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Changes in Immunological and Hematological Parameters of Female Residents Exposed to Volatile Organic Compounds in the City of Kaohsiung, Taiwan

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Abstract

The objective of this study was to assess the effects, if any, of volatile organic compounds (VOCs) in the ambient air of Kaohsiung, Taiwan, on certain hematological and immunological parameters of 153 female study participants. The major source of VOCs was vehicle emissions. The participants were selected from three areas, each area at a different distance from a freeway. Results indicated that total concentrations of VOCs and a subgroup of 25 VOCs (VOC₂₅) ranged from 250 to 335 ppb and 89 to 113 ppb, respectively. The distribution of VOC concentrations did not correlate with distance from the freeway. The participants living in the area with higher VOC concentrations had significantly higher abnormalities of white blood cells (WBC) and hemoglobin (Hb). In addition, IgG and IgA counts were significantly lower for the participants in the area with higher VOCs than for participants in the area with lower VOCs. This finding indicates that VOCs in ambient air may suppress immunological variables.

Introduction

Volatile organic compounds (VOCs) are a mixture of various kinds of polycyclic aromatic, aliphatic, alicyclic, and halogenated hydrocarbons. Major constituents include benzene, toluene, xylene, styrene, naphthalene, pyrene, benzo(a)pyrene, butane, octane, hexane, and trichloroethylene. The growing number of automobiles has contributed to significant emissions of VOCs from automobile exhaust into the ambient air, especially in urban areas (Chen, Lai, & Ho, 2003; Hsieh, Chang, & Kao, 1999). Some researchers have found that the concentration of VOCs in ambient air has been highly correlated with traffic density (Bahrami, 2001).

Animal studies have confirmed a dysfunction of the immune system as a result of high VOC exposure (Robinson, Shah, Wong, & Farris, 1997; Farris, Robinson, Wong, Hahn, & Shan, 1997; Snyder & Valle, 1991; Aoyama, 1986). As for humans, some studies on benzene-induced effects have shown adverse immune functions, including alterations of serum immunoglobulins (Dimitrova, Kostadinova, Marinova, Popov, & Panev, 2005), development of antibodies (Dimitrova et al.), and decreased T-lymphocyte numbers (Moszczynsky & Lisiewicz, 1984). These studies have mainly focused on occupational exposure to workers. To date, however, there have been only a limited number of studies

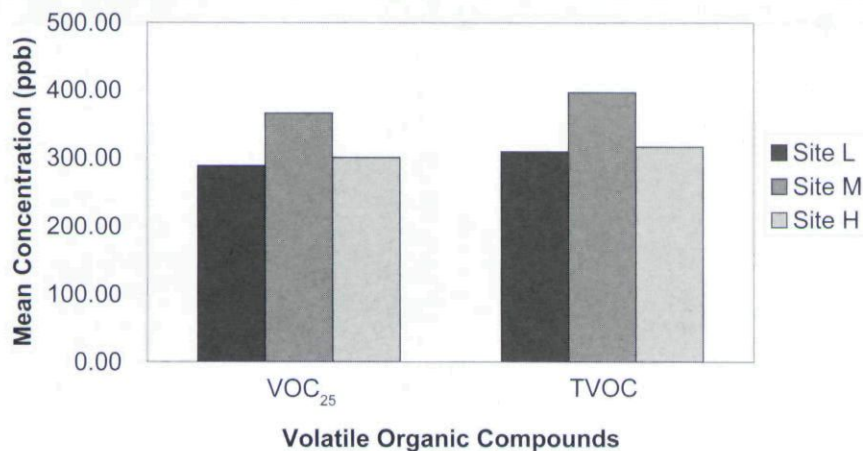
on biomonitoring of human populations exposed to VOCs from automobile exhaust.

Completed blood count values have been used to assess health effects from volatile pollutant exposure. In most previous studies, investigators have reported positive associations between hematological changes in workers and children and VOC exposure (Lee, Yoo, Lee, Kim, & Kim, 2002; Georgieva, Lukanova, Panev, & Popov, 1998; Shaham, Levi, Gurvich, Shain, & Ribak, 2000). However, no such association has been observed in other studies (Collins, Ireland, Easterday, Nair, & Braun, 1997). So far, most studies have emphasized the effects of VOCs on human health through occupational exposure. Furthermore, studied subjects have consisted mainly of men and children (Georgieva et al., 1998; Lee et al., 2002).

Many residents in Taiwan live adjacent to major roadways. Traffic on these primary arteries has become more congested because of the increasing number of motor vehicles in use and the volume of traffic. Policy makers and scientists have recognized the importance of assessing the potential impact of air pollutants from automobile emissions on human health. During the past decades, several investigators conducted studies on this matter; however, most studies mainly focused on the impact on respiratory and cardiovascular systems (Yang et al., 2002; Xu, Dockery, & Wan, 1991). At the time of those studies, limited data were available related to VOCs associated with immunological and hematological parameters of residents, particularly female individuals.

FIGURE 1

Distribution of VOC Concentrations in the Three Study Sites



Overall, the aim of the study reported here was to determine whether there were any significant adverse immunological and hematological health outcomes for female residents who were environmentally exposed to VOCs from automobile emissions. Particular objectives were to 1) assess the distribution of VOCs in selected study areas, 2) measure levels of certain immunological and hematological parameters in the female population, and 3) conduct statistical analysis to determine possible association between immunological and hematological outcomes and VOC concentrations.

Materials and Methods

Study Area

Kaohsiung, the second largest city in Taiwan, is located in the southern region of the island with an area of 153.6 km² and a population of 1.49 million divided into 12 districts. Sen-Ming District was chosen as the study area because of its high population density, lack of industry pollution sources, and the intersection of the Zhong-Shan freeway. This freeway was built in 1978 and is the only freeway that connects the city of Keelung (in the north of Taiwan) with Kaohsiung.

Three study sites in the target area were selected on the basis of traffic flow and distance from the freeway. The authors measured density of traffic by counting the number of automobiles and trucks passing through the sampling sites during the hours of peak, average, and low traffic. The sites were designated

as Site H, Site M, and Site L. Site H, at the intersection of Zhong-Shan Freeway and Chiu-Ju road, covers a total land area of 367,000 m², with a traffic density of 2,936 vehicles per day. Site M, located approximately 400 m from the freeway, spans 278,000 m², with a traffic density of 980 vehicles per day. Site L, which had lower traffic density than either Site H or Site M, is located approximately 600 meters from the freeway and covers a total of 240,000 meter². The traffic density for Site L totaled 302 vehicles per day.

Study Participants

The authors randomly selected participants for the study on the basis of address and house location obtained from the Resident Registry Database in the city administration office and a map of the study area. Potential participants were excluded from the study if they 1) had had previous exposure to fumes, environmentally or occupationally; 2) smoked and consumed alcohol regularly; 3) had been diagnosed with or had a medical history of hematological and immunological diseases, abnormal liver function, or unusually high protein levels in urine; 4) were less than 21 or more than 50 years of age; or 5) had lived in the area for less than two years. A total of 153 female participants were finally enrolled. Among them, 57, 51, and 45 female subjects were located in Site H, Site M, and Site L, respectively. The participants were informed of the nature of the study and potential benefits and risks. Informed consent was obtained from each participant before enrollment in the study.

Questionnaire

A questionnaire was developed to assess the demographic data, basic physical conditions, and medical history of the participants. Some questions were added concerning working conditions, related hematological diseases, and previous work history. The demographic data included gender, age, length of residence, type and length of occupation, work history, and level of education. Life habits noted were smoking, drinking, exercise, and use of drugs. The last part of the questionnaire focused on medical history, including respiratory diseases, cardiovascular diseases, allergies, circulation diseases, and others.

Interviewers administered the questionnaire to each respondent. Interviewers were trained before the study through lecture sessions and practice administration of the questionnaire. The survey, including the administration of questionnaires, was carried out between June 2001 and December 2002.

VOC Concentrations

A 4-mm Carbotrap 300 absorption tube (Supelco, United Kingdom) connected to a small pump was used for sampling air (Supelco Company). The pump was operated at 80 mL per minute. A thermal desorption unit (Model 890/891, Supelco, 1993) double-stage desorber was used to transfer compounds from the 2-mm Carbotrap to a gas chromatograph (GC) or a GC-mass spectrometer (MS) (National Institute of Occupational Safety and Health [NIOSH], 1997). The temperature of desorption was 310°C, and the flow rate of the inert carrier gas (helium) into the GC-MS was 2 mL per minute.

A Varian 3700 gas chromatograph equipped with a flame ionization detector was used for quantitative measurements. Separation of the compounds was achieved with a VOCOL capillary column (Supelco) of 60 m × 0.25 mm with a 1.50- μ m film. This column has a programmed temperature of 35°C for 4 minutes, followed by an increase to 200°C at a rate of 4°C per minute, and finally, during analysis, a constant temperature of 200°C for 4 minutes. The U.S. Environmental Protection Agency Method TO-15 (U.S. Environmental Protection Agency, 1999) recommends sampling of VOCs with a sorbent tube and analysis with thermal desorption-GC/MS. Before FID analysis, species were identified through GC/mass spectrometry (MS, Shimadzu GC-14A/GCMS-QP2000A). The GC/FID outputs were then connected to the desktop computer and

analyzed with Hewlett-Packard GC Chem-Station software for peak identification and integration.

A total of 37, 43, and 35 samples were taken in areas of high, medium, and low traffic, respectively, during different hours (between 6:00 a.m. and 2:00 p.m. or 2:00 p.m. and 10:00 p.m.). To monitor the traffic flow, the automobiles and trucks passing through the sampling sites were counted during hours of peak, average, and low traffic.

To ensure the integrity of the results, quality control was conducted throughout the course of the study. To calibrate a GC mechanism, the authors spiked clean absorbent tubes with six levels of standard samples, all with a coefficient of determination, R^2 , above .995. At the beginning of the day, the authors analyzed a laboratory blank trap and a field blank trap to check trap contamination. When the quantitative response differed by more than 20 percent from that predicted by a specified calibration equation, a new calibration equation was determined. One duplicate sample was collected for every 10 samples. If an average of the sample measurement and the corresponding duplicate measurement differed by more than 20 percent, the corresponding measurement was not included when VOC concentrations were calculated.

Blood Sampling and Analysis

A 20-mL peripheral blood sample was taken from each individual. Of this sample, 10 mL was collected in ethylene diamine tetra-acetic acid (EDTA) for total and differential blood cell counts, with 5 mL allowed to clot for the measurement of serum immunoglobulin. The serum samples for immunoglobulin were frozen the same day and were kept at -70°C until they were analyzed. All blood samples were collected between 9:00 a.m. and 11:00 a.m. The collected samples were transported to a laboratory for analysis within 12 hours. All of the blood tests were analyzed in the laboratory of the Department of Clinical Pathology in Kaohsiung Medical University Hospital.

Immunoglobulin Determination

To determine serum concentrations of IgG, IgA, and IgM, the authors used the radial immunodiffusion test with NOR-Partigen Immunodiffusion plates. The IgE serum level was determined by the radioimmunoassay test according to Wahyuni and co-authors (2003).

Hematological Determination

Six hematological parameters were assessed: WBC, red blood cells (RBC), hemoglobin

TABLE 1

Demographic Characteristic and Disease History*

Characteristic or Disease	Site H (N = 57)	Site M (N = 51)	Site L (N = 45)
Age ≥ 40 years (%)	100.0	100.0	100.0
Duration of residence ≥ 10 yr (%)	100.0	100.0	100.0
Education >high school (%)	52.6	66.7	60.0
Previous occupational exposure to fumes (%)	15.8	5.9	17.8
Smoker in house (%)	52.6	47.1	55.6
Drinking (%)	7.0	3.9	6.7
Sport habits (%)	75.4	78.4	80.0
Respiratory diseases (%)	5.3	13.7	8.9
Liver diseases (%)	5.3	17.6	15.6
Cardiovascular diseases (%)	15.8	11.8	15.6
Blood diseases (%)	5.3	7.8	8.9
Allergen diseases (%)	17.5	23.5	13.3
Other diseases (%)	10.5	9.8	8.9

*For all values, $p < .05$.

(Hb), platelets, neutrophils, and lymphocytes. A cytometer tallied the counts of these parameters. The counts were compared with normal ranges of the variables. Results were labeled as abnormal when the counts were out of bounds of the normal range and normal, when the counts were within the normal range. The normal ranges for WBC, RBC, Hb, platelets, neutrophils, and lymphocytes are 4~10 (1,000/mL), 4.0~5.6 (M/mL), 32~53 (percent), 150~450 (1,000/mL), 37~75 (percent), and 12~50 (percent), respectively. Blood samples were drawn on three occasions during the 12-month study period. The average concentration of each studied parameter was computed. To calculate the percentage of abnormal cases, the authors divided the cases of abnormal counts by the total cases of abnormal and normal counts.

Data Analysis

Multiple logistic regression analysis was used to estimate the effect of VOCs on the risk of abnormal counts in study participants. Odds ratios (ORs) and 95 percent confidence intervals (CIs) were computed. Difference in hematological parameter levels and VOC concentrations were detected with the Wilcoxon rank sum test. All analyses were adjusted for age (<50 and >21 years) of age, presence of household smokers, and previous occupational fume exposure. The analyses were performed with Statisti-

cal Package for the Social Sciences (SPSS) software. Probability values of less than .05 were considered statistically significant.

Results

Sixty-four hydrocarbons, identified as total VOCs, were detected in the ambient air of the city of Kaohsiung, Taiwan. Several hydrocarbons did not, however, show up consistently in the samples taken. In addition, the concentrations of some hydrocarbons fluctuated greatly in the readings taken. To prevent the outlier effect on the mean value, the authors eliminated concentrations greater than 5 standard deviations from statistical analysis. On the basis of consistency of presence and the negation of the outlier effect, a total of 25 VOCs, labeled as VOC₂₅, were kept in the study. Figure 1 shows the distribution of VOC concentrations within the three study sites. Site M has the highest total VOC and VOC₂₅ concentrations. Statistical analysis indicated that VOC concentrations in Site M were significantly higher than those in both Site H and Site L ($p < .01$).

Table 1 shows demographic data for the participants living in the three designated areas. Participants in the three groups were comparable with respect to educational level, presence of a smoker in the house, and previous occupational exposure to fumes and possible VOC exposure. All three groups had a very low percentage of identified respiratory, liver, cardiovascular, allergy, and other diseases.

TABLE 2

Serum Immunoglobulin Concentrations of Study Subjects in Site H, Site M, and Site L

Immunoglobulin	Site H		Site M		Site L		p
	Mean		Mean		Mean		
	Arithmetic	Geometric	Arithmetic	Geometric	Arithmetic	Geometric	
IgG ($\mu\text{g/dL}$)	1363.78	1339.03	1235.02	1215.09	1316.23	1290.51	.064
IgA ($\mu\text{g/dL}$)	282.37	260.42	223.15	206.72	341.80	315.18	.000*
IgE (IU/dL)	69.40	38.21	72.31	41.55	74.50	44.67	.678

* $p < .01$.

TABLE 3

Hematological Abnormality of Study Subjects at Site H, Site M, and Site L

Hematological Abnormality	N (%)	OR ^a (95% CI ^b)
White blood cell		
Site H	3 (5.3)	2.892 (0.245–34.099)
Site M	8 (15.7)	10.131 (1.044–98.340)**
Site L	1 (2.2)	1
Red blood cell		
Site H	9 (15.8)	0.640 (0.213–1.926)
Site M	16 (31.4)	1.690 (0.627–4.552)
Site L	10 (22.2)	1
Hemoglobin		
Site H	16 (28.1)	2.802 (0.906–8.670)
Site M	17 (33.3)	3.593 (1.184–10.908)*
Site L	6 (13.3)	1
Platelet		
Site H	3 (5.3)	2.293 (0.216–24.176)
Site M	0 (0.0)	—
Site L	2 (4.4)	1
Neutrophils		
Site H	0 (0.0)	—
Site M	4 (7.8)	7.882 (0.473–131.423)
Site L	1 (2.2)	1
Lymphocyte		
Site H	0 (0.0)	—
Site M	5 (9.8)	2.493 (0.287–21.634)
Site L	2 (4.4)	1

^aOdds ratio.^b95% confidence interval.* $p < .05$.** $p < .01$.

Means of IgG and IgA of participants living in Site M were less than those living in Site H and Site L. Multiple regression analysis indicates that IgA was significantly lower for

participants in Site M than for participants in both Site H and Site L. No significant difference, however, was found in IgE among participants in the three study areas (Table 2).

Table 3 shows the changes in certain hematological parameters of study subjects. The percentage of abnormal results for WBC, RBC, Hb, neutrophils, and lymphocytes was higher among residents in Site H than among those in both Site M and Site L. Multiple logistic regression analysis indicated that percentages of abnormal results for WBC (OR: 10.13; 95 percent CI: 1.04–98.34) and Hb (OR: 3.59; 95 percent CI: 1.18–10.91) among study subjects in Site M were significantly higher than those among study subjects in Site H and Site L.

Discussion

The distribution of VOC concentrations did not correlate with the distance of the study area from the highway. This finding differed from the anticipated results and those of other studies. Possible reasons for the finding were location of buildings and widths of roads. The roads with higher traffic density were wider (approximately 60 meters) and had fewer surrounding buildings, leading to easy dispersal of pollutants; the roads in the middle-traffic-density area were narrower (approximately 12 meters) with houses clustered close together. As a result, the pollutants may have required more time for dispersion. Another possible reason could be the types of business activities in the area. In Site M, there were many shops and stores, such as dry cleaners and gas stations, that could possibly contribute to VOC concentrations. Furthermore, weather could contribute to the finding; however, no data are available to show the effect of weather on the distribution of VOCs. Lee and co-authors (2002) found that hematological changes among children exposed to VOCs differed according to the month of year; they found that the direction of wind and atmospheric pressure influenced the concentration of VOCs in ambient air (Lee et al.).

In the study reported here, the misclassification of exposure was minimized because all study subjects had permanently lived in the same area for at least the past 10 years. In addition, the response rate from the study subjects was favorably high. Furthermore, there was a careful examination of personal and occupational histories of those subjects to minimize pre-exposure to VOCs from other sources. The study also controlled for several possible confounding factors, such as age, smoking habits, and alcohol consumption.

The study had certain limitations. First, it considered only female residents. This fact may restrict somewhat the generalization of these findings to all individuals residing in Taiwan, but it should not affect the internal validity of the study.

Second, the size of the study population was small, the period of biological monitoring was limited, and levels of VOCs were relatively low. As a result, it was impossible to generate a dose-response relationship between VOCs and immunological and hematological parameters.

Third, the findings did not provide any clue about specific VOCs affecting the changes in immunological and hematological parameters since VOCs are a mixture. A total of 64 compounds were detected. Among them,

25 compounds were present consistently in all samples collected. Bogadi-Sare and co-authors (2000) reported that exposure to benzene at concentrations lower than 15 ppm can induce depression of the circulating B-lymphocyte level in workers (Bogadi-Sare et al., 2000). Zeman found significantly lower levels of CD3 lymphocyte phenotype in workers exposed to benzene concentrations of up to 10 ppm (1990). By contrast, other investigators found that benzo(a)pyrene and benzene did not contribute to observed effects on the immune systems of humans (Hadnagy, Stiller-Winkler, & Idel, 1996).

Fourth, the study did not consider other air pollutants besides VOCs. One study has suggested that permanent exposure to increased levels of airborne particles might lead to irritation of the airway in association with activation of the immune system; however, gaseous air pollutants such as sulfur dioxide, carbon monoxide, nitrogen monoxide, and nitrogen dioxide are not factors responsible for immunological alterations (Hadnagy et al., 1996). Since no other major industrial sources of VOCs existed close to the study area, it can be assumed that automobile exhaust fumes were the major contributor to local VOC concentrations. In this context, it is inferred that immunological ef-

fects among participants in the three study regions were not caused by another external source of VOCs.

Results showed that environmental exposure to VOCs could suppress some immunological parameters of female residents. This finding was in line with findings of other studies, which have reported that long-term exposure to polyaromatic hydrocarbons in workers or high concentrations of n-pentane resulted in significant observed immunosuppression (Szczeklik et al., 1994; Karakaya, Yucesoy, Burgaz, Sabir, & Karakaya, 1996). In addition, VOCs can affect abnormality of certain hematological variables. For future studies, the frequency of blood sampling and the number of study subjects should be increased. In addition, more research should be done to assess the factors contributing to distribution of VOCs in the area.

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