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EXPOSURE TO MUTAGENIC CHEMICALS IN FOUNDRY AND URBAN ENVIRONMENTS

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Abstract. The study was aimed at the estimation of occupational exposure to mutagenic substances in a piston-ring foundry. The following samples were examined: solid phase of aerosol from the foundry and from different places of urban environment together with the foundry workers' urine collected during the 8-hour shift. The mutagenic substances were extracted from the collected material with acetone or concentrated with XAD-2 resin. The mutagenic property was estimated with the Ames' test using S. typhimurium strain TA98 without and with S9 fraction. The highest mutagenic activity was found at the following work-posts: caster, moulder, steerer of an induction furnace, and smelter and in the office rooms and in the flat occupied by heavy smokers. The mutagenic activity of aerosol at some other productive work-posts in the foundry was similar to the mutagenic activity of aerosol in the office and flat rooms occupied by nonsmokers or in the street in Lodz. The mutagenic activity of urine from foundry workers was not correlated with the level of the occupational inhalation exposure to the mutagenic substances, however, the mutagenic activity of urine from smoking workers was about 10—20 times higher than from nonsmokers.

INTRODUCTION

Several reports have demonstrated the increased cancer risk, particularly of lung cancer, among foundry workers (7, 15, 21, 27). Foundry air, in addition to the inorganic contaminations, such as silica dust and metal fumes, contains complex mixtures of organic compounds. These include polycyclic hydrocarbons, which are formed by the decomposition of organic additives used in the molding sand (23, 24), and chemical ingredients (e.g. epichlorhydrin, formaldehyde, phenol) used in the manufacturing of kernels and cores (4). Although different foundries are not

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exactly alike in physical structure or methods of production, the data cited above indicate the possibility of occurrence of various chemical carcinogens in a foundry work environment. A large percentage of carcinogens are also mutagens in the Salmonella/microsome test (22, 25). Thus, in the present study, we applied this test for the evaluation of the biological reactivity of foundry air contaminants and the mutagenic activity in urine of foundry workers, taking smoking habits into account.

MATERIAL AND METHOD

1. Air sampling. Our study was performed in a foundry manufacturing piston rings of various types. The airborne particulate samples were collected with high-volume samplers (Staplex pumps) on glass fiber filters (Whatman, type GF/A). Stationary long-term sampling was performed within the breathing zone of workers at 8 work-posts in foundry bays and in 4 foundry management rooms. Additional samples of an ambient particulate matter were extracted at various sites in Lodz in order to get a background level of indoor and outdoor particle mutagenicity. Sampling flow velocity varied between 32 and 45 m^3/h and was measured every hour with an anemometer to obtain a mean flow velocity and a total volume of sampled aerosol. In most cases the sampling lasted 2 hours.

2. Extraction. In the initial experiments the effectiveness of the extraction procedures was evaluated. The particulate matter collected on filter at the work-post where molten metal was being poured into moulds was extracted with acetone for 3 hrs in a Soxhlet apparatus or for 2 hrs in an ultrasonic cleaner. Since the resulted mutagenicity of both extracts was similar, the extraction in an ultrasonic cleaner was later used for the sake of time. This method of extraction is employed in several other laboratories (12, 17, 19, 26). The choice of extraction solvent was based on the results of another experiment in which particles collected during the metal pouring process were extracted in an ultrasonic cleaner with one of the following solvents: methanol-water (85:15), benzene-methanol (60:40), acetone, methanol, cyclohexane and methylene chloride. The highest mutagenic activity showed acetone extracts (Table 1). Thus, during further study, the filters were extracted with acetone for 2 hrs in an ultrasonic cleaner. Insoluble particles were separated by filtering the extract through a column filled with XAD-2 resin. Substances absorbed by the resin were eluted with an additional portion of acetone. Then both portions of the acetone were joined and evaporated to dryness at a temperature of 70°C in an argon atmosphere. The residues were

dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C. Each particulate matter sample was tested in a mutagenicity assay in at least two different doses.

3. Urine samples. 8-hour urine samples were collectd into 500 cm³ polyethylene flasks containing a drop of formaldehyde from 16 smoking and 11 nonsmoking workers. The urine was filtered through Whatman No. 1 filter paper and adjusted to pH-7. The creatinine concentration was

TABLE 1. Mutagenicity of Different Solvent Extracts of Airborne Particles Collected at the Caster Work Site in the Piston Ring Plant

Solvent	Amount of dust, of which extracted substances were added on the plate	Mean number of revertants induced by extracted substances from sampled dust in S. typhimurium TA98	
	mg	−S9	+S9
Methanol:water (85:15, v/v)	10	94	328
	5	55	201
	1	29	63
Benzene:methanol (60:40)	10	105	337
	5	96	299
	1	27	70
Acetone	10	118	359
	5	78	228
	1	36	87
Methanol	10	104	228
	5	55	164
	1	32	56
Cyclohexane	10	36	100
	5	44	41
	1	18	16
Dichloromethane	10	73	166
	5	49	119
	1	24	45

measured using the Folin method (16). The urine mutagenicity was analyzed from samples concentrated with the nonpolar XAD-2 resin according to the methods of Yamasaki and Ames (31). The concentrated urine samples were tested at several doses ranging from 3.125 cm³ to 25 cm³ of the original volume of urine.

4. Assay of mutagenicity. Mutagenicity was determined by the Salmonella plate incorporation method (18). Assays were performed with the tester strain TA98 kindly donated by B. N. Ames (University of Califor-

nia, Berkeley, CA). Samples were studied on duplicate plates both with and without metabolic activation. The 9000 g supernatant rat liver homogenate (S9) was prepared as described by Ames et al (1). The amount of S9 added to the plates was 50 µl. The reference tests were performed with 4-nitro-o-phenylenediamine (3 µg/plate). The levels of the spontaneous reversion rates (his+revertants/plate) varied in different days from 21 to 26 (TA98, −S9) and from 32 to 36 (TA98, +S9). The number of spontaneous revertants has been substracted from the test result to obtain the number of induced revertants. In order to standardize the results the numbers of revertants induced by substances isolated from 1 mg of collected airborne particles or from particulate matter enclosed in 1 m^3 of air sampled at different sites were calculated. The mutagenic activity of urine was expressed as a number of revertants induced by substances excreted in urine simultaneously with 1 mM of creatinine.

RESULTS

The particulate matter concentration in the air has been presented in Tables 2 and 3. The highest dust concentrations were found in a foundry bay (1.92—3.68 mg/m^3), much lower in foundry office rooms (0.11—1.61 mg/m^3) and the lowest ones in the indoor and outdoor air at different urban locations (0.06—0.16 mg/m^3). The mutagenic activity of substances extracted from the dust samples was even more variable (Table 2 and 3). In most cases the extracted substances required meta-

TABLE 2. Mutagenic Activity of Airborne Particulate Matter Collected from the Indoor and Outdoor Air at Different Urban and Work Environments [a)]

Location	Aerosol concentration	S. typhimurium TA98			
		revertants/mg		revertants/m^3 air	
		−S9	+S9	−S9	+S9
Foundry:					
— technical section office	1,61	1	18	1	29
— power engineer office	0,11	22	404	2	44
— safety and work hygiene office	0,15	39	172	6	29
Smokers' flat	0,12	36	397	4	49
Nonsmokers' flat	0,06	47	42	3	3
Office (nonsmokers only)	0,08	28	19	2	2
Laboratory (nonsmokers only)	0,15	45	100	7	15
Lodz street	0,16	22	57	4	9
Lodz suburb (garden allotment)	0,11	3	37	0	4

a) — mean number of spontaneous revertants without S9 amounted to 23—26 and with S9 to 36.

bolic activation with an S9-mix fraction in order to induce reverse mutations in S. tymphimurium TA98. The highest airborne particulates mutagenicity was found in samples taken in the offices or rooms where cigarette smoking was very frequent (172—397 rev/mg), although, due to low dust concentrations, the air mutagenicity was increased to a lesser extent there (29—49 rev/m³) (Table 2). The mutagenic activity of substances extracted with acetone from dust samples taken at different

TABLE 3. Mutagenic Activity of Airborne Particulate Matter Collected at the Work-Posts in the Foundry [a)]

Work-post	Dust concentration at the work-post	S. typhimurium TA 98			
		revertants/mg		revertants/m³ air	
		−S9	+S9	−S9	+S9
Smelter	3,19	1	8	3	25
Steerer of the induction furnace	1,92	3	16	6	31
Caster at deriving of cast iron from the furnace to the vat	2,48	2	13	5	33
Caster at pouring moulds	3,68	3	11	10	40
Moulding sand manufacturer	3,50	0	1	2	4
Machine moulder	2,09	3	18	7	37
Grinder	2,19	2	3	4	6
Sorter	3,22	0	1	0	4

a) — mean number of spontaneous revertants without S9 amounted to 23 and with S9 to 32.

urban locations (19—57 rev/mg) was much higher than those taken at various work-posts in a foundry bay (1—18 rev/mg) (Table 2 and 3). However, due to the much higher particulate matter concentration in the air of the foundry bay as compared with that at urban sampling locations, the air mutagenicity on some work-posts was considerably increased. A raised level of air mutagenicity was found at all work-posts connected with such processes as smelting, casting and moulding (25—37 rev/m³) (Table 3). The mutagenic activity of aerosols sampled at the workplaces for cast grinding and sorting and for manufacturing of moulding sand was much lower (4—6 rev/m³) (Table 3) and comparable with that found in outdoor urban locations (4—9 rev/m³ (Table 2).

TABLE 4. Mutagenic Activity in Air of Workplaces and in Urine of Smoking and Nonsmoking Workers in the Piston Ring Foundry

Occupation	No. of revertants TA98/m³ of air	No. of revertants TA98/mmol creatinine	
	+S9	smokers +S9	nonsmokers +S9
Foundry bay office clerks	68	199, 359	51, 45
Moulder	47	976	25
Power engineering workers	44	429	31
Caster	33	1120, 666, 586, 57, 2147	—
Steerer of the induction furnace	31	165	—
Technical section workers	29	56[a], 849	105, 19
Safety technicians	29	985	45
Smelter	25	847	26
Grinder	6	1324	—
Sorter	4	583	64
Moulding sand manufacturer	4	—	30

a) — each number in this and other rows is urinary mutagenicity of one person.

The cigarette-smoking workers had considerably higher urine mutagenicity than their non-smoking colleagues (Table 4). However, no correlation was found between the level of urine mutagenic activity of workers and the magnitude of air mutagenicity at their usual workplaces.

DISCUSSION

The results obtained indicated that genotoxic, potentially carcinogenic substances are present in the airborne particulate matter occurring in the occupational environment of a piston rings foundry. This finding is in agreement with data from studies of other foundries (13, 14, 24). It has been shown in our data that samples obtained from the workplaces where casting and melting operations were carried out exhibited high mutagenic activity. The relatively high mutagenicity of aerosol at the moulding bench was most probably connected with the proximity of this workplace to the casting area, since the manufacture of moulding sand using similar raw materials, but located much further away in an other part of foundry bay, did not lead to increased mutagenic activity of aerosol. Thus, the analysis of variation of aerosol mutagenicity within foundry bay supports the hypothesis that casting and, to a lesser extent, melting operations are emission sources of mutagenic substances into the environment. During casting, large quantities of various polynuclear

aromatic hydrocarbons are synthesized, then vaporized and absorbed into airborne particles (11, 23). This class of organic compounds is known to be highly mutagenic in Salmonella typhimurium TA98 after metabolic activation by the S9 preparation (20). Polynuclear aromatic hydrocarbons do not seem to be the only group of chemicals responsible for mutagenicity of foundry particulate matter. Skytta et al (24) found a high correlation between benzo(a)pyrene concentrations and the mutagenic activity of aerosol in only one of two iron foundries. Only a certain part of the total mutagenic activity of chemicals absorbed on foundry air particles could be attributed to polynuclear aromatic hydrocarbons (13). The other mutagenic chemicals have not yet been identified.

The highest mutagenicity of airborne particles sampled in different foundry offices is related to the contamination of air with cigarette smoke. Tobacco smoke contains more than 3000 different compounds, some of which are mutagens and/or carcinogens (10). The addition of S9-mix was necessary to reveal the mutagenic activity of tobacco smoke (2). The level of mutagenic activity found in samples obtained in the foundry office, where employees used to smoke cigarettes, was similar to that in the smokers' flat. In the foundry bay office located behind and above the level of electric-induction furnaces, the air was most probably additionally polluted with their effluent gases and fumes. The high mutagenic activity of particulate matter, in rooms inhabited by cigarette smokers, has already been noted (30). This finding may be causally related to the increased risk of lung cancer in passive smokers, reported in some papers (8, 9). Limited information suggests that there is a correlation between the bacterial mutagenicity level of airborne particles and lung cancer incidence (14, 28).

The biological monitoring of mutagenic activity in urine was suggested in order to detect and assess the occupational exposure to genotoxic chemicals. The urine mutagenic activity of workers at work in the chemical or rubber industry is considerably higher than after several days or a longer break (3, 4, 29). Falck et al (6) have demonstrated the increased urine mutagenicity (TA98, +S9) in smokers working in the rubber industry (717 rev/mmol of creatinine) in comparison with control smokers (298 rev/mmol of creatinine). In nonsmoking workers employed in three different foundries, the mean mutagenic activity (TA98, +S9) of substances extracted from urine depended on the type of foundry and varied from 941 ± 567 to 174 ± 159 rev/mmol of creatinine (5).

In this study, however, no correlation was found between the mutagenicity of airborne particles in the occupational environment and the mutagenic activity in the workers' urine. This may be due to a low level of occupational exposure to genotoxic chemicals rather than to inter-

individual variation in xenobiotic metabolism. Smoking appeared to be a strong confounding factor in the assessment of occupational exposure to genotoxicants based on an evaluation of mutagenic activity in the urine.

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