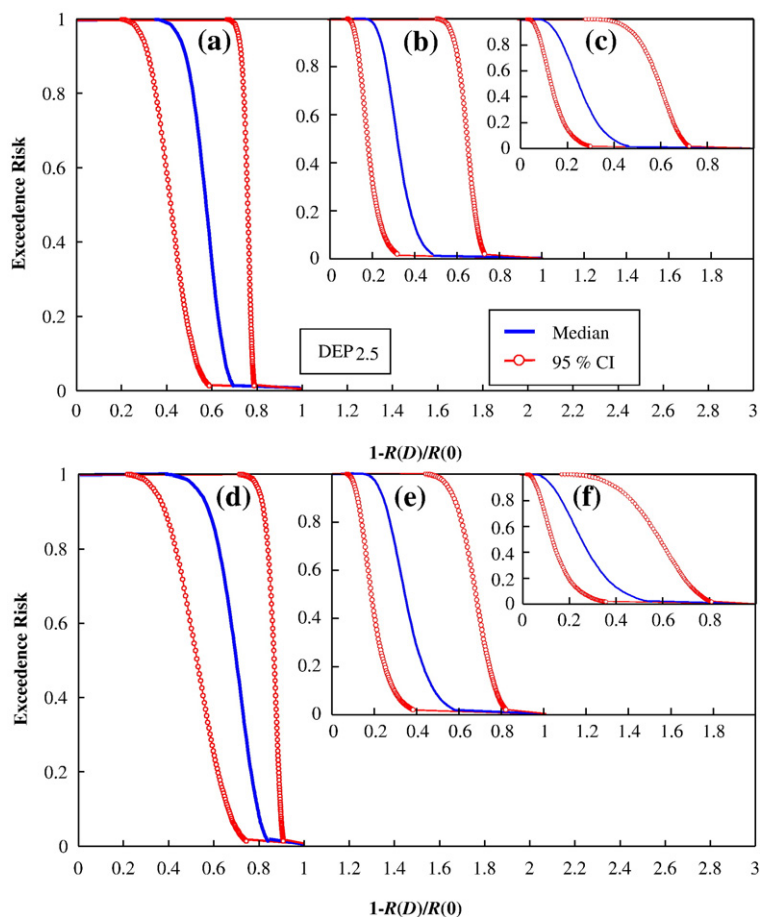


Fig. 6. Estimate exceedence risk curves with 95% confidence interval in THP-1 and BEAS-2B cells, respectively, for (a, d) *driver*; (b, e) *homemaker*; and (c, f) *student* exposed to $\text{DEP}_{2.5}$ for a 2-year duration in northern Taiwan.

responses and significant outcomes (Halliwell and Gutteridge, 1999). The antioxidant response was found for cellular redox homeostasis in tier 1 (lower amount of OS). The inflammation and cytotoxicity responses were found in tier 2 (medium amount of OS) and tier 3 (high amount of OS) (Halliwell and Gutteridge, 1999; Nel et al., 2006). Li et al. (2002) observed that the cytotoxicity response in THP-1 cells and cell death in BEAS-2B cells occurred at a DEP dose equal to or greater than 25 and $10 \mu\text{g ml}^{-1}$, respectively. These critical points for DEP dose were estimated to be 137.5 mg ($R=0.58$) and 55.0 mg ($R=0.32$) for THP-1 and BEAS-2B cells, respectively (Fig. 5).

3.3. Risk analysis

3.3.1. Regional assessment

For $\text{DEP}_{2.5}$, the median response in THP-1 cells for *driver* in northern Taiwan was, at 0.57, near the critical 0.58 (Fig. 6a) and hence this setting is at risk of $\text{DEP}_{2.5}$ -

induced oxidative stress in these cells. The upper band of the 95% CI for *homemaker* and *student* also encompasses the critical 0.58 level (Fig. 6b, c) and hence these settings also are at potential risk of $\text{DEP}_{2.5}$ -induced oxidative stress. The median response in BEAS-2B cells was above critical (0.32) for *driver* in all three regions of Taiwan (Fig. 6d–f), sometimes substantially, again identifying risk of $\text{DEP}_{2.5}$ -induced OS for the *driver* settings and furthermore that risk is greater than for THP-1 cells. For $\text{DEP}_{0.18}$, median responses in THP-1 cells indicated insignificant risk of OS for *driver* in northern Taiwan (Fig. 7a) and no risk for *homemaker* and *student* (Fig. 7b, c), whereas those in BEAS-2B cells indicated a significant risk of OS for *driver* (Fig. 7d), and a potential risk for *homemaker* and *student*, the upper bands of the 95% CI of which again encompass the critical level (Fig. 7e, f).

The results show that there is a potential risk of OS from exposure to $\text{DEP}_{2.5}$, via exceedence of the critical levels ($R > 0.58$ in THP-1 and $R > 0.32$ in BEAS-2B), in

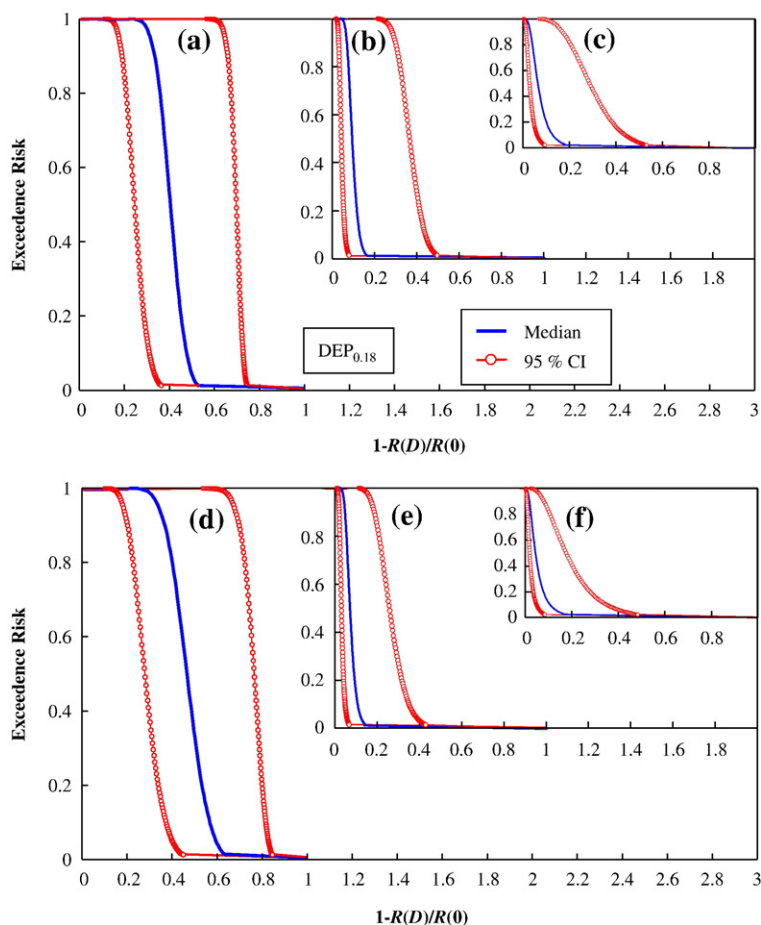


Fig. 7. Estimate exceedence risk curves with 95% confidence interval in THP-1 and BEAS-2B cells, respectively, for (a, d) *driver*, (b, e) *homemaker*, and (c, f) *student* exposed to $DEP_{0.18}$ for a 2-year duration in northern Taiwan.

all settings investigated, indicated by the 95% CIs (Table 4), whereas the risk from exposure to $DEP_{0.18}$ is largely limited to *driver* in northern Taiwan. The potential risk to *driver* from $DEP_{2.5}$ in northern Taiwan is approximately 2–3 times that to *homemaker* or *student*, while the potential risk from $DEP_{0.18}$ is 4–11 times higher (Fig. 3). Comparing the potential risk in THP-1 and BEAS-2B cells for the same occupational settings in different Taiwan regions indicates that the OS risk from $DEP_{2.5}$ and $DEP_{0.18}$ is in the order northern Taiwan > southern Taiwan \approx central Taiwan (Table 4).

3.3.2. Population level assessment

The complete process of risk assessment for an integrated data conducted from Taiwan is shown in Fig. 8. Fig. 8a, indicating that the model input data (PM) which result from MC simulation and all of them are considered as lognormal distributions (LN(GM, GSD)). The $PM_{2.5}$ values are LN(34.7, 1.6), LN(59.4, 1.5) and

LN(133.5, 1.7) in indoor, outdoor, and in-traffic settings, respectively, whereas the $PM_{0.18}$ values are LN(5.37, 1.6), LN(7.63, 1.6) and LN(48.8, 2.2) in these three environmental settings. Fig. 8b shows the external DEP concentrations for *driver* (D), *homemaker* (H) and *student* (S) settings. The daily median $DEP_{2.5}$ and $DEP_{0.18}$ concentrations with 95% CI for *driver* setting are 0.584 (0.219–1.298) and 0.26 (0.07–1.18) mg, respectively.

The external DEP in the other two settings (*homemaker* and *student*) are insignificantly different each other and less than that in *driver* setting. Fig. 8c presents the $DEP_{2.5}$ and $DEP_{0.18}$ daily doses deposited on the lung tissue, the influential $DEP_{2.5}$ (include BB, bb, and AI regions) are 157.6, 92.4, and 84.3 $\mu\text{g m}^{-3}$ for *driver*, *homemaker* and *student* settings, respectively, whereas the $DEP_{0.18}$ doses are only 58.8, 22.6, and 20.4 $\mu\text{g m}^{-3}$. Results show that the daily doses for *driver* setting are significant than the other two settings.

Table 4

Cumulative dose–response with 95% confidence intervals (CIs) in THP-1 and BEAS-2B cells for *driver*, *homeworker*, and *student* exposed to DEP_{2.5} and DEP_{0.18} for 2-year duration

	THP-1 cell		BEAS-2B cell	
	Median	(95% CI)	Median	(95% CI)
DEP _{2.5}				
Northern Taiwan				
<i>Driver</i>	0.57	(0.42–0.76)	0.70	(0.53–0.87)
<i>Homeworker</i>	0.32	(0.18–0.65)	0.34	(0.19–0.68)
<i>Student</i>	0.25	(0.13–0.60)	0.25	(0.13–0.59)
Central Taiwan				
<i>Driver</i>	0.35	(0.20–0.67)	0.39	(0.22–0.72)
<i>Homeworker</i>	0.26	(0.14–0.61)	0.27	(0.14–0.61)
<i>Student</i>	0.20	(0.10–0.54)	0.18	(0.09–0.49)
Southern Taiwan				
<i>Driver</i>	0.46	(0.30–0.72)	0.55	(0.36–0.81)
<i>Homeworker</i>	0.29	(0.16–0.63)	0.31	(0.16–0.65)
<i>Student</i>	0.22	(0.11–0.57)	0.22	(0.11–0.54)
DEP _{0.18}				
Northern Taiwan				
<i>Driver</i>	0.40	(0.25–0.70)	0.47	(0.28–0.77)
<i>Homeworker</i>	0.09	(0.04–0.37)	0.07	(0.03–0.26)
<i>Student</i>	0.07	(0.03–0.29)	0.04	(0.02–0.18)
Central Taiwan				
<i>Driver</i>	0.12	(0.06–0.43)	0.10	(0.05–0.33)
<i>Homeworker</i>	0.03	(0.02–0.18)	0.02	(0.01–0.08)
<i>Student</i>	0.04	(0.01–0.13)	0.01	(0.01–0.05)
Southern Taiwan				
<i>Driver</i>	0.09	(0.04–0.35)	0.07	(0.03–0.24)
<i>Homeworker</i>	0.03	(0.02–0.18)	0.02	(0.01–0.08)
<i>Student</i>	0.02	(0.01–0.12)	0.01	(0.01–0.05)

Fig. 8d presents the risk for *driver* setting expose to ambient population level in overall Taiwan region. Based on the assessment for 2-year duration, the median cumulative dose–responses with 95% CI in THP-1 and BEAS-2B cells for *driver* exposed to DEP_{2.5} are 0.27 (0.14–0.61) and 0.28 (0.14–0.61), respectively, whereas the corresponding values expose to DEP_{0.18} only fall on 0.18 (0.09–0.51) and 0.16 (0.08–0.46).

3.4. Improvement strategy

Our results show that *driver* in northern Taiwan is at the highest risk from DEP_{2.5} exposure. Based on sensitivity analysis, the time-spent-specific air breathed rate and PM_{2.5} or PM_{0.18} concentrations for in-traffic activity are the most two factors affecting the daily cumulative dose of DEP_{2.5} or DEP_{0.18}. The daily cumulative dose of DEP_{2.5} is correlated with time spent in the higher PM_{2.5} concentrations of the in-traffic activity, as shown in sensitivity analysis. Results also show that the internal DEP_{0.18} daily exposure (median=0.132

with 95% CI=0.094–0.186 mg) is the largest fraction of DEP_{2.5} daily exposure (median=0.232 with 95% CI=0.144–0.388 mg) for *driver* in northern Taiwan. Therefore, the potential risk from DEP-induced ROS could clearly be reduced by reducing the time spent in the in-traffic activity (Table 2).

The second strategy is to reduce the PM level from roadside or underpass (i.e. in-traffic activity), yet this is an expensive and time-consuming task. We must provide a lot of methods to decrease the pollutants emitted from vehicle exhaust, including emission standard legislation (Maga, 1982), improvement of traffic types (ex. changes in traffic volumes, speed, vehicle age distributions, diesel-vehicle fractions and congestion levels) (McCarthy et al., 2006), and improvement of fuel use (Sharp, 1998; Swanson et al., 2007). Many researches have shown a less decrease in PM emissions with biodiesel, whereas the other pollutants (NO_x and OC) may increase (Sharp, 1998; Swanson et al., 2007).

ITRI (2007) suggested that the biodiesel can be mixed with fossil diesel or directly used by diesel engines with no engine adjustment necessary. According to ITRI's evaluation, there are 30–50% and 5–15% of PM reduction for pure biodiesel (B100) and the blend of 20% biodiesel with 80% fossil diesel (B20), respectively (ITRI, 2007). Although this improvement of fuel use can reduce the PM level from in-traffic activity, the emerging pollutants emitted from biodiesel-burned vehicles should be studied more detail. The relationships between biodiesel exhaust emissions and human health are not clear so far (Swanson et al., 2007). Based on the knowledge of biodiesel technology, we cannot assess the human health induced by biodiesel through our proposed model. The major reason is that the selected dose–response profiles or significant endpoints may be changed.

3.5. Model improvement

In our proposed model, DEP (and/or PM) dose is association with OS response, whereas the particle size, surface area, and chemical composition impact on the mode and type of lung injury sustained from PM (Donaldson et al., 1998). Smaller particulates have higher mobility, are more easily inhaled, and may cause a larger fraction deposition in human lung and penetrate the alveolar epithelial barrier into the blood/lymphatic system (Biswas and Wu, 2005). Nemmar et al. (2003) showed that while fine particulate inhalation can induce lung inflammation, ultrafine can additionally induce thrombosis. The exact surface area for measured DEP is very difficult to obtain and, moreover, only PM mass

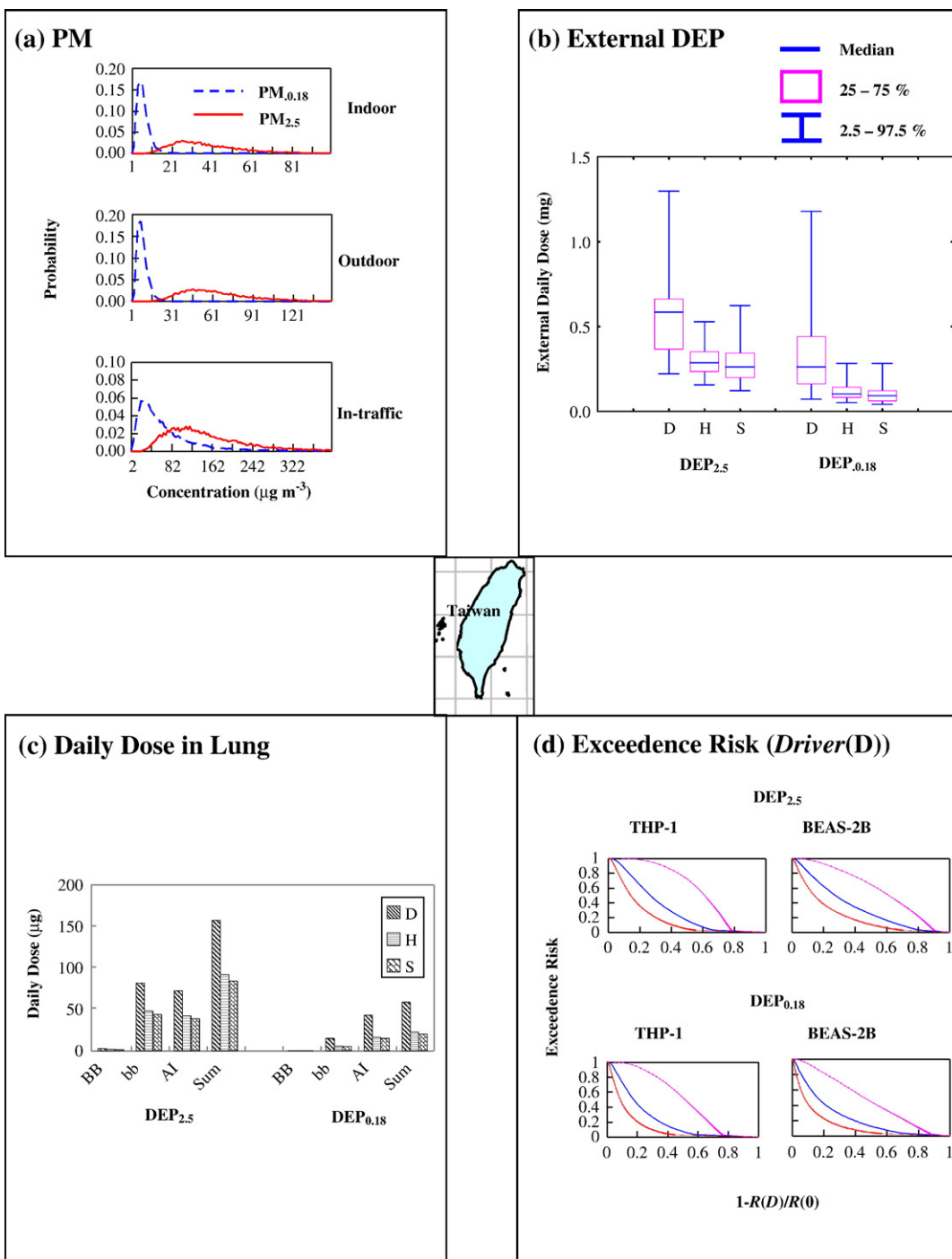


Fig. 8. Model inputs and outputs based on an integrated Taiwan setting. (a) PM, (b) External DEP, (c) Daily Dose in Lung, and (d) Exceedence risk.

concentrations were known in the original data sources for this study.

Furthermore, no dose–response profiles with GSH/GSSG indicator based on surface area and number concentration were found. For these reasons, we focused

on the mass concentration of the DEP dose in this study, yet maybe more suitable effects/endpoints correlated to surface area-based or number concentration-based of DEP or their compositions can be found in near future. Currently, our proposed model should be used cautiously,

whereas they can be enhanced more reliable and valuable based on overcoming more knowledge gaps.

The GSH/GSSG ratio was able to describe the OS state for exposure to DEP, detailed indexes of lung toxicity including inflammation, cytotoxicity, parenchyma change, and general toxicity potencies should be introduced in the future (McDonald et al., 2004). To date, many researches have indicated that the DEP-induced health effect from *in vitro* and *in vivo* studies, including lung tumor rate (Mauderly et al., 1987; Heinrich et al., 1995), inflammatory and genotoxic effects (Dybdahl et al., 2004). Our proposed endpoint (GSH/GSSG ratio in human cells line) is selected to prevent the predicament of species differences. Our explanations are (i) we should take into account the modifying factors when the effects conducted from the rodent studies are selected and (ii) we are intended to assess the preliminary effect on human lung epithelial cells and macrophages expose to ambient particles (PM and/or DEP). Our models have combine the appropriate dose–response relationships and critical concentrations from Li et al. (2002) with the hierarchical oxidative stress (OS) model from previous studies (Halliwell and Gutteridge, 1999; Nel et al., 2006) to predict the OS potential risk exposing to atmospheric particles. Although many *in vivo* studies provide useful information in rat model, there are inadequate dose–response profiles for employing in our study.

4. Conclusions

In conclusion, we have quantified the effective DEP exposure in atmospheric environment and assessed probabilistically the human OS risk from DEP-induced ROS in selected occupational settings. We employed an HRT model to successfully estimate the DEP_{2.5} and DEP_{0.18} cumulative doses deposited in lung compartments and applied DEP dose–response profiles based on mass to evaluate simultaneously the OS risk for human macrophage cells (THP-1) and human bronchial epithelial cells (BEAS-2B). The government or decision makers are encouraged to use these results to initiate and set an environmental quality standard or emission standard for diesel exhaust particulate. In future work, we may incorporate the surface area and/or number concentration-based DEP dose–response profiles to precisely describe the risk potential induced by DEP.

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