RESEARCH ARTICLE

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Evaluation of Cytochrome P4502E1 mRNA Expression and Its Effects in Antioxidant Defenses, and Cell Toxicity in Printing Workers

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Abstract

Background: Cytochrome P4502E1 (CYP2E1) metabolizes environmental toxins, however, compound metabolism can produce oxidative stress, causing in-cell toxicity and sometimes transformation. **Aim:** To evaluate CYP2E1 gene expression and its effects in antioxidant defenses, and cell toxicity in printing workers. **Methods:** The hierarchical method of health and chemical risk was used to evaluate chemical exposure in workplace. Blood samples and buccal epithelial cells were obtained from printing workers, and workers without any history of occupational exposure to chemicals (control group). Gene expression of CYP2E1, and antioxidant enzymes Superoxide dismutase (SOD) and Catalase (CAT) from leukocytes were evaluated. Hematic analysis and cell-free DNA from plasma were analyzed. Frequencies of cells with micronuclei (MN) and nuclear abnormalities from buccal epithelial cells were explored. **Results:** Evaluation of chemical exposure in working place demonstrated that ethyl alcohol, isopropyl alcohol, and isophorone represent 91% of the accumulated potential risk. CYP2E1 expression showed a 2.5-fold overexpression in the printing workers compared to the control group. SOD expression showed a 0.5-fold lower level in the printing workers than the control group, and CAT expression showed no differences between groups. Lower red blood cell and platelet values were detected in the printing workers than in the control group, and cell-free DNA plasma concentration was 3-fold higher in the printing workers than in the control group. The printing workers showed a higher frequency of cells with MN and nuclear anomalies than the control group. **Conclusion:** CYP2E1 overexpression triggers antioxidant defenses and toxic cell effects in printing workers.

Keywords: cytotoxicity- genotoxicity- oxidative stress- CYP2E1

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Introduction

Within the printing industry, high levels of chemical compounds including ozone (O3) and solvents, which are complex chemical mixtures containing many different hydrocarbon types, such as carbonyls, alcohols, alkanes, alkenes, esters, aromatics, ethers, and amides, are emitted while using cleaning agents, inks, alcohol, and other solutions to moisten the printing plates and these chemicals evaporate and become incorporated into environmental air as volatile organic compounds (VOCs) (Prica et al., 2016; Lyu et al., 2021; Mendoza-Cantú et al., 2006).

Some reports have described how chemical compounds in the printing industry may affect the health of workers, inducing mainly some symptoms such as irritation of the eye and skin, respiratory discomfort, headache, nausea, fatigue, or dizziness (Alabdulhadi et al., 2019; Decharat, 2014). Some authors have also reported cardiopulmonary function changes (Lyu et al., 2021) and neurobehavioral effects (Song et al., 2015).

Meanwhile, the International Agency for Research on Cancer (IARC) suggested in 1996 that chemicals used in the printing industry produced cancer (IARC,1996), and some authors have suggested and provided evidence that printing industry workers are at increased risk of several types of cancer (Yamada et al., 2015; Ito et al., 2016; Kubo et al., 2017; Kvam et al., 2005), with a latency period of 15-25 years (Kvam et al., 2005).

Cytochrome P450 family 2 subfamily E member 1

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(CYP2E1) enzyme metabolizes numerous low molecular weight toxicants, which are solvents, industrial monomers and cancer suspect agents (Gonzalez, 2005; Chen et al., 2019). Some studies have suggested that VOCs metabolized by CYP2E1 induce oxidative stress (Kin et al., 2011), which can lead to damaged mitochondria, DNA modification, lipid peroxidation, elevated cytokine production and even cell death and various pathological conditions (Gonzalez, 2005) whose alteration is important because of a possible association with the development of diseases and is also involved in the bioactivation of a number of low molecular weight cancer suspects (Guengerich, 2020).

Some reports using biomarkers have shown pathological alterations in occupational printing workers exposed to chemical compounds, including cytotoxic and genotoxic damage by increase micronuclei frequency and presence of sister chromatid exchange in peripheral blood (Aksoy et al., 2017; Hammer, 2002; Sellappa et al., 2017), DNA degradation (Sul et al., 2002), increase of chemical metabolites derived from biotransformation of VOCs in urine samples (Hormozi et al., 2019; Takeuchi et al., 2015), modulation of oxidative markers and inflammatory cytokine in blood samples (Mourad, 2021; Guo et al., 2020; Khatri et al., 2017), and alteration in blood count (Celik et al., 2013; Song et al., 2017). However, the association of modulation of CYP2E1 and its effects in printing workers has not been studied and its study is limited to a mouse model (Yanagiba et al., 2016), and biomarker analysis is necessary to detect pathological alterations during an early stage in health workers, as well as to improve conditions in order to prevent damage to health. Therefore, in this study, we evaluated the expression level of CYP2E1 and its effects in antioxidant defenses, and cellular toxicity from the blood and buccal epithelium of printing workers in a printing press in Mexico City.

Materials and Methods

Study Population

This cross-sectional study included 47 non-smoking subjects, divided into two groups: the exposed group, comprising 25 workers with occupational exposure to chemical risk in a printing press in Mexico City, and the unexposed group comprising 22 workers without any history of occupational exposure to chemical risk.

The research protocol and the informed consent forms were approved by the Bioethics Committee of "ENMH-IPN" (Approval number CBE/022/2019). The subjects signed informed consent and completed a questionnaire relating to demographic data, work conditions and clinical symptoms.

Evaluation of chemical exposure in the workplace

The chemical compounds used in the workplace were evaluated according to the hierarchical assessment method of chemical risk developed by the National Institute for Safety and Health at Work. It is a semiquantitative method in which the risk potential is calculated from the categorization of a number of variables. The variables considered are the danger associated with the chemical agents, and the potential exposure calculated from the amount and frequency of use (Aguilar et al., 2010).

Gene expression of Cytochrome P4502E1 (CYP2E1), Superoxide Dismutase (SOD) and, Catalase (CAT) from leukocytes

RNA was extracted from leukocytes using the trizol method (Invitrogen, Life technologies CA, USA). The RNA was treated with DNAse I (Promega), and then cDNA synthesis was performed using the SuperScript First-strand kit (Invitrogen, Life technologies CA, USA). The amplification of SOD, CAT, and CYP2E1 was performed by qRT-PCR in the Stratagene Mx- 3005P (Agilent Technologies, CA, USA) using SYBR Green PCR Master Mix (Applied Biosystems CA USA). Primers were CYP2E1 forward: (5'AGAGATGCCCTACATGGATGCT 3'), CYP2E1 reverse: (5'GGGCACGAGGGTGATGAAC 3'); SOD forward: (5' CAGGGCATCATCAATTTCGA 3'), SOD reverse: (5' TGCTTCCCACACCTTCAC 3'); CAT forward: (5' CTGGAGAAGTGCGGAGATTCA 3'), CAT reverse: (5' AATGCCCGCACCTGAGTAAC 3'), and the endogenous gene GAPDH forward: (5' CGGACTTCCTCGGTGATACC 3'), GAPDH reverse: (5' CAATGCCGGCCTTAGCAT 3'). The analysis and comparison of expression levels of SOD, CAT, and CYPE21 between groups were evaluated using the 2-ΔΔ CT method (Livak and Schmittgen, 2001).

Blood analysis: Hematological parameters and Cell-free DNA plasma concentration

Blood samples were extracted by venipuncture, collected into EDTA tubes, and analyzed in an automated hematology analyzer (Sysmex XN-1000, Kobe JA). After plasma was separated by centrifugation, the DNA was extracted using the phenol-chloroform-isoamyl alcohol reagent (Invitrogen, Life technologies CA, USA). DNA concentrations were evaluated using an Epoch spectrophotometer (BioTek, VT, USA).

Buccal micronuclei assays and nuclear anomalies

Buccal epithelial cell samples were obtained after rinsing the oral cavity with water, gently scraping the right and left cheek with a cytobrush, and the samples were then spread on clean slides. The slides were fixed with methanol for 10 minutes and Feulgen staining was conducted. After staining, the slides were examined under an optical microscope. The slides were prepared in triplicate for each subject, with 2000 cells per preparation. The mean frequency was determined for both MN and nuclear anomalies.

Statistical analysis

Normality tests were performed using the Shapiro-Wilk method. The data are expressed as the mean ± standard deviation (SD), and a comparison of variables between groups was performed by t-test for independent samples using the SPSS Statistic software version 23 (SPSS Inc, Chicago, IL). A p-value of < 0.05 was considered significant.

Results

Study Population

Table 1 summarises the characteristics of the study population and their clinical symptoms.

Evaluation of chemical compounds in the workplace

Regarding the evaluation of chemical compounds in the workplace, it was found that the most common substances used were VOCs for inks, including ethyl alcohol, isopropyl alcohol, isophorone, ethyl acetate, dipropylene glycol monomethyl ether, and propyl acetate.

Following the hierarchical method for evaluating chemical risk assessment of the National Institute of Safety and Health at Work (INSHT), and on consideration of the hazard class, quantity, frequency, and exposure class of each substance, it was concluded that ethyl alcohol, isopropyl alcohol, and isophorone represent 91% of the accumulated potential risk.

Gene expression of CYP2E1, SOD, and CAT

The results of the gene expression of CYP2E1, SOD, and CAT, are shown in figure 1. We detected a significant difference between groups for CYP2E1 and SOD gene expression. CYP2E1 expression showed a 2.5-fold higher level in the printing worker group compared to the control group; SOD expression showed a 0.5-fold lower level in the printing worker group than the control group. No statistically significant difference between groups was detected in the CAT expression level.

Hematological analysis and Cell-free DNA plasma concentration

The results of the hematological analysis and plasma free DNA are summarized in table 2. With respect to hematological parameters, both groups showed values within the normal parameters, although the printing worker group showed lower red blood cell, hemoglobin,

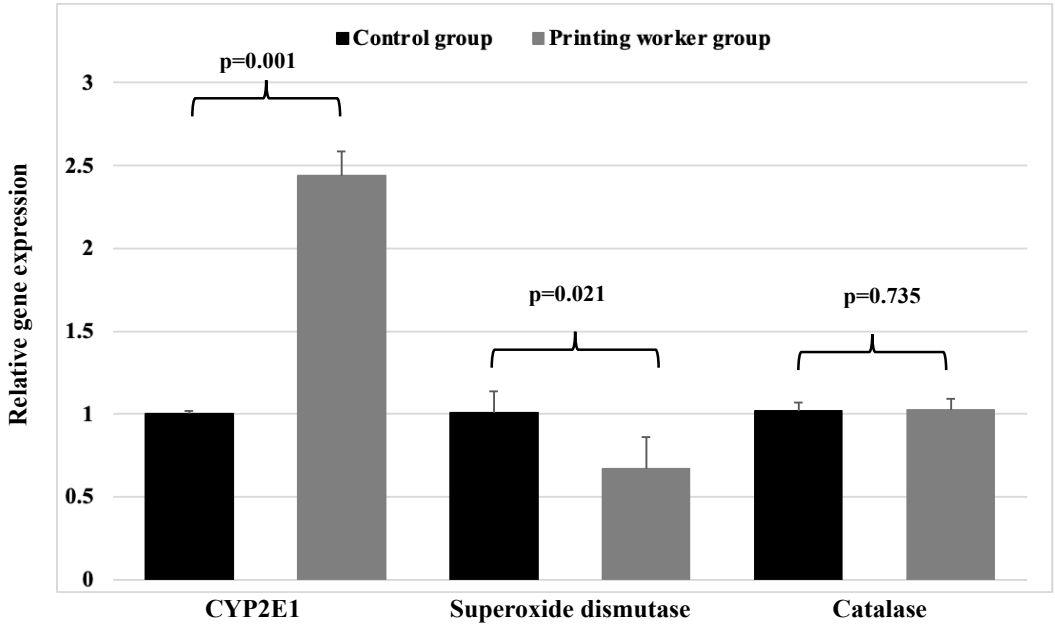

Figure 1. Relative Gene Expression of CYP2E1, SOD, and CAT in Groups Study

Table 1. Characteristics of The Study Population

Characteristic	Printing worker group N= 25	Control group N=22	p*
Age (years) Mean ± SD	35.5 ± 11.05	35.6 ± 13.2	0.461
Male/Female	16/9	14/8	
Working time (year) Mean ± SD	6.9 ± 6.0	6.6 ± 3.6	0.212
Hours per week worked	40	40	
Alcohol consumption	10/25(40%)	10/22 (45%)	
Symptoms	15 (60%)	0 (0%)	
Feeling tired n (%)	6 (24)	0	
Headache n (%)	3 (12)	0	
Sore throat n (%)	2 (8)	0	
Dizziness n (%)	2 (8)	0	
Irritation of eyes n (%)	1 (4)	0	
Others n (%)	1 (4)	0	

*Statistical significance was assumed when p<0.05

Table 2. Hematological Parameters and Cell-free DNA Plasma Concentration

Parameter	Normal value	Printing worker group n=25	Control group n=22	p*
		Mean ± SD	Mean ± SD	
White Blood Cells (x10^9/L)	3.5 - 10.8	6.8 ± 1.9	7.1 ± 1.8	0.639
Red Blood Cells (x10^9/L)	4.4 - 6.2	5.1 ± 0.4	5.3 ± 0.5	0.028
Hemoglobin (g/dL)	13.8-18.7	14.4 ± 0.9	15.9 ± 1.2	<0.0001
Hematocrit (%)	35.4 - 49.4	43.8 ± 5.4	47.5 ± 3.4	0.001
MCV (fL)	84.1 - 100	86.7 ± 3.9	87.7 ± 2.4	0.287
MCH (pg)	27.1 - 33.5	28.2 ± 1.1	28.2 ± 0.9	0.867
MCHC (%)	32 - 35	32.6 ± 0.5	32.7± 0.9	0.841
Platelets (x10^9/L)	147 - 431	206 ± 41.9	258 ± 59.7	0.001
Cell-Free DNA (ng/µl)		131.1 ± 109.3	38.5 ±15.6	<0.0001

*T test for independent samples. Statistical significance was assumed when p<0.05

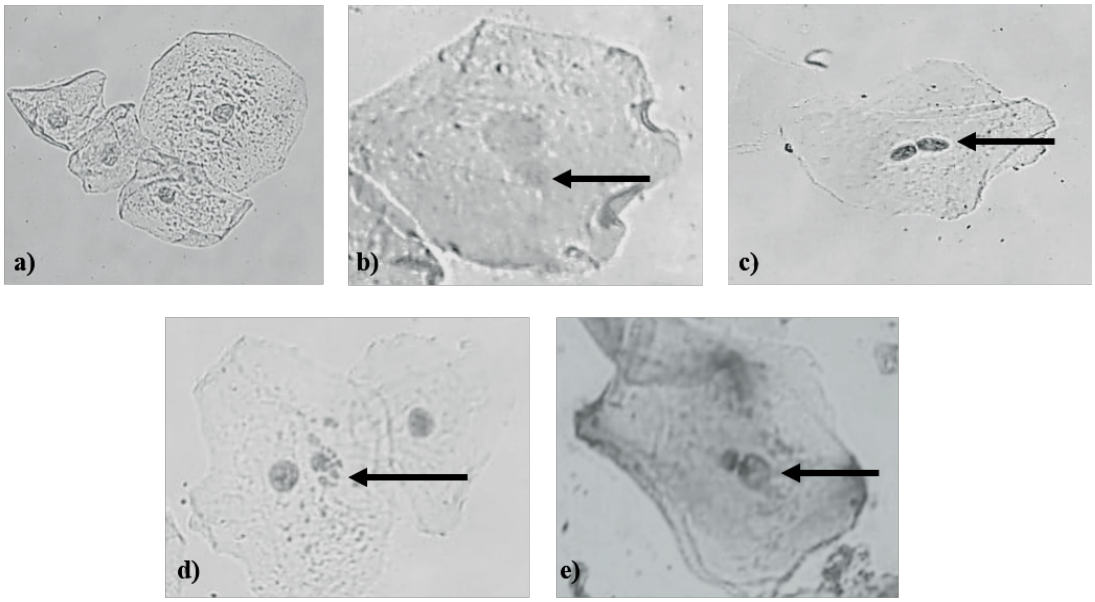

Figure 2. Main Nuclear Anomalies Seen in Buccal Epithelial Cells. a, Normal cells; b, Cell with micronucleus (arrow); c, Binucleated cell; d, Cell with karyorrhexis (arrow); e, Cell with nuclear bud.

hematocrit, and platelet values than the control group. The plasma free DNA plasma concentrations were 3-fold higher in the printing worker group than the control group.

Buccal micronuclei assays

The results of the buccal epithelial cell analysis are summarized in table 3 and figure 2. The printing workers group showed significantly higher frequencies of cells with MN, cells with nuclear buds, binucleated cells, and karyolytic cells, than the control group. Figure 2 shows the main nuclear anomalies seen in exfoliated buccal cells analyzed here.

Discussion

In this study, we evaluated the expression level of CYP2E1 and its effects in antioxidant defenses, and cellular toxicity in printing workers. The study population worked 40 hours per week, had an average work history of 8 years, were young, and were predominantly male.

Table 3. Frequency of Micronucleus and Nuclear Anomalies in Oral Mucosa Cells

Characteristics	Printing worker group N= 25	Control Group N= 22	p**
	Mean* ± SD	Mean* ± SD	
Micronuclei	2.17 ± 1.5	0.32 ± 0.47	< 0.001
Binucleation	5.64 ± 3.2	0.90 ± 0.56	< 0.001
Karyorrhexis	0. 08 ± 0.21	0.02± 0.10	0.125
Karyolyisis	0.55 ± 0.63	0.09 ± 0.33	0.002
Condensed chromatin	0. 03 ± 0.15	0.00 ± 0.00	0.182
Lobed core (broken eggs)	0.32 ± 0.48	0.04 ± 0.15	0.01

*, Mean value in 1000 cells;**, T test for independent samples; statistical significance was assumed when p<0.05

The printing worker group presented mucosa irritation, headache, and tiredness symptoms, which have been ascribed in printing workers to exposure with VOCs and their synergistic interaction when mixed (Alabdulhadi et al., 2019; Decharat, 2014).

The evaluation of potential chemical risk in the workplace, according to the hierarchical method of health risks, showed that ethyl alcohol, isopropyl alcohol, and isophorone represented 91% of the accumulated potential risk. In concordance with this conclusion, a recent study showed that alcohols are the most prevalent class of VOCs in the printing industry (Alabdulhadi et al., 2019). In addition, a recent report has determined quantitatively different volatile organic compounds from blood samples from printing press workers and has evidenced higher concentrations of ethyl alcohol and isopropyl alcohol than other VOCs (Yaqub et al., 2020).

The analysis of CYP2E1 gene expression in the study groups revealed a higher CYP2E1 expression level in the printing worker group than the control group, and this indicates a detoxifying activity in printing workers. This finding is supported by Yanagiba and colleagues (2016), who demonstrated in a mouse model that the oxidative metabolism of 1,2-Dichloropropane, a solvent which is the main component of the cleaner used by offset printing companies, is exclusively catalyzed by CYP2E1, and this step is indispensable for the manifestation of liver damage (Yanagiba et al., 2016).

Moreover, the results are consistent with other authors, who have demonstrated induction of CYP2E1 gene expression in workers of other industries following exposure to toluene (Mendoza-Cantú et al., 2006), trichloroethylene (Xu et al., 2016), pesticides (Sharma et al., 2013), styrene (Prieto-Castelló et al., 2010) and vinyl chloride monomer (Wang et al., 2008). However, the overexpression levels showed variation, which could be due to the differences in exposure time, chemical concentration, and specific type of chemical compound.

Because it is known that CYP2E1 is capable of inducing reactive oxygen species (ROS), which can initiate the disease process in chronic exposure (Gonzalez, 2005), we therefore evaluated the SOD and CAT gene expression, being antioxidant enzymes that have been used as indicators of oxidative stress (Ighodaro and Akinloye, 2018). The results showed that while SOD expression was 0.5-fold lower in the printing worker group than the control group, no significant changes in CAT gene expression between groups was detected.

The study of SOD and CAT antioxidants in printing workers is limited (Huang et al., 2005), although reports in other industries have been documented, with findings of reduced antioxidant activity in workers exposed to chemical compounds (Huang et al., 2005; Xotlanihua-Gervacio et al., 2005; Costa-Amaral et al., 2019). In this study, a low SOD level was detected in printing workers, which could reflect a dejected mechanism to remove oxidants and may be associated with a higher level of oxidative stress from constant exposure to chemical compounds.

The imbalance between SOD and CAT level expression could be due to consecutive enzyme action, in which CAT completes the detoxification process initiated by SOD, the first detoxification enzyme and the most powerful antioxidant in the cell (Ighodaro and Akinloye, 2018).

To evaluate the cytotoxic and genotoxic effects in these workers, hematological analysis was carried out, and both groups showed values within the normal parameters, although the printing worker group showed lower red blood cell and platelet values than the control group, indicating cytotoxic damage. Other reports on printing workers have also described similar red blood cell alterations, as evidenced by reticulocytosis (Song et al., 2017), elevated red blood cell distribution (Celik et al, 2013), and low hemoglobin and hematocrit levels (Loh et al., 2003). However, no findings of low platelet values have been reported previously.

In line with the reduction in hematological parameters from the printing worker group, cell-free DNA in plasma showed a 3-fold higher value in the printing worker group compared to the control group, indicating cell damage. Although no findings of cell-free DNA have been reported in the plasma of printing workers, one report detected around 1.5 -fold higher cell-free DNA in plasma from a group exposed to car paints compared to a non-exposed group (Villalba-Campos et al., 2016). The variability of effects could be due to the differences in exposure time, chemical concentration, and specific type of chemical compounds.

The buccal mucosa contains cells that are the first to encounter different potentially carcinogenic products, and these cells are susceptible to damage by these agents before manifesting any systemic condition (Thomas et al., 2009) so we accordingly analyzed MN cells and nuclear abnormalities from oral mucosa in workers. The Buccal MN assay has been used to measure biomarkers of DNA damage (MN and/or nuclear buds), cytokinetic defects (binucleated cells) and proliferative potential (basal cell frequency), and/or cell death (condensed chromatin, karyorrhexis, pyknotic, and karyolytic cells) (Thomas et al., 2009). We detected that the printing worker group had significantly higher frequencies of cells with MN, cells with nuclear buds, binucleated cells, and karyolytic cells than the control group, indicating DNA damage, cytokinetic defects, and cell death in the printing worker group. MN assays have been explored in textile printing workers from peripheral blood lymphocytes, and similarly the authors founded a high frequency of MN in printing workers compared to a control group (Aksoy et al., 2006; Sellappa et al., 2010).

The oxidative stress, cytotoxic and genotoxic effects in printing workers evidenced here demonstrates the potential health risk for these workers, and highlight the importance of the introduction of eco-friendly replacements for toxic substances when possible, and the introduction of prevention measures at the workplace, including wearing protective equipment, and improvement of ventilation systems.

Important limitations in this study include a low size sample and the lack of quantitative assessment of chemical compounds.

Finally, because solvents used in the printing industries are usually mixtures of chemicals rather than a singles

substance, the results should be interpreted with caution.

Author Contribution Statement

Brenda Ivonn Rodríguez Romero: MSc conceived the study and collected the data analyzed the data and drafted the manuscript; Maritere Domínguez Rojas: PhD collected data; Maria Olivia Medel Flores: PhD collected data; Nadia Pérez-Vielma: PhD analyzed the data and drafted the manuscript; Mario Mendoza-Garrido: MSc collected data; Virginia Sánchez Monroy: PhD conceived the study analyzed the data and drafted the manuscript. All authors read and reviewed the final manuscript.

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Ethical Declaration

The research protocol was approved by the Bioethics Committee of "ENMH-IPN" (Approval number CBE/022/2019) as thesis of student Brenda-Ivonn Rodríguez-Romero

Approval

The research protocol was approved by the Bioethics Committee of of "Escuela Nacional de Medicina y Homeopatía- Instituto Politécnico Nacional (ENMH-IPN)" (Approval number CBE/022/2019).

Conflict of Interests

The Authors declare that there is no conflict of interest.

References

Aguilar FJ, Bernaola AM, Gálvez PV, et al (2010). Hierarchization of risks in "Chemical Risk Systematics for Hygienic Evaluation". 1st ed. Madrid, España: INSHT, pp 51-4.

Aksoy H, Yilmaz S, Çelik M, Yüzbaşioglu D, Unal F (2006). Genotoxicity study in lymphocytes of offset printing workers. *J Appl Toxicol*, **26**,10-5.

Alabdulhadi A, Ramadan A, Devey P, Boggess M Guest M (2019). Inhalation exposure to volatile organic compounds in the printing industry. *J Air Waste Manag Assoc*, **69**, 1142-69.

Celik A, Aydin N, Ozcirpici B, et al (2013). Elevated red blood cell distribution width and inflammation in printing workers. *Med Sci Monit*, **19**, 1001–5.

Chen J, Jiang S, Wang J, et al (2019). A comprehensive review of cytochrome P450 2E1 for xenobiotic metabolism. *Drug Metab Rev*, **51**, 178-95.

Costa-Amaral IC, Carvalho LVB, Santos MVC, et al (2019). Environmental assessment and evaluation of oxidative stress and genotoxicity biomarkers related to chronic occupational exposure to Benzene. *Int J Environ Res Public Health*, **16**, 2240.

Decharat S (2014). Prevalence of acute symptoms among workers in printing factories. *Adv Prev Med*, **2014**, 854052.

Gonzalez FJ (2005). Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *Mutat Res*, **569**, 101-10.

Guengerich FP (2020). Cytochrome P4502E1 and its roles in desease. *Chem Biol Interact*, **322**, 109056

Guo NL, Bello D, Ye Q, et al (2020). Pilot deep RNA sequencing of worker blood samples from Singapore printing industry for occupational risk assessment. *NanoImpact*, **19**, 100248.

Hammer KD (2002). Metabolite ratio of toluene-exposed rotogravure printing plant workers reflects individual mutagenic risk by sister chromatid exchanges. *Mutat Res*, **519**, 171-7.

Hormozi M, Mirzaei R, Nakhaee A, et al (2019). Quantification of urinary metabolites of toluene and xylene isomers as biological indices of occupational exposure in printing industry workers. *Health Scope*, **8**, e8296.

Huang PL, Zhang L, Zhang SH, Wang YM (2005). Effect of pollutants of press-pack industry on antioxidants level of worker. *Wei Sheng Yan Jiu*, **34**, 747-8.

Ighodaro OM, Akinloye OA (2018). First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defense grid. *Alexandria J Med*, **54**, 287-93.

International Agency for Research on Cancer (IARC) (1996). Printing processes and printing inks, carbon black and some nitro compounds, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 65. Lyon, France: World Health Organization.

Ito Y, Nakaya T, loka A, et al (2016). Investigation of spatial clustering of biliary tract cancer incidence in Osaka, Japan: Neighborhood Effect of a Printing Factory. *J Epidemiol*, **26**, 459-63.

Khatri M, Bello D, Martin J, et al (2017). Chronic upper airway inflammation and systemic oxidative stress from nanoparticles in photocopier operators: Mechanistic insights. *NanoImpact*, **5**, 133-45.

Kim JH, Moon JY, Park EY, Lee KH, Hong Y (2011). Changes in oxidative stress biomarkers and gene expression levels in workers exposed to volatile organic compounds. *Ind Health*, **49**, 8-14.

Kubo S, Takemura S, Tanaka S, et al (2017). Occupational cholangiocarcinoma caused by exposure to 1,2-dichloropropane and/or dichloromethane. *Ann Gastroenterol Surg*, **2**, 99-105.

Kvam BM, Romundstad PR, Boffetta P, Andersen A (2005). Cancer in the Norwegian printing industry. *Scand J Work Environ Health*, **31**, 36-43.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, **25**, 402–8.

Loh CH, Shih TS, Liou SH, et al (2003). Haematological effects among silk screening workers exposed to 2-ethoxy ethyl acetate. *Occup Environ Med*, **60**, E7.

Lyu L, Li Y, Ou X, et al (2021). Health effects of occupational exposure to printer emissions on workers in China: Cardiopulmonary function change. *NanoImpact*, **21**, 100289.

Mendoza-Cantú A, Castorena-Torres F, Bermúdez de León M, et al (2006). Occupational toluene exposure induces cytochrome P450 2E1 mRNA expression in peripheral lymphocytes. *Environ Health Perspect*, **114**, 494-9.

Mourad BH (2021). Prevalence of work-related musculoskeletal disorders among Egyptian printing workers evidenced by using serum biomarkers of inflammation, oxidative stress, muscle injury, and collagen type I turnover. *Toxicol Ind Health*, **37**, 9-22.

Prica M, Kecić V, Adamović S, Radonić J, Turk Sekulić M (2016). Occupational exposure to hazardous substances in printing industry. Proceedings of 8th International Engineirng

Symposium at Banki, pp 1–8.
Prieto-Castelló MJ, Cardona A, Marhuenda D, Roel Jm, Corno A (2010). Use of the CYP2E1 genotype and phenotype for the biological monitoring of occupational exposure to styrene. *Toxicol Lett*, **192**, 34-9.
Sellappa S, Prathyumnan S, Joseph S, Keyan KS, Balachandar V (2010). Genotoxic effects of textile printing dye exposed workers in India detected by micronucleus assay. *Asian Pac J Cancer Prev*, **11**, 919-22.
Sharma RK, Upadhyay G, Siddiqi NJ, Sharma B (2013). Pesticides-induced biochemical alterations in occupational North Indian suburban population. *Hum Exp Toxicol*, **32**, 1213-27.
Song H, Yu IT, Lao XQ (2015). Neurobehavioral effects of occupational exposure to organic solvents among male printing workers in Hong Kong. *Arch Environ Occup Health*, **70**, 147-53.
Song SH, Kang SK, Choi WJ, et al (2017). Reticulocytosis in screen-printing workers exposed to 2-butoxyethanol and 2-ethoxyethanol. *Ann Occup Environ Med*, **29**, 54.
Sul D, Lee D, Im H, et al (2002). Single strand DNA breaks in T- and B-lymphocytes and granulocytes in workers exposed to benzene. *Toxicol Lett*, **134**, 87-95.
Takeuchi A, Ogawa Y, Endo Y, et al (2015). Evaluation of urinary cyclohexanediols and cyclohexanol as biomarkers of occupational exposure to cyclohexane. *J Occup Health*, **57**, 365-70.
Thomas P, Holland N, Bolognesi C, et al (2009). Buccal micronucleus cytome assay. *Nat Protoc*, **4**, 825-37.
Villalba-Campos M, Ramírez-Clavijo SR, Sánchez-Corredor MC, et al (2016). Quantification of cell-free DNA for evaluating genotoxic damage from occupational exposure to car paints. *J Occup Med Toxicol*, **11**, 1–9.
Wang AH, Zhu SM, Qiu YL, et al (2008). CYP2E1 mRNA expression, genetic polymorphisms in peripheral blood lymphocytes and liver abnormalities in Chinese VCM-exposed workers. *Int J Occup Med Environ Health*, **21**,141-6.
Xotlanihua-Gervacio MDC, Guerrero-Flores MC, Herrera-Moreno JF, et al (2018). Micronucleus frequency is correlated with antioxidant enzyme levels in workers occupationally exposed to pesticides. *Environ Sci Pollut Res Int*, **25**, 31558-68.
Xu X, Ke Y, Yuan J, et al (2016). Trichloroethylene-induced hypersensitivity dermatitis was associated with hepatic metabolic enzyme genes and immune-related genes. *Toxicol Res (Camb)*, **5**, 633-40.
Yamada K, Kumagai S, Kubo S, Endo G (2015). Chemical exposure levels in printing and coating workers with cholangiocarcinoma (third report). *J Occup Health*, **57**, 565-71.
Yanagiba Y, Suzuki T, Suda M, et al (2016). Cytochrome P450 2E1 is responsible for the initiation of 1,2-dichloropropane-induced liver damage. *Toxicol Ind Health*, **32**, 1589-97.
Yaqub G, Hamid A, Khan N, et al (2020). Biomonitoring of workers exposed to volatile organic compounds associated with different occupations by headspace GC-FID. *J Chem*, **2020**, 6956402.