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CORRELATION BETWEEN POLYCYCLIC AROMATIC HYDROCARBONS CONCENTRATION AND AIRBORNE PARTICLE MUTAGENICITY IN THE RUBBER FACTORY

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Abstract. The study was undertaken to evaluate the correlation between benzo[a]pyrene and coal tar pitch volatiles concentrations and mutagenic activity of airborne particles sampled at different workplaces of the factory producing various types of tires. The solid phase of aerosols was collected on Whatman glass-fiber filters using Staplex pumps. Coal tar pitch volatiles (CTPVs) were extracted from sample filters using ultrasonic-benzene extraction and determined by the gravimetric method. Benzo[a]pyrene (BaP) analysis was performed using high performance liquid chromatography with a spectrofluorimetric detector. The mutagenic substances were extracted from collected material with acetone. The mutagenic properties were estimated with the Ames' test using S. typhimurium strain TA98 without and with S9 fraction. At nearly all worklplaces the concentrations of BaP and CTPVs were within the range of 4–61 ng/m^3 and 0.11–1.26 mg/m^3, respectively. Only at weighing were they much higher and amounted to 172–2261 ng/m^3 for BaP and 3.05–4.07 mg/m^3 for CTPVs. The highest exposure to mutagenic airborne particulate matter was found at weighing (1500 rev/m^3), the mixers loading level (> 500 rev/m^3) and the carbon black station (> 150 rev/m^3). The air mutagenic activity at other workplaces, especially at the extruder mill of the mixer (>90 rev/m^3), the two-roll mill of mixers (> 70 rev/m^3), mixer I loading (> 70 rev/m^3), calendering (> 70 rev/m^3) and fender vulcanizing (> 80 rev/m^3) was even much more higher than that found in the urban indoor and outdoor air (2–9 rev/m^3). Relatively high correlation coefficients were found for indirect air mutagenicity (rev/m^3, +S9) and the concentrations of BaP and/or CTPVs, but these parameters don't have a normal distribution. However, after transforming empirical data into their logarithms, the correlation between air mutagenicity and BaP or CTPVs concentrations was not found significant.

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INTRODUCTION

Epidemiological studies have revealed the excess cancer risk of workers in the rubber industry, however, most of them have not provided data on specific occupational exposure or they have used job descriptions as a substitute for exposure categories (5). The chemical composition of volatile material during rubber curing is not well defined and it has been shown that minor changes in rubber stock formulations and heating temperature give rise to marked changes in the composition of the emitted fumes (11). Due to lack of adequate data on real occupational exposure to fumes a direct causal link between usage of certain chemicals and the excess of distinct cancer types has not been established. Benzo[a]pyrene has been found as an airborne contaminat of the working environment in several rubber plants. It is known that the working environment in the rubber industry is contaminated by various chemical exhibiting mutagenic and/or carcinogenic activity (5, 10). The mutagenicity of airborne particulates collected in several workplaces in tire plants is much higher than that in urban indoor or outdoor air (2). The chemicals responsible for this increased air mutagenicity are not known. In this study an attempt was made to determine the correlation between coal tar pitch volatiles and benzo[a]pyrene concentration with airborne particles mutagenicity in the tire factory.

MATERIALS AND METHODS

Sampling

Stationary sampling was performed within the breathing zone of workers at different work sites of a factory producing various types of tire. Airborne particulate matter was collected at each work site simultaneously with two Whatman glass-fiber filters (type GF/A): one for coal tar pitch volatiles (CTPVs) and benzo[a]pyrene (BaP) determination and a second for mutagenicity assessment of airborne particulates. For the determination of CTPVs, air was drawn with pump AS-50 through a filter with a volume velocity of $3m^3/h$ for 80 min. Three to four such air samples were taken on each work site. Samples for mutagenicity assessment were collected using high volume Staplex pumps (sampling volume 60-200m^3, sampling 2-4h).

Methods of determinig coal tar pitch volatiles and benzo[a]pyrene

Filtres were extracted in 20 ml of benzene for 20 min. in an ultrasonic bath. The extracts were dried at 40°C under a nitrogen flow until they reached a constant mass in order to determine CTPVs (7). Polycyclic aromatic hydrocarbons (PAHs) were isolated from non-PAH compounds present in the obtained benzene soluble fraction by column chromatography on silica gel (6). The PAH fractions of the samples were analysed quantitatively for BaP by high performance liquid chromatography (HPLC Varian 2510) with spectrofluorimetric detector 2070 (steel column 25 cm × 4.6 mm, packed with dichrosorb ODS-120T, mobile phase-metanol: water (96:4)). The detection limit for BaP was 1 ng/m^3 for an air sample of 4 cm^3 and for CTPVs – 0.03 mg/m^3.

Table 1. Concentrations of benzo[a]pyrene and coal tar pitch volatiles and mutagenic activity of the aerosol collected at work-posts in the rubber factory

Work-post	Period of sampling (season, year)	Benzo[a]pyrene concentration at the work-post (geometric-mean) ng/m³	Coal tar pitch volatiles (CTPVs) concentration at the work-post (geometric-mean) mg/m³	Mutagenic activity rev/m³ S.typhimurium TA98 -S9	Mutagenic activity rev/m³ S.typhimurium TA98 +S9
Weighing	Spring, 89	2261	4.07	0	1500
	Spring, 90	172	3.05	18	28
Mixers loading level (first stage mixing)	Spring, 88	30	0.67	0	531
	Spring, 89	61	0.87	0	23
	Spring, 90	46	0.54	22	40
Carbon black station	Autumn, 87	17	-	21	158
	Spring, 88	20	0.42	8	55
	Autumn, 87	10	-	4	89
Extruder mill of mixers	Spring, 88	-	0.63	21	94
	Spring, 89	35	1.26	3	23
	Spring, 90	20	0.28	14	34
Two-roll mill of mixers	Spring, 88	16	0.57	7	71
	Spring, 90	46	0.49	25	62
„Bizon" harvester tire vulcanizing	Spring, 90	9	0.34	11	34
Mixer I loading (Second mixing operation)	Spring, 88	10	0.50	27	74
	Spring, 89	7	-	7	56
	Autumn, 87	10	-	6	37
Calendering	Spring, 88	12	0.24	3	21
	Spring, 89	16	-	6	77
	Spring, 90	14	0.31	4	24
Fender vulcanizing	Spring, 88	22	0.13	3	88
Tractor tire vulcanizing	Autumn, 87	4	-	1	10
	Spring, 88	-	0.11	3	25
	Spring, 90	21	0.42	7	1
Tire tread extruder	Spring, 88	11	-	3	13
Lorry tire vulcanizing	Spring, 90	27	0.28	9	9

Mutagenicity assessment

The substances adsorbed onto dust or fume particles were extracted with acetone for 30 min in an ultrasonic cleaner. Acetone was then evaporated at a temperature of 70°C in an argon atmosphere and the residue dissolved in dimethylsulphoxide. Mutagenicity was determined by the Salmonella plate incor-

poration method (8) with the tester strain TA98 known to be sensitive to mutagens present in rubber additives and vulcanization (4). The details of the experimental procedure and part of the results were published previously (1). The number of revertants induced with and without the S9 fraction by substances isolated from particulate matter enclosed in 1 m^3 of air sampled at different workplaces was calculated as an index of airborne particle mutagenicity.

RESULTS AND DISCUSSION

In most of the work-posts BaP concentrations were within the range of 4 to 61 ng/m^3 and they were higher than the concentration of BaP (from < 1 to 5 ng/m^3) in the rubber factory in the United Kingdom (9). Only during the weighing operation was the BaP concentration much higher, amounting to 172 – 2261 ng/m^3 (Table I). Similarly CTPVs concentrations were within 0.11 – 1.26 mg/m^3 except in the weighing operation where they were 3.05 to 4.07 mg/m^3. Taking into account concentration combined with BaP and CTPVs, operations such as mixer loading, extruding, milling, and some vulcanizing operations produce more pollution than calendering, tire tread extruding, or tractor tire vulcanizing (Table 1).

Table 2. Analysis of probability distribution of benzo[a]pyrene and coal tar pitch volatiles (CTPVs) concentrations and mutagenic activity of airborne particles sampled in the rubber factory

Variable	Skewness*	Kurtosis*	Shapiro-Wilk statistics	Significance
BaP conc.	4.86	23.70	0.2525	< 0.0100
CTPV conc.	2.61	6.41	0.6008	< 0.0100
rev/m^3, +S9	4.34	19.86	0.3777	< 0.0100
rev/m^3, −S9	0.96	-0.36	0.8566	< 0.0100
ln (BaP conc.)	2.28	7.27	0.7980	< 0.0100
ln (CTPV conc.)	0.75	1.02	0.9354	0.2842
ln (rev/m^3, +S9)	0.04	2.91	0.9298	0.0876
ln (rev./m^3, −S9)	-0.20	-0.50	0.9435	0.2821

* – Values for skewness and kurtosis are 0 in a normal distribution

The direct mutagenicty of airborne particulate matter was low in all periods of sampling. The addition of S9-mix produced increases in the mutagenicity of airborne particles in nearly all samples collected in 1987 – 90 (Table 1). The highest exposure to mutagenic airborne particulate matter was found at the weighing (1500 rev/m^3), at the mixers loading (first stage mixing) (> 500 rev/m^3) and at the carbon black station (> 150 rev/m^3). The air mutagenic activity at nearly all workplaces, especially at the extruder mill of mixers (> 90 rev/m^3), fender vulcanizing (80 rev/m^3), calendering (> 70 rev/m^3), mixer I loading (second stage mixing) (> 70 rev/m^3), two-roll mill of mixers (> 70 rev/m^3), harvester tire vulcanizing (> 30 rev/m^3) and tractor tire vulcanizing (> 20 rev/m^3) was still much higher than that found in the urban indoor and outdoor air, which amounted to 2 – 9 rev/m^3 (2). Next all collected sets of data

were tested for type of probability distribution. Analysis of concentrations of BaP and CTPVs distribution and direct or indirect air mutagenicity was performed on observed values and transformed into their natural logarithms. For each data set, the coefficient skewness, kurtosis and Shapiro-Wilk statistics (3) were calculated in order to assess a departure of an empirical distribution from a normal one.The results are shown in Table 2. The distributions of nontransformed data were not normal since the coefficients of skewness and/or kurtosis were too high and the values of Shapiro-Wilk statistics were too low. We therefore must reject the hypothesis that the observed values of tested parameters have normal distribution. The transformed values, on the other hand, were characterized by low skewness and/or kurtosis coefficients and high Shapiro-Wilk statistics, so we cannot reject the null hypothesis except BaP concentration. The linear regression equations and correlation coefficients are shown in Table 3. Relatively high correlation coefficients were found for indirect air mutagenicity (rev/m^3, +S9) and BaP concentration or CTPVs concentration. However, it is hard to accept a statistically significant correlation between these parameters since they do not have a normal distribution. Moreover, when the empirical data were transformed into their logarithms in order to get the normal distribution, we did not see a significant correlation between air mutagenicity and BaP or CTPVs concentrations.

Table 3. Correlation between benzo[a]pyrene concentration or coal tar pitch volatiles concentration and mutagenic activity of airborne particles in the rubber factory

Regression equation	Correlation coefficient	Significance	Sample size
rev./m^3 (+S9)=50.502+0.637 BaP conc.	0.938	< 0.001	24
rev./m^3 (+S9)=−53.982+247.87 CTPV conc.	0.729	< 0.001	19
rev./m^3(−S9)=9.154−0.0037 BaP conc.	−0.207	0.166	24
rev./m^3(−S9)=10.378−0.802 CTPV conc.	−0.093	3.353	19
ln (rev./m^3, +S9)=2.203+0.492 ln (BaP conc.)	0.442	0.015	24
ln (rev./m^3, +S9)=4.177+0.619 ln (CTPV conc.)	0.378	0.056	19

CONCLUSIONS

1. The work environment in the rubber industry is contaminated with polycyclic aromatic hydrocarbons and chemicals exhibiting mainly an indirect mutagenicity in the Salmonella typhimurium/microsome test.

2. Benzo[a]pyrene and other polycyclic aromatic hydrocarbons might be responsible for only a part of indirect air mutagenicity on different work-posts in the rubber industry.

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