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Occupational Diseases and Illnesses among Workers in Paint Industry in Pathumthani Thailand

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Abstract

Over the past several decades, around the world, Thailand has seen tremendous growth in key industries such as tourism, healthcare, petrochemicals, and oil. More than four million people worldwide are exposed to different chemicals due to different industries. Volatile organic compounds, in particular, are one of the important chemicals in the oil and paint industry. Volatile substances are present in workplaces and urban areas in varying concentrations. The compounds of Pb with Cr (chromium), Mo (molybdenum) Cl (chlor) have been extensively used as "chrome" pigments. PbCrO4 compound is used extensively in the paint industry to obtain the yellow-chrome colour, Pb (OH) 2·2PbCO3 is used to obtain white tin colour, while Pb3O4 is used to obtain red tin colour.

Key words: Paint industry, Workers, illness and Occupational diseases

Introduction

An overview of color exposure can be obtained from human tissues and fluids such as blood, hair, teeth, bone and urine. Screening for and diagnosing lead exposure But it can also be used as a direct indicator related to the amount of lead entering the body. In general, it is known that components of color exposure can pose health risks, such as central and peripheral nerve damage. Cardiovascular system hematopoietic system, kidneys, digestive system and reproductive system It has been listed as a carcinogen. Research on dye-treated hematology is very important. Because red blood cells in the bone marrow and blood are the main targets in adults. The paint may cause hemolysis and inhibit hemoglobin formation. Paints and its components can induce G-6PD deficiency and pyrimidine- 5'-nucleotidase inhibition in mature erythrocytes. This leads to a decrease in the lifespan of red blood cells and increases the vulnerability of red blood cell membranes. Decreases the number of red blood cells According to Barbosa 2005, the enzyme deficiency is characterized by the presence of basophilic erythrocytes. A study conducted by Richard 2006 showed that lead can inhibit heme synthesis through inhibition of the enzymes δ-ALAD, coproporphyrinogen and ferrochelatase, which causes the concentration of hemoglobin in the blood to decrease To complete the study results, enzyme concentration should be measured (Meyer, 2008).

According to a recent study, occupational exposure to paint may lead to an increased risk of several cancers including lung cancer bladder and pancreas and tumors in the lymphatic and hematopoietic system. The findings are consistent with a 1989 report released by the International Agency for Research on Cancer that classified painting as a cause of occupational-related cancer. It provides further evidence that the risk of certain cancers is increased by exposure in the paint manufacturing process. However, occupational exposure in paint manufacturing is not classed as a carcinogen (Brown, 2002; Lundberg, 1998; IARC, 1989). Thousands of chemicals are used in the manufacture of paint products, such as pigments, extenders, binders, additives and solvents (toluene, xylene, ketones, alcohols, esters and glycol). (Ether) Paint production workers may be exposed to chemicals found in paint products, although the pattern and level of exposure to each material may differ from that of the painter, exposure period and industry.

Little information is available about the genetic effects associated with color exposure to explain positive and negative outcomes. Higher values of chromosomal aberrations (CAs), sister chromatid

exchange (SCE), micronucleus (MN) (in lymphocytes and oral mucosa cells) and DNA damage were detected. Tests of comet in white blood cells have been reported for workers exposed to automotive coatings and painters in general. In addition, Diaz et al. described an increase in MN in the peripheral lymphocytes and oral mucosa of paint industry workers in Cuba that dust and fumes of lead-containing paints destroy chromosomes This resulted in a substantial increase in the level of inheritable CA in painters. the contrary Cárdenas-Bustamante examined the levels of exposure to organic solvents and their associated genetic effects in paint factory workers using cytogenetic monitoring (MN test and comets), with no statistical difference in genetic biomarkers between the exposed and non-exposed workers (Madhavi 2008).

Cytogenetic analysis of peripheral blood lymphocytes has been recognized as a suitable technique for the biological investigation of genetic damage in somatic cells since the early 1970s. In the present study, we used the MN assay in stripped epithelial cells and single-cell gel electrophoresis (SCGE) or comet assays, Due to the advantages of these systems in screening for DNA damage caused by mutations in the environment. MN are acentric chromosome fragments or whole chromosomes delayed during mitotic cellular division, and appear in the cytoplasm of interphase cells as small additional nuclei. The MN test is faster and easier than metaphase analysis and it can be used both *in vivo* and *in vitro* in a variety of cells. This assay has also been shown to be a reliable and sensitive biomarker for human biomonitoring. The frequency of MN in human exfoliated cells is considered a useful biomarker of genotoxic effects in populations exposed to genotoxicants, through direct contact with ingested or inhaled compounds (Collins, 2008).

In brief, in the Comet assay, a simple and sensitive method for studying DNA damage and repair, cells are embedded in agarose on a microscope slide. Lyophilized with detergent and high salt to form supercoiled nuclei, loops of nuclear matrix-linked DNA are then electrophoresised in an alkaline medium.

In cells with increased DNA damage, the result is a comet-like structure as DNA migrates from the nucleus which is an event that can be observed under a microscope. The intensity of the comet's tail relative to its head reflects the amount of DNA fragmentation. A possible basis for this is that the loop with the divider loses supercoiling and is free to extend towards the anode as with other genotoxicity tests the comet test does not predict the risk of individual cancers. Rather, it is a useful tool to assess the effects of genotoxicity early. It can also be corrected due to exposure from work or the environment. (Møller, 2009)

Materials Methods

Study population and sample collected

This study was approved by the institute Ethical Committee on Research and informed written consent was obtained from each individual prior to the start of the study. The study included 63 workers of a paint manufacturing company employed in the sectors where they were occupationally exposed to solutions containing organic mixtures. The control group consisted of 30 healthy workers with no occupational exposure

All participants were fully informed about the purpose of the study. Written informed consent was obtained from each participant after the consent form was read by the participants. The consent form was in Thailand, the local language and in English, and it stated that the participation was completely voluntary and that the participant could withdraw at any time from the study. Confidentiality was maintained throughout the study. During data collection, each person was identified by giving them a unique identification number. The participant was required to enter their name only while signing for written consent.

All the individuals examined in the study were required to answer a Thai version of a questionnaire from the International Commission for Protection against Environmental Mutagens and Carcinogens

and participate in a face-to-face questionnaire which included standard demographic data (age, gender,) as well as questions relating to medical issues (exposure to X-rays, vaccinations, medications), life style (smoking, coffee, alcohol, diet,) and their occupation (number of hours worked per day, time exposed to organic solvents, use of protective measures). Individuals were selected for the two groups (control and paint exposed) in such a manner so as to ensure that except for occupational exposure to organic solvents, there were no marked differences between the members of the groups. Individuals who smoked more than five cigarettes per day for at least 1 year were considered smokers. The characteristics of the two groups are presented in.

Blood and urine samples were obtained from individuals in the two groups on the same day at the end of a normal shift during the workers' periodical medical examinations. All blood samples were collected using venipuncture and heparinized vacutainers and processed as quickly as possible to avoid the damage associated with storage. The blood cell samples were transported to the university laboratory at or below 8°C and processed within 5 h of collection.

Hippuric acid analysis

As a biomarker of toluene exposure, 50 mL of urine samples were collected at the end of the working day and analyzed for hippuric acid (HA) using high performance liquid chromatography (HPLC) with UV-VIS detector in a commercial laboratory

Analysis of Hematological parameters

The following hematological markers were measured: leukocytes (granulocytes, lymphocytes, and monocytes), erythrocytes, hemoglobin, hematocrit. All blood tests were analyzed in a laboratory according to standard hematological methods.

Genotoxicity tests

Comet assay in peripheral blood leukocytes

The alkaline Comet assay was performed as described by Singh *et al*., with the modifications suggested by Tice *et al*. Samples of 5 μL of whole peripheral blood were embedded in 95 μL of 0.75% low-melting point agarose and added to a microscope slide (two slides per donor) precoated with normal agarose (1.5% buffer solution). When the agarose solidified the slides were placed in lysis buffer (2.5 MNaCl, 100m MEDTA and 10 mM Tris; pH 10.0–10.5) containing freshly added 1% (v/v) Triton X-100 and 10% (v/v) dimethylsulfoxide (DMSO) for a minimum of 1 h and a maximum of 2 weeks. After treatment with lysis buffer, to allow DNA unwinding, slides were incubated in a freshly made alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA; pH > 13) for 20 min in a horizontal electrophoresis tank and the DNA was electrophoresed for 20 min at 25 V (0.90 V/cm) and 300 mA. Every step was carried out under indirect yellow light. After electrophoresis, slides were washed three times in a neutralization buffer (0.4 M Tris; pH 7.5) for 5 min, rinsed three times in distilled water, and left to dry overnight at room temperature. Slides were stained with silver nitrate as previously described by Villela *et al*. the slides were fixed for 10 min in trichloroacetic acid 15% w/v, zinc sulfate 5% w/v, glycerol 5% v/v, rinsed three times in distilled water, and dried for 2 h at 37°C. The dry slides were re-hydrated for 5 min in distilled water, and then stained (sodium carbonate 5% w/v, ammonium nitrate 0.1% w/v, silver nitrate 0.1% w/v, tungstosilicic acid 0.25%, formaldehyde 0.15% w/v, freshly prepared in the dark), and constantly shaken for 35 min. The stained slides were rinsed twice with distilled water, and submerged in the stop solution (acetic acid 1%), rinsed again, and immediately coded for analysis in an optical microscope. Images of 100 randomly selected cells were analyzed per individual. Cells were scored visually into five classes, according to tail size and shape (from undamaged – 0, to maximally damaged – 4), and a value (damage index (DI)) was assigned to each Comet according to its class. DI thus ranged from 0 (completely undamaged: 100 cells×0) to 400 (with maximum damage: 100 cells×4.The damage frequency (DF) (%) was calculated based on the percentage of damaged cells (0–100%). International guidelines and recommendations for the Comet assay consider that visual scoring of comets is a well-validated evaluation method. It

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has a high correlation with computer-based image analysis. Negative controls were processed together with workers' samples and analyzed by one investigator. (Carrano, 1988; Singh, 1988; Tice, 2000; Villela, 2006)

Comet assay in epithelial buccal cells

Buccal mucosa cells were obtained by swabbing the left inner cheek with a cervical brush. The cells were washed with a phosphate buffer solution and centrifuged at 800 rpm for 10 min. Then, 20 μL of the pellet was resuspended in 80 μL of 0.75% low-melting point agarose. The Comet assay was then performed as described above.

MN test in epithelial buccal cells

An exfoliated buccal mucosa cell were collected by swabbing the right inner cheek of the individuals with a moistened wooden tongue depressor and was smeared over clean slides containing two drops of physiological solution. Cells were fixed in a methanol–acetic acid (3:1) solution for 10 min, dried at 50°C in a chamber for 5 min, and stained with Giemsa 5% (phosphate buffer solution, pH 5.8). Then the slides were washed in distilled water and stained with Fast green for 1 min, washed again, and stained with total Giemsa for 1 min. After this, they were washed in distilled water again and dried at room temperature.

The criteria used for MN analysis were those of Tolbert *et al*. and Titenko-Holland *et al*., i.e. for a structure to be considered as a micronucleus it must be: (a) less than one third of the diameter of the main nucleus; (b) be in the same plane of focus as the main nucleus e; (c) have the same color, texture, and refraction as the main nucleus; (d) have a smooth oval or round shape; and (e) be clearly separated from the main nucleus. Only cells that were not smeared, clumped or overlapped, and those who contained intact nuclei were included in the analysis. According to Tolbert *et al*. and Gomez-Arroyo *et al*.,exfoliated buccal cells undergo degenerative processes which can produce anomalies that are difficult to distinguish from MN (binucleates, pycnosis, karyorrhexis, and karyolysis). In our study, these were excluded from the micronucleus analysis and all the slides were coded to blind analysis. The MN frequency was estimated based on the number of normal exfoliated buccal cells counted using a bright-field Zeiss microscope at a magnification of 1000×. For each volunteer, 2000 buccal cells (i.e. 1000 from each of the duplicate slides) were scored. (Tolbert, 1992; Titenko-Holland, 1998; Gomez-Arroyo, 2000)

From our knowledge Cytogenetic data relevant to paint industry workers in Thailand have not been published with reference to Pathumthani area. Therefore, the objective of this study was to assess the genotoxicity risk of these workers using the comet leukocyte assay and oral mucosa cells and MN assay in oral mucosa cells.

Statistical analysis

The normality of variables was evaluated by the Kolmogorov–Smirnov test; χ^2 and t-tests were used to compare the demographic characteristics of study populations. The statistical analysis of differences in HA, MN test, and DNA damage measured by the Comet assay were carried out using the non-parametric Mann–Whitney U-test. Correlations between different variables were determined by the Spearman rank correlation test, when appropriate. The critical level for rejection of the null hypothesis was considered to be a two-tailed *P* value of 5%. All analyses were performed using Graph Pad Prism for Windows, Graph Pad Software, San Diego, California, USA,

Results

Table 1: demographic characters of exposed group in paint industry.

Age	Number of cases n (%)
20-25	9 (14.28)
26-30	14 (22.22)
31-35	22 (34.92)

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36-40	6 (9.52)
41-45	2(3.17)
46-50	1 (1.587)
51-55	0
56-60	0
61 and above	0
Gender	
Male	56(88.89)
Female	7(11.11)
Smoking habit	
Smokers	49(69.84)
Non smokers	14(22.22)
Duration of exposure years	
1-5	21(33.33)
6-10	27(42.86)
11-15	11(17.46)
16-20	4(6.35)

The main characteristics of the two groups studied are presented in table 1. No significant differences were observed between the mean age of subjects in the different groups (Student's t-test). Smoking habits, in the exposed group just three subjects smoking an average of 9.42 ± 3.98 cigarettes per day, while in the control group all individuals were non-smokers. The duration of exposure in the exposed group was 9.86 ± 5.11 years, ranging from 1 to 20 years. Analysis of questionnaires revealed that all paint industry workers used silicone gloves to prevent skin contact with organic solvents, glasses, and breathing masks. We also observed that the work areas in the company were equipped with ventilation devices.

Table 2 Haematological parameters in study groups (Mean ± SD)

Parameter	Control group 30	Exposed group 63	*P*
Granulocytes (%)	54.18±8.21	50.89±6.91	0.0464*
Lymphocytes (%)	42.71±8.1	40.09±6.93	0.1102
Monocytes (%)	3.26±0.97	3.46±1.54	0.5164
Leukocyte count ($x10^6$/ µl)	5.64±3.72	6.55±1.14	0.0780
Erythrocytes (x 10^3/ µl)	3.64±0.10	3.66±1.10	0.9213
Hematocrit (%)	45.24±2.67	44.11±2.54	0.0516
Haemoglobin (g/dl)	14.69±1.56	13.65±1.62	0.0043*
Students *t* test			
Hippuric acid (g/g creatinine)			
($P \leq 0.05$, Mann–Whitney U-test).	0.19±0.10	0.39±0.21	0.0001**

In relation to the hematologic parameters, Table 2 reported that no significant differences were found between the two groups and both groups exhibited normal hematological values similar to reference values observed in the literature for other populations as described by several investigators. The comparison of the mean values (g/g creatinine) of urine HA level of the control and exposed group is shown in table 2, A significant increase in HA levels was observed in the exposed group relative to

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the controls, Hippuric acid (HA) concentration (mean ± SEM) in urine of control and exposed group. Significant difference relative to the control group at $P \leq 0.05$ (Mann-Whitney U-test, two-tail)

Table 3 Mean values (± SD) obtained by MN test and Comet assay in control (n =30) and exposed (n= 63) groups

	MN in 2000 epithelial buccal cells	Comet test in 100 epithelial buccal cells		Comet essay in 100 leukocytes	
		Dqamage frequency	Damage index	Damage frequency	Damage index
Control group	3.12±2.54	12.65±8.91	17.95±17.65	2.82±2.47	2.78±2.54
Exposed group	6.72±73.89	24.63±17.36	35.63±31.24	6.44±5.12	6.95±6.26

Table 3 presents data obtained using three cytogenetic assays for the control and exposure groups and analyzed using the Mann-Whitney U-test for testing micronuclei in buccal cells. No significant differences were found between workers in the regulated industry and the paint industry.

The comet assay in buccal epithelial cells showed that the observed DI and DF in the exposed group were significantly higher than those in the control group ($P \leq 0.05$). The Comet test data in peripheral blood leukocytes showed that the two analytical parameters of this test (DI and DF) were significantly greater than those for the control group. There were also no significant differences between smokers and non-smokers in the exposure groups for all cytogenetic analyses.

Discussion

Many methods and techniques have been developed to track human populations exposed to mutagens in the environment. The traditional approach is to use existing blood cells (such as lymphocytes and red blood cells) as biomarkers to record mutagenic effects. Although long-term disease from the affected blood cells is not expected, long-term disease is not expected. But it is generally accepted that blood cells can be used as sentinel cells to provide early warning signals for adverse health effects. It is also suitable to determine whether the biomarker effects observed in blood cells are consistent with existing target cells. In this paper, we present a study using two cell types and two genotoxicity assays to assess the occupational risks of workers in the paint industry. Paint industry workers are exposed to a complex mix of organic solvents, heavy metals such as lead, zinc, chromium, cadmium and other compounds. Many others have mutagenic properties such as phthalic acid and chlorophenol. Since the main solvent contained in the organic solvent mixture used in the paint production process is toluene. We therefore analyzed the urinary HA concentrations of workers in the paint industry. Our data indicate a higher mean concentration of toluene present in the urine of these workers relative to the control group. And these levels confirmed exposure to toluene in paint industry workers. However, the observed HA values for all subjects may be considered low according to NR-7 (up to 1.5 g/g creatinine). However, Pelclova et al. found similar results in printers exposed to toluene in Poland. The urinary HA of this cohort of printers was significantly higher than that of the control group. However, the level remained below the Czech limit for occupational preferences (i.e., 2.5 g/L of urine). In addition, de Rosa et al. conducted a follow-up of volunteers working at printing companies exposed to toluene. Using urine samples collected before and after shifts to determine HA, they found several correlations between the levels of HA in urine and environmental samples of toluene collected at the company required for evaluating even low levels of toluene exposure (Norma, 1994, Pelclova 2000). Some of the biological effects of exposure to organic solvents are hematological alterations. These effects may result in reduced production of red blood cells, white blood cells and platelets. Beving et al with painters in Sweden. The values obtained in our study for hematological parameters showed no significant differences between the control and exposure groups.(Descatha 2005, Pinto 2000, Beving 1991)

The influence of age, gender and smoking on DNA damage is a well-known issue in industry investigations. However, in this study these factors may be excluded. in both groups similar average age everyone is a man and only three of the workers had a habit of smoking. Although in this case The values obtained from the cytogenetic test were not different from the control group. Similarly, Silva et al. 1996 concluded that smoking behavior was not a significant factor in terms of the production of different types of chromosomal abnormalities. Found in a career pursuit with a car mechanic. The results obtained in this study demonstrated no exposure-related induction of MN in buccal epithelial cells of workers exposed to solvents in the paint manufacturing industry. Although there have been few studies with paint workers. But data reported using these cells indicate positive results. The use of the MN assay in exfoliated cells has increased dramatically. This is because it is considered a useful biomarker of genotoxic effects in populations exposed to genotoxic agents through direct exposure to ingested or inhaled compounds. Keeping in mind that epithelial cells are highly proliferative and account for more than 90% of cancers, the use of these cells in bioassays can be very useful. On the other hand, the comet test values for paint industry workers were significantly higher than those for the control group in both blood and buccal cells. A positive result in the Comet test is not always consistent with a positive result in the MN test, especially when exposure to genotoxic agents is low. Comet tests tend to detect more defects than MN tests. Positive results in the Comet assay and MN assay are due to different mechanisms. MN assay detects an injury that can survive at least one mitotic cycle. Whereas the comet test identifies a fixable injury or an alkali-labeled location for this reason, Goethem et al.recommend both the MN test and the Comet test. (Goethem 1997, Vrzoc 1997)

Possibility of cytogenetic damage in different occupations The increasing use and diversity of solvents has raised concerns about the efficacy of organic solvents. Possible risks of exposure in the workplace several previous reports have pointed to detrimental effects on those exposed to the paint and its components. Madhavi et al. reported that exposure to occupational lead-containing paints was associated with an increased frequency of CA in workers compared to controls. A follow-up study was designed by Pinto et al. to determine the occupational exposures of outdoor painters. Painters showed more CA and SCE in lymphocytes and MN in oral epithelial cells than in the control group. Diaz et al. analyzed MN lymphocytes and oral mucosa cells in 21 Cuban paint industry workers. Work and control group another study reporting CA in lymphocytes from car painters in Brazil showed that the frequency of aneuploidies and chromosomal deletions in the peripheral lymphocytes of car painters was significantly higher than in the control group important. The MN test and the Comet test were applied to the peeled buccal cells to assess the genetic risk associated with exposure to 10 automotive painters by Martino-Roth with significant occupational exposure in both the MN test and the Comet test. On the other hand, Cardenas-Bustamante investigated the degree of exposure to organic solvents and associated genetic effects in paint factory workers. MN frequencies in lymphocytes and DNA damage were identified using Comet tests. They found no statistical differences in genetic biomarkers between exposed and non-exposed workers. A major problem in interpreting bio monitoring studies is estimating the level of exposure. Misuse or misuse can lead to significant levels of exposure and under various exposure conditions using several different biomarkers. In this context, it is not surprising that the results obtained by different authors also showed variability. It is therefore difficult to determine the genetic damage of any chemical or compound. Therefore, the DNA damage observed in our study and in all of the above should not be caused by a single compound. It is a cumulative effect of many chemicals used in paint products. Transplacental and lactational exposure arsenic causes significant alterations in steroidogeneic enzymes (Reddy 2010) and chromium hazards to aquatic animals and fishes (Reddy 2017)

Conclusion

In this study Even though paint workers say they use adequate personal protective equipment. However, an increased urinary HA concentration was also observed in conjunction with an increased Comet test value, both for buccal leukocytes. It was evident that the organic solvent levels in the samples were low. This corresponds to non-mutagenicity and the presence of genotoxicity in cells only. It was concluded from this study that occupational exposure to paint may lead to a slightly increased risk of genetic damage among workers in the paint industry due to these considerations and the complex composition of many paints and solvents. Therefore, sanitary measures have been introduced. A better understanding of the parameters involved in cytogenetic damage will greatly reduce the uncertainty in carcinogenic risk assessment among paint industry workers.

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