INDUCED SPUTUM PARTICLES IN BERYLLIUM-EXPOSED DENTAL TECHNICIANS REFLECT HYGIENE AND OXIDATIVE STRESS

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BACKGROUND

Chronic Beryllium Disease (CBD) and Beryllium Sensitization (BeS) are caused by occupational exposure to Beryllium (Be). CBD is caused by prolonged occupational exposure to beryllium and is characterized by a wide variety of clinical presentations, mostly pulmonary. Despite health warnings, occupational exposure to beryllium continues to result in increasing beryllium-related health effects in a multitude of industries worldwide, as evidenced by many epidemiological studies over the past decade. The only currently available noninvasive technique that retrieves cells and soluble factors from the lung is induced sputum (IS). Accumulated data suggest that particulate matter (PM) exposure may lead to pulmonary inflammation and oxidative stress. In addition, beryllium stimulates the formation of reactive oxygen species. Heme oxygenase 1 (HO1) plays a key role in the reduction of oxidation and inflammation.

In the last years we have shown the application of IS technique in occupational exposures. We hypothesize that quantitative and qualitative analysis of particles recovered by IS and evaluation of detoxification enzyme such HO1 can serve as method for biologically monitoring of healthy workers exposed to hazardous dusts. This could protect exposed workers from developing beryllium-related health effects (CBD or BeS).

OBJECTIVE

- To use particle size distribution (PSD) and shape parameters in IS to bio monitoring dental technicians.
- To investigate the Heme Oxygenase one (HO-1) molecular pathway in IS of dental technicians.

METHODS

Study Population: 83 dental technicians were included in a cross-sectional study.

Pulmonary Function testing (PFT) Pulmonary Function Tests (PFTs) were performed using standard protocols according to American Thoracic Society (ATS) guidelines.

Sputum induction & processing

After pretreatment with a short acting beta-2 agonist, 3% saline was administered by an ultrasonic nebulizer for up to 20 min. Viscid selected portions (plugs) were treated with dithiothreitol [DTT (Sputalysin); Calbiochem Corp., San Diego, CA, USA] and 400 non squamous cells were counted in cytospin slides Giemsa stained [1, 2].

Particle size distribution (PSD)

was performed according to a laser technique based on the Time of transition Theory (TOT) (Figure 1) using an Eyetech Analyzer (Donner Technologies, Israel) in ranges of 0.5-60 [3].

Particle shape image analysis (SIA)

Was performed by video system and based on principles of image analysis in which images of the moving particles are captured with a sensitive charge-coupled device (CCD).

- Image analysis software defines the parameters of each particle under assumption of particles being not spherical in which equivalent diameters differ from each other (Figure 2).

The main parameters studied were:

CO	Convexity	Figure 3
GL	Gray Level	Figure 3
CI	Circularity	Figure 3
AF	Average Ferret	Figure 4
AR	Aspect Ratio	Figure 5

Be lymphocyte proliferation test BeLPT

BeLPT was performed as previously described [4]. Mononuclear cells were separated from blood sample using density Ficoll gradient and incubated at 37ºC for 4 and 6 days with 0, 1, 10, and 100 μM of BeSO4. A SI >2.5 was considered abnormal.

RNA extraction and real time polymerase chain reaction (RT PCR)

RNA extraction was done on the sputum cell by TRI reagent-chloroform solutions. RT-PCR was carried out with 0.5 μg of total RNA that was extracted from the sputum cells. Quantitative RT PCR (qRT PCR) was carried out using Applied Biosystems AB7500 SYBER Green PCR.

Questionnaire

Evaluated questionnaire of Tel Aviv University epidemiology department include demographic, occupational and health data. In addition, occupational questionnaire was used.
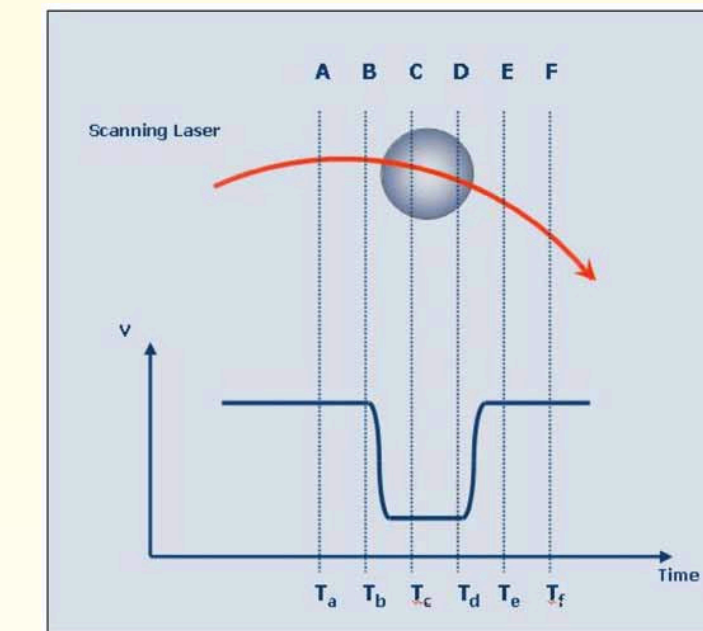
Statistical methods

Associations between the presence of positive BeLPT or exposure (yes/no) and various covariates were tested by the Fisher exact test for categorical variables and the t test for continuous variable. Analysis of variance (ANOVA) was used for comparisons of categorical variables. Logistic regression analyses were performed to evaluate the relation between exposure to PSD and shape. Models were adjusted for demographics, smoking and work-related factors. All statistical analyses were performed using the SPSS software version 15.0 for Windows. a P value < 0.05 was considered significant.

RESULTS

- Demographic parameters are shown in Table 1. The study population included 69 males (83%) and 14 females (17%). Seventy one percent (n=59) of the study population reported on Be exposure.
- PFT: subjects were divided into two groups: exposed (yes/no according to self report) (Table 2a) and BeLPT results (Table 2b). PFT were within normal values for all exposed and unexposed individuals. According the BeLPT results: significantly decline was found in all the CBD PFT's parameters (Table 2b).
- PSD analysis: The 92% for particles under 5μ was set up as cut off for % particles in IS samples (Figure 6). This found to have an OR-3.81(1.04-14.7) for the risk to develop (+)BeLPT test (BeS or CBD) (figure 7).
- SIA: GL was associated with Be exposure (OR 0.957 p=0.018). Significant differences in the IS GL were found between non-exposed and exposed workers (83.8±21 vs. 69.1±16.7, respectively, p=0.006)(figure 8). The GL of particles was significantly higher in workers exposed to dust vs fumes (83.43±18.48 vs 66.7±15.8, p=0.001) (fig 9A), and the GL of the workers who used hoods or combination of hood and personal masks was significantly lower than workers who used no protection (63.9±13 and 72.9±21 vs 83±18, p=0.007) (fig 9B).

- HO1 gene expression. A high expression of the HO1 gene was associated with a normal CD4/CD8 ratio (i.e., <2.5) (0.065 ± 0.08 vs 0.05 ± 0.09, P = 0.042) (Figure 10). This HO1 gene expression was more likely to be higher in the combined fume- and dust-exposed group compared the group exposed solely to dust (0.08 ± 0.1 vs 0.04 ± 0.07, respectively) (P = 0.015) (Figure 11).

Table 2a: Pulmonary Function Tests Of Dental Technicians Who Were Or Were Not Exposed To Beryllium.

	N	FEV1%	FVC%	FEV1/FVC	DLCOSB%
Be exposure (yes)	59	93.1±21.3	93.7±22.6	81.4±6.4	86.8±19.3
Be exposure (no)	21	94.2±15.2	94±14	83.3±7.1	92±16.02
		P=0.92	P=0.45	P=0.05a	P=0.14

Analysis of Variance for differences between exposed vs unexposed. aFEV1/FVC P =0.05 for exposed versus unexposed.
FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; DLCOSB = diffusion lung CO single breath;

Table 2b: Pulmonary Function Tests Of Dental Technicians With Negative BeLPT, Beryllium Sensitization, And Chronic Beryllium Disease

	N	FEV1%	FVC%	FEV1/FVC	DLCOSB%
Negative	65	97.3±16.4	98.7 ±15	81.6 ±6.7	89.8 ±16.7
BeS	9	90±12	88.5 ±11	83.7 ±3.6	93.5 ±13.8
CBD	6	54±24.2b	56.7 ±23c	82 ±8.8	56 ±16d
		P<0.001	P<0.001	P=0.82	P<0.001

Analysis of Variance and multiple comparisons for differences between the BeLPT test results groups: bFEV1 P <0.001, cFVC P <0.001 and dDLCOSB P <0.001 for CBD versus the negatives and BeS.
FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; DLCOSB = diffusion lung CO single breath.

Figure 1: Particles Size Distribution (Psd) The Tot Theory.

Since the laser beam rotates with a constant speed, the duration of the obscuration provides a direct size measurement of each particle under assumption of sphericity particles.
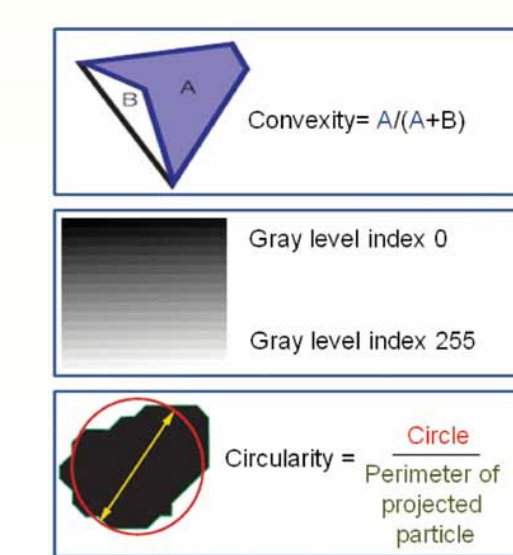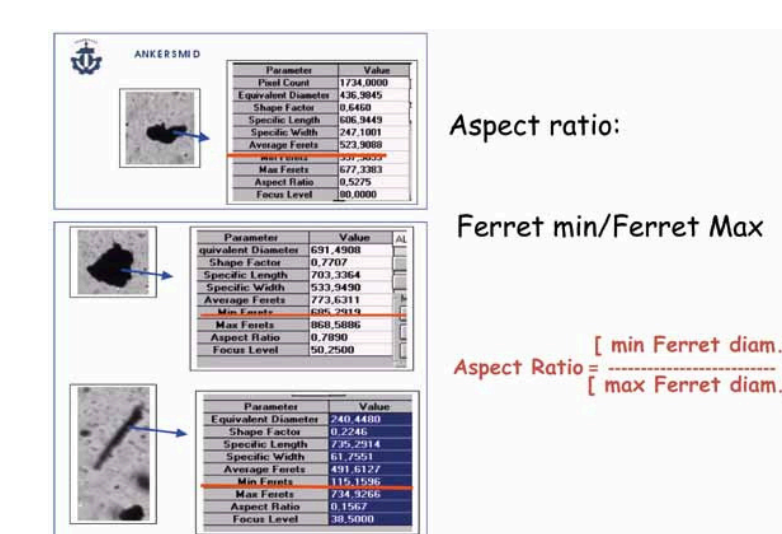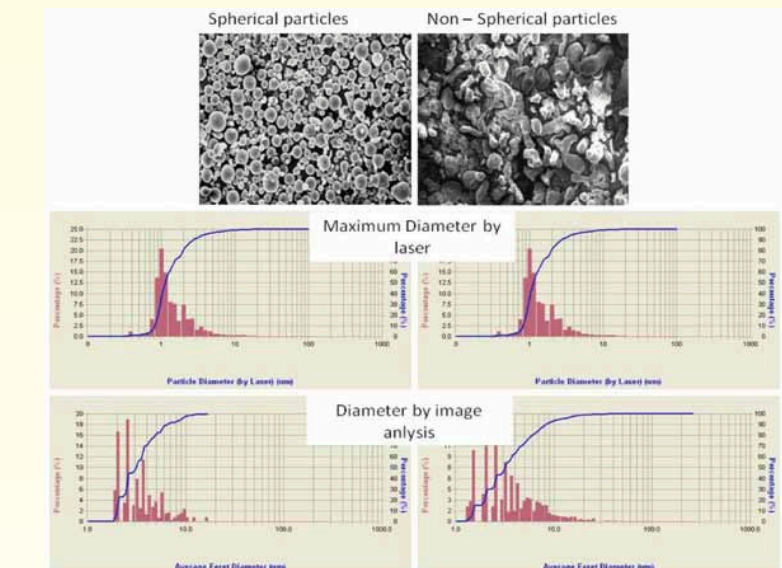
Figure 2: Particle Shape Image Analysis (Sia)

Characterization of non-spherical particles by two dimensional shape information. Dynamic Image Analysis uses digital video microscopy to capture optimal particle images for processing. Acquired images are processed using sophisticated image analysis procedures and No assumption of particle sphericity is required.

Figure 3: Particle Shape Image Analysis (Sia) Parameters

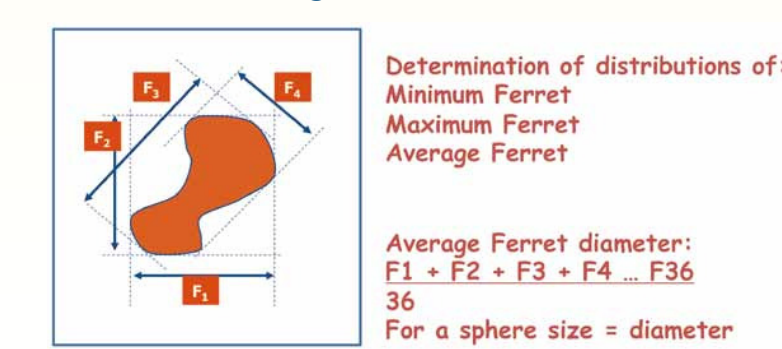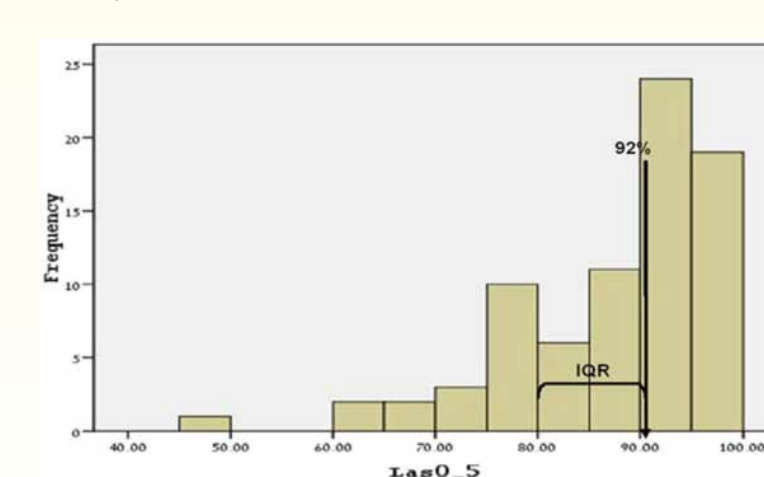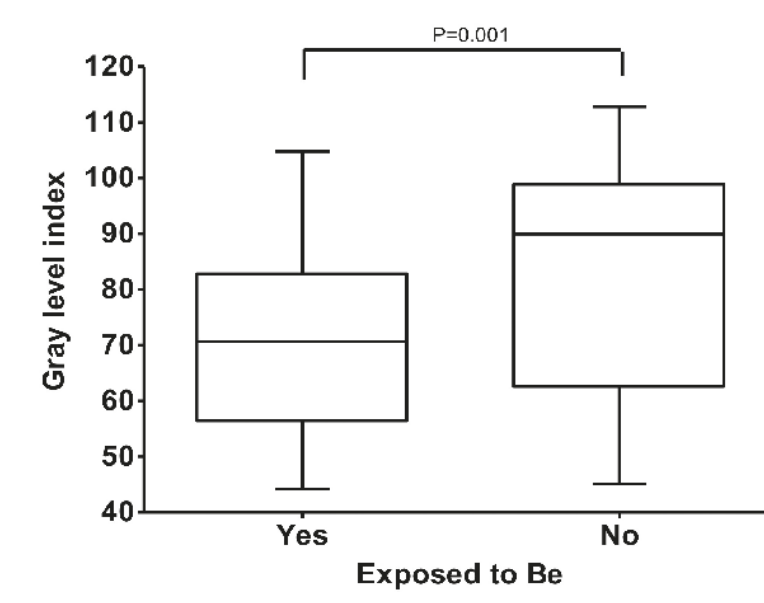### Figure 4: Particle Shape Image Analysis (Sia) Average Ferret Diameter

Figure 5: Particle Shape Image Analysis (Sia) Aspect Ratio

Table 1: Demographic And Exposure Assessment Of The Study Population (N=83)

	Male N=69 (83%)	Female N=14 (16.9%)	Total
Age (Year)	41.6±11.8	37.8±10.5	41±11.6
Exposure (Year)	19.3±10.8	16±10.7	18.8±10.8
Smoking (Yes)	29%*	14.2%*	26% N=22
Working with Be (Yes)	72%*	77%*	71% N=59

* Percentage of all "Yes" responses from the total subjects according to gender.
Working with Be was evaluated by questionnaire.

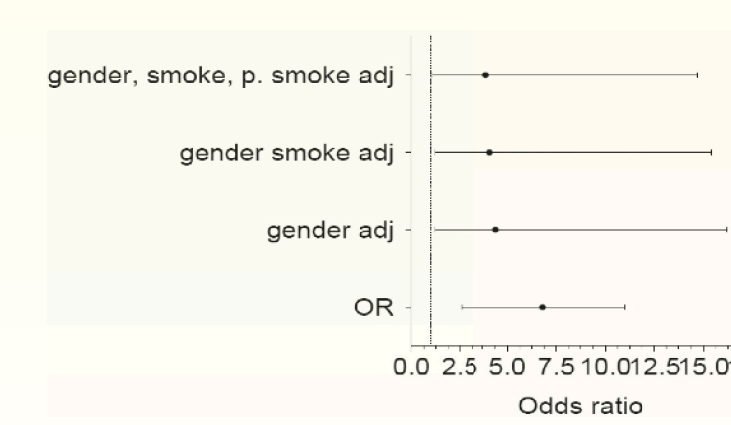### Figure 6: PSD Under 5μ As Binary Parameter By Using The Interquartile Range (IQR)

PSD performed by Eyetech particles scanner. PSD under 5μ was divided into binary parameter by using the interquartile range (IQR). The 92% for particles under 5μ was set up as cut off for % particles in IS samples. 44 patients were classified under the IQR and 33 above. Y axis represent the frequency (%) of subjects for each interval of PSD under 5μ (X axis).

Figure 7: The Association Between PSD Under 5μ And The Risk For Development Positive Belpt Response.

Logistic regression was performed to evaluate the risk (OR) of positive BeLPT test by percentage of small particle under 5μ. Y axis reveal the basic odds without adjustment (OR), and specific adjustment for gender, smoking habits and passive smoking on the basic odds.

Figure 8: Gray Level (GL) In The Be Exposed Worker Comparing Non-exposed

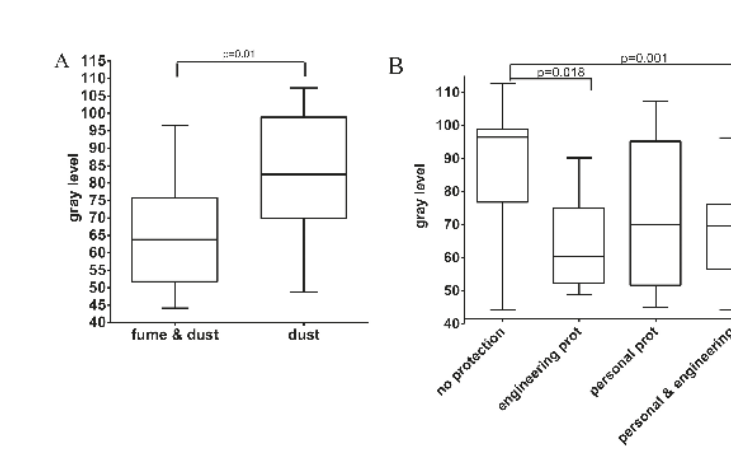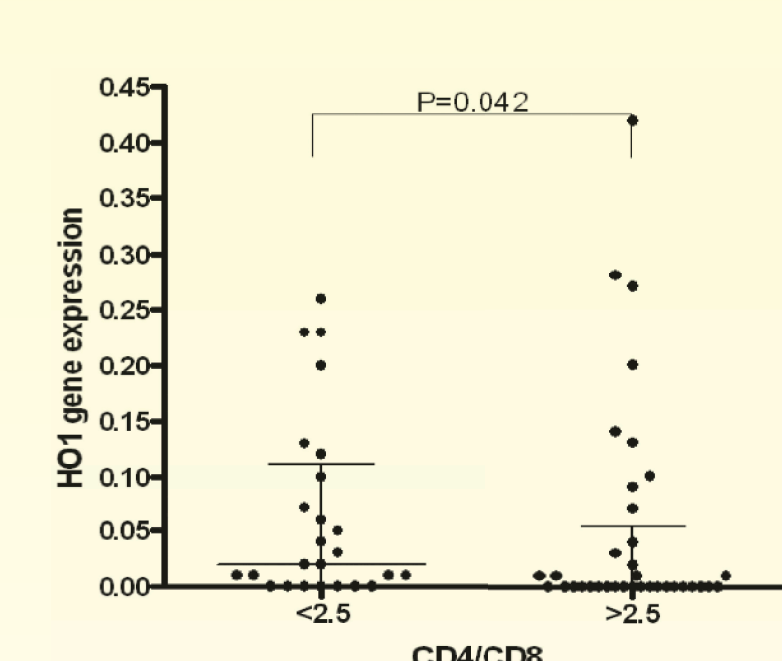Y axis reveal the gray level index ranged from 0 (opaque) to 255 (translucid) it's the most significant parameter of all studied SIA Eyetech parameters.

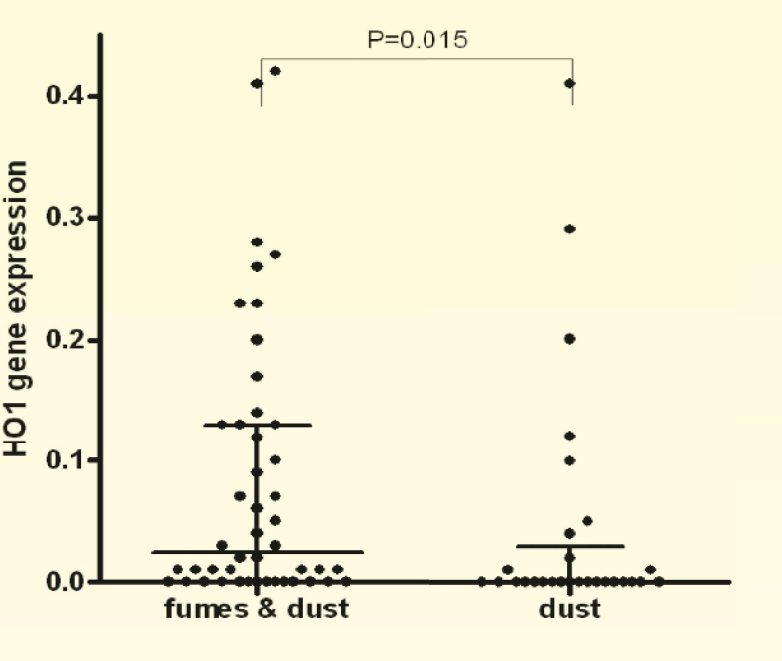### Figure 9: Gray Level (GL) Distributions According The Hygienic Condition.

Y axis reveal the gray level index ranged from 0 (opaque) to 255 (translucid). Fume- Melting or hitting metals byproducts. Engineering protection- using hood and pumps in the workplace. Personal protection- Personal mask using.

Figure 10: HO1 Gene Expression According The T Cell Subpopulations.

HO1 gene expression vs T cell subtypes in the induced sputum (IS) samples of the studied dental technicians. HO1 gene expression was evaluated by real-time PCR (Δ∆ct method) normalized by an internal gene (GAPDH). The T cell subpopulation was measured by FACS. The median and interquartile ranges are shown. (nonparametric Mann-Whitney test).

Figure 11: HO1 Gene Expression According Fume And Dust Exposure

HO1 gene expression in dental technicians exposed to beryllium dust and fumes. Gene expression was evaluated by qRT-PCR. The median and interquartile ranges are shown. Nonparametric (Mann Whitney) P = 0.015 for dust- vs fume-exposed subjects.

CONCLUSIONS

1. Hygienic condition of the workplaces significantly modulates the type of particles present in the airways of exposed workers (reflected in the GL).
2. GI is the most sensitive parameter influenced by these hygienic conditions.
3. Particulate matter under 5μ is highly associated with the risk to developed positive response in BeLPT test.
4. Biological monitoring based on PSD and SIA may be useful to detect impending and existing CBD.
5. Fume exposure promoted oxidative stress that resulting in a defense response expressed by HO1 gene expression and it plays an important role in modulating immune reactions induced by T cell subpopulations.

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Reference

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