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MEDICAL
SURVEILLANCE
STUDIES OF WORKERS
EXPOSED TO LOW
LEVEL BENZENE

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The paper presents the results of an investigation of haematotoxicity in workers exposed to low benzene concentrations. Forty-seven female workers in the shoemaking industry, exposed to solvent mixture and twenty-seven non-exposed controls were examined. Benzene concentrations in the working atmosphere ranged from 1.9 to 14.8 ppm. Significant differences in the levels of benzene in blood and phenols in pre- and post-shift urine between the exposed and control groups confirmed benzene exposure. Haemoglobin level and mean corpuscular haemoglobin concentration were significantly lower, and mean corpuscular volume was higher in the shoemaking workers than in controls. In the subgroup of shoemaking workers exposed to benzene concentrations of 5 ppm or lower, no differences in haematological parameters were found. In conclusion, exposure to a benzene concentration lower than 5 ppm does not appear to produce an increased level of abnormal haematological outcomes detectable in routine medical surveillance. The results of the study corroborate the present maximum permissible concentrations (5 ppm) as a protective limit preventing the onset of haematotoxic non-leukemogenic effects of chronic benzene exposure.

Key terms:
haematotoxicity, low-level exposure, occupational exposure, routine blood tests

Benzene has long been associated with haematopoietic disorders, ranging from mild peripheral blood cytopenias to fatal aplastic anaemia and leukaemia. Most of these effects, however, develop in individuals exposed to relatively high benzene concentrations (≥ 100 ppm) (1, 2). It is assumed that benzene exposure below

30 ppm does not have an adverse effect on health (1). However, the exposure level hazardous in terms of incurring haematotoxic non-leukemogenic and clinically inapparent effects is not known. Data are lacking concerning the potential "practical threshold level" below which adverse effects do not occur in exposed individuals. This critical information could be the basis for improvement of the legislation on permissible exposure limit in the working environment.

The difficulty in occupational health protection is not only in determining the allowable exposure level, but also in deciding on the mode of screening and routine medical surveillance of exposed workers to be used. A major problem in using laboratory tests in occupational health practice is that they are designed for use in a clinical setting and for the purpose of detecting persons who have been seriously affected. An occupational health-oriented approach needs to be able to detect subtle changes in the group level using tests sensitive enough to detect changes in the early subclinical phase of the disease. In the case of benzene, this is complicated by the fact that there is no specific end-point indicative of benzene haematotoxicity. The usual approach in the medical surveillance of benzene exposed workers is to obtain standard blood counts, including red blood cell count, haematocrit, haemoglobin, red cell indices, platelet count, white blood count and differential white cell count.

Studies of subjects exposed to very high concentrations of benzene showed the most significant changes in quantitative haematological parameters (3, 4). However, in studies of haematological outcomes in the benzene exposure of 30 ppm and lower, only alterations in qualitative parameters, such as mean corpuscular volume, were seen (5-11).

The incidence and degree of haematotoxic effects are presumed to correspond to the risk of human acute myelogenous leukaemia. Furthermore, because an animal model for benzene-induced acute myelogenous leukaemia has not been identified, benzene haematotoxicity could be taken to be surrogate for acute myelogenous leukaemia (5). Consequently, there is a need to investigate indicators of early benzene haematotoxicity to obtain a basis not only for the assessment of haematological hazard due to low-level benzene exposure but also for reducing the risk of benzene-induced leukaemia. The aim of this study was to determine more precisely:

- the haematotoxic effects at low-level benzene exposure, detectable by standard haematological methods,
- the validity of biomarkers of effect in low-level benzene exposure,
- the efficiency of the present maximum permissible concentration (5 ppm) to prevent haematotoxic non-leukemogenic effects of chronic benzene exposure.

SUBJECTS AND METHODS

Early indicators of benzene haematotoxicity at an exposure level below 15 ppm were investigated in 47 female workers aged 23-53 years (mean 38) employed in a shoe manufacturing factory, who were exposed to solvent mixtures for the period of 1-33 years (mean 17). A group of 27 healthy women, aged 29-46 years (mean 38), employed in the confectionery industry, served as controls. Benzene was present as a contaminant in glues, cleaners and paints. The control group was not exposed to solvents, including benzene or to any other haematotoxic agent. The groups did not differ with regard to age, lifestyle or the number of current smokers.

Benzene and toluene concentrations in the air of the working environment were measured continuously during the work shift in the middle of the working week. Air sampling was performed at 10 production areas using sampling tubes with Casella sampling pumps. Benzene and toluene concentrations were measured by gas chromatography (13). To examine internal exposure, benzene and toluene in blood and phenol concentrations were analysed in pre- and post-shift urine samples. Blood samples were taken before the work shift in the middle of the working week and urine samples before and at the end of the same working day. Benzene and toluene concentrations in blood were measured by head-space gas chromatography (14) and phenol in urine by the gas chromatographic method (15). Blood samples obtained for the analysis of complete blood cell count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocytes and serum iron were collected at the time of benzene analysis and were processed within three hours of collection.

Finally, statistical analyses were performed using Mann-Whitney U test and Student's t-test to determine significant differences in haematological parameters between the exposed and control groups.

RESULTS

Benzene concentrations in the working air ranged between 1.9 and 14.8 ppm. Significant differences in exposure biomarkers between the exposed and control groups confirmed benzene exposure (Table 1). Although all the investigated haematological parameters were within clinically normal ranges, haemoglobin level ($P=0.02$) and MCHC ($P=0.0002$) were significantly lower and MCV ($P=0.03$) was higher in benzene exposed workers than in controls. Analysis of haematological outcome in a subgroup exposed to an ambient benzene level of below 5 ppm showed no differences in the above parameters (Table 2).

Table 1. Benzene exposure biomarkers in shoemaking workers and controls

Benzene exposure	Controls	Shoe workers
Duration (y)($\bar{X} \pm SD$)	-	17.0 \pm 9.7
Benzene in working atmosphere (ppm)(range)	-	1.9-14.83
Benzene in blood (mg.L ⁻¹)	0.000 (0.000-0.009)	0.005* (0.000-0.030)
Phenol (mg.g ⁻¹ creatinine)		
- in pre-shift urine	2.197 (0.504-9.770)	3.830* (1.660-20.400)
- in post-shift urine	2.759 (0.540-6.960)	5.240* (2.150-27.310)

Results of benzene in blood and phenols in urine are presented as median (range in parentheses)
* P < 0.01 (Mann-Whitney U test)

Table 2. Haematological outcomes in shoemaking workers with respect to benzene exposure levels

Haematological parameter	Controls	Shoe workers	
		Ambiental benzene (ppm) 1.9-14.8	<5
Haemoglobin	133 \pm 9.03	129 \pm 10.8**	131 \pm 9.68
MCV	95.3 \pm 5.26	98.1 \pm 5.46*	97.8 \pm 5.13
MCHC	332 \pm 12.7	320 \pm 23.3**	328 \pm 29.5

MCV=mean corpuscular volume, MCHC=mean corpuscular haemoglobin concentration
Results are presented as mean and standard deviations *P<0.05 **P<0.01 (Student's t-test)

DISCUSSION

Animal studies of benzene haematotoxicity indicate little or no effect at exposure levels below 30 ppm. Although no haematological abnormalities were seen in short-term investigations of low-level benzene exposure (16, 17), in long-term studies with benzene exposure of several months decreased blood cell counts were found even when benzene concentrations were below 10 ppm (18). However, because of the differences in the metabolic pathway and benzene kinetics in various animal species, and in individual sensitivity to benzene toxicity in humans, it is difficult to obtain a reliable low-dose extrapolation of animal study results to

man. Therefore, epidemiological studies of haematological damage in persons exposed to low-level benzene exposure are needed.

The importance of a medical surveillance programme in occupational health protection is generally recognized. However, screening procedures vary and results are difficult to interpret. Of particular controversy is the utility of routine blood screening for early detection of blood dyscrasia. A small number of cohort studies have reported on the haematological effects of benzene as monitored by routine peripheral blood counts (Table 3).

Table 3. Consolidated presentation of early indicators of benzene haematotoxicity

Reference	Benzene exposure (ppm)	Haematological outcomes				
		RBC	WBC	PI	HB	MCV
Greenburg and co-workers (3)	11-1061	-	-	-	-	+
Aksoy and co-workers (4)	30-210	0	-	-	-	?
Townsend and co-workers (5)	<2-30	-	0	?	0	0
Fishbeck and co-workers (6)	>25	0	0	0	-	+
	10	0	0	0	0	0
Tsai and co-workers (7)	<0.1-25	0	0	0	0	?
Hancock and co-workers (8)	1.9-31.5	0	0	?	0	?
Yardley-Jones and co-workers (9)	1-10	0	0	0	0	+
Kipen and co-workers (10)	75	-	-	?	-	?
	15-20	0	0	?	0	?
Collins and co-workers (11)	0.001-1.40	0	0	0	0	0
Bogadi-Šare and co-workers	1.9-14.8	0	0	0	-	+
	<5	0	0	0	0	0

RBC=red blood cell count; WBC=white blood cell count; PI=platelet count; Hb=haemoglobin concentration; MCV=mean corpuscular volume

- the benzene-exposed group has lower value than the control group
- + the benzene-exposed group has higher value
- 0 no difference between the exposed and control groups
- ? data either not reported or the tests not done

Decreased count of all three blood cell types was found only in persons with high benzene exposure (3, 4, 10). Although *Townsend and co-workers* detected a slight decrease in RBC values in workers exposed to up to 30 ppm, they did not consider this to be clinically significant (5). Subsequent studies found no alterations in blood cell counts in benzene exposure below 25 ppm (6-11). In our study, likewise, changes in blood cell counts were not found in benzene

exposure of between 2 and 15 ppm. These results confirm the assumption that in view of the large bone marrow reserve, haematopoietic stem cells can be affected without an apparent decrease in the circulating blood count below the clinically accepted normal range. Therefore, the use of peripheral blood cell count is not a sufficiently sensitive indicator for detecting early benzene haematotoxicity (19).

In addition to quantitative alterations, there is evidence that benzene also leads to qualitative abnormalities in circulating blood cells. Such changes may precede an overt decrease in cell counts and therefore be useful for the detection of early benzene toxicity. Thus, an increased red cell mean corpuscular volume was seen in persons exposed to low-level concentration of benzene (6, 9). Our study also demonstrated that increased red cell mean corpuscular volume is a possible indicator of haematological damage in low-level benzene exposure.

Some authors found latent detectable haematopoietic changes in workers exposed to benzene concentrations exceeding 25 ppm, but not in benzene exposure below that level (6, 10). These findings are consistent with the existence of a possible threshold level for non-leukemogenic haematotoxic effects. In our study, decreased haemoglobin level and MCHC and increased MCV were found in benzene exposure up to 15 ppm, but no differences were shown when ambient benzene decreased to 5 ppm. This suggests that the possible threshold level for the benzene induced non-leukemogenic effects is lower than previously reported (Table 3). Consequently, exposure to a benzene concentration below 5 ppm does not appear to produce an increased level of abnormal haematological outcomes detectable in routine medical surveillance. This result supports the rationale for establishing the threshold for benzene workplace concentration at 5 ppm, as recently adopted in Croatia by the legislation on maximum permissible concentrations (20).

CONCLUSION

Routine annual blood tests may be sufficient to detect subclinical changes in haematological outcomes of benzene exposed workers. Haematological tests should be performed during preplacement examinations and periodic assessments including legally required examinations. Haematological outcomes previously associated with excessive benzene exposure are recommended in health surveillance of benzene exposed workers. Such measures include red blood cell count, white blood cell count and differential, haemoglobin, platelet count and red blood indices, including mean corpuscular volume. No single finding or constellation of findings in these monitoring techniques is pathognomonic of benzene haematotoxicity, and therefore must be carefully interpreted in terms of the individual or group at risk.

The "no effect" exposure level for benzene blood effects in humans is established by different government authorities. In Croatia, this level is 5 ppm. Available evidence and the results of this study suggest that this benzene level is sufficient to

protect against overt symptomatic haematotoxic effects and is safe enough to represent maximum permissible concentrations in occupational settings.

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Sažetak

ZDRAVSTVENI NADZOR NAD OSOBAMA PROFESIONALNO IZLOŽENIMA NISKIM KONCENTRACIJAMA BENZENA

U zaštiti zdravija osoba profesionalno izloženih benzenu nuždan je zdravstveni nadzor kojim je moguće otkriti rane pokazatelje oštećenja krvotvornog sustava. U članku su prikazani rezultati ispitivanja krvotvornog sustava u radnica izloženih niskim koncentracijama benzena. U istraživanje je bilo uključeno 47 radnica obučarske industrije i 27 zdravih ženskih osoba. Benzen u zraku radnih prostora iznosio je 1,9-14,8 ppm. Izloženost benzenu potvrđena je značajno višim koncentracijama benzena u krvi i fenola u mokraći prije i poslije radne smjene u radnica obučarske industrije. U njih je nađen niži hemoglobin i srednja koncentracija hemoglobina u eritrocitu te viši srednji volumen eritrocita u usporedbi s osobama poredbene skupine. Međutim, ova odstupanja nisu potvrđena i u skupini radnica obučarske industrije izloženih benzenu ispod 5 ppm. Prema ovim rezultatima, izloženost benzenu koncentracije 5 ppm i niže ne uzrokuje odstupanja koja je moguće utvrditi rutinskim krvnim testovima. To potvrđuje maksimalno dopustivu koncentraciju od 5 ppm benzena kao zaštitnu granicu za sprečavanje hematotoksičnih neleukemogenih učinaka benzena.

Ključne riječi:

hematotoksičnost, izloženost niskim koncentracijama, profesionalna izloženost, rutinski krvni testovi

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