

Mutagenic exposure in the rubber manufacturing industry: an industry wide survey

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Abstract

Mutagenic exposure conditions in several rubber manufacturing companies ($n = 9$) in The Netherlands were studied. Mutagenicity of total suspended particulate matter in air (TSPM) and of wipe samples from possible contact surfaces were measured in the Ames mutagenicity assay with *Salmonella typhimurium* YG1041 in the presence of a metabolic activation system. Large differences in median mutagenicity of TSPM samples were observed between companies (range 49–1056 rev/m³) and to a lesser extent between production functions (range 129–402 rev/m³). The production function curing revealed overall the highest TSPM mutagenicity levels. Forty-one percent of the surface wipe samples revealed mutagenic activity ranging from 26 to 665 rev/cm². Mixing had the largest proportion of positive samples resulting in a median surface mutagenic contamination of 39 rev/cm². Surface mutagenic contamination, averaged per department/company combination, showed only a weak correlation with TSPM mutagenicity ($r = 0.28$, $P = 0.05$). Company, production function and total soluble matter (e.g. mass collected upon extraction with organic solvents with different polarity) explained 79 and 81% of the variability in mutagenicity of TSPM and surface contamination levels, respectively. ‘Company’ was identified as the most important exposure determinant for mutagenic activity in TSPM and surface wipe samples. This indicates the importance of company specific determinants like production volume and rubber chemicals used for the encountered mutagenic exposure conditions. Detection of substantial mutagenic activity on possible contact surfaces supports furthermore the potential importance of the dermal route in the uptake of genotoxic compounds of workers in the rubber manufacturing industry. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Epidemiological studies among workers in the rubber industry have shown an excess cancer risk with most consistent results for bladder, laryngeal and lung cancer and leukemia [1]. Unfortunately,

these epidemiological studies did not provide information associating specific job-related exposures with the observed cancer risks. Most of these studies used job titles and work areas as proxy of exposure, due to the general absence of detailed exposure assessment.

A number of reports on exposure measurements among rubber manufacturing workers have been published. Traditionally, these surveys focussed on exposure to airborne particulate matter and solvents

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[2–5]. More recently, compound(s) specific studies were conducted of exposure to nitrosamines and polycyclic aromatic hydrocarbons [6–8]. However, it has in general not been possible to identify specific chemicals responsible for the increase in malignant neoplasms in the rubber manufacturing industry, therefore, estimation of the integrated genotoxic potency of the exposure, without the need for analytical methods to identify each genotoxic compound separately, could possibly yield valuable exposure information.

In several studies in the rubber manufacturing industry, exposure to genotoxic compounds has been described by measuring mutagenic activity of airborne particulates and fumes and mutagenicity in urine of workers [6,9–12]. These studies were typically performed in only one company and focussed on the mixing and curing department as these production functions were thought to represent worst case situations. Therefore, little is known about the range and variation in genotoxic exposure levels between companies and production functions.

Several studies have addressed the possible relevance of dermal exposure in the rubber manufacturing industry [5,11,13]. Direct evidence for the importance of the dermal exposure route was found in a study by Bos et al. [12] in an aircraft tire retreading company. In this study a relation was found between dermal exposure to cyclohexane soluble matter (CSM) and urinary mutagenicity. Recently, a dermal exposure pathway analyses, carried out in the same companies as the present study, showed that personal dermal CSM exposure was related to the level of CSM contamination of possible contact surfaces [14]. Therefore, additional assessment of mutagenic activity of these contaminated surfaces could give insight in the potential role of the dermal route for uptake of genotoxic compounds.

This paper describes the result of an industry wide survey of mutagenic exposure conditions in the rubber manufacturing industry in The Netherlands. Mutagenicity of total suspended particulate matter (TSPM) and surface contamination was measured in the Ames mutagenicity assay with *S. typhimurium* YG1041 in the presence of a metabolic activation system. The influence of company, production function and several exposure indices on the variance in mutagenic activity was subsequently studied.

2. Material and methods

2.1. Location

The actual field study was conducted from January 1997 to July 1997 in nine rubber manufacturing companies in The Netherlands (three rubber tire, five general rubber goods and one retreading company). All production functions (e.g. compounding and mixing, pre-treating, moulding, curing, finishing, shipping, engineering service and laboratory) were included in the survey. General characteristics of the companies and production functions studied are presented elsewhere [15,16]. Information on rubber chemicals used was collected in companies with a compounding and mixing department ($n = 5$) based on chemical inventory registries and a walk through survey. All samples and additional information were collected during the course of 1 week per company.

2.2. Air monitoring

Eight hour total suspended particulate matter (TSPM) exposure was measured on random days with a high-volume sampler at a flow rate of $0.9 \text{ m}^3/\text{min}$ in combination with Whatman GF/A glass fiber filters with a diameter of 12.5 cm [17]. Flow rate was measured before and after sampling and the accepted range was set between 0.8 and $1.0 \text{ m}^3/\text{min}$. Samples were discarded if the measured flow rate did not meet the a priori accepted range. All samples were conditioned (e.g. constant temperature and relative humidity) at least 24 h before weighing and analyzed gravimetrically in a conditioned weighing room at a temperature of $20 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity. Subsequently, mass of particulate matter was used to calculate TSPM exposure (mg/m^3). On average 1.8 repeated TSPM samples were collected per sample site for each department within a company, which were then pooled for further analyses [14].

2.3. Surface contamination

Surface contamination was determined by obtaining wipe samples of potential contact surfaces. Potential contact surfaces were identified based on interviews and observations of the workers while executing their specific tasks using the following criteria: wipe

location should be a potential dermal contact site; site is regularly and frequently involved in the handling of chemicals and/or rubber products; wipe location is sufficiently large to accommodate a wipe of 100 cm² (rubber compounds and products ($n = 12$), machines and tools ($n = 57$), control panels ($n = 4$), workbenches ($n = 31$)). Samples were taken by a modification of the OSHA wipe sampling procedure [14,18]. In the modified procedure, a surface area of 100 cm² of a potential contact surface was chosen as sampling area. Each area was wiped three times consecutively with Clean cylce™ wet VDU wipes (Inmac) containing 70% water and 30% isopropyl alcohol. A consistent sampling area was maintained by use of a template. The same wipe pattern, applied with maximum operator pressure, was adhered throughout the study. Repeated samples of the same surface were pooled and stored at -20°C before analyses.

2.4. Analytical analyses

2.4.1. Extraction procedure

Samples (filters and VDU wipes) were extracted consecutively with cyclohexane, dichloromethane and methanol (Merck, Darmstadt, Germany) as described previously [19]. In short, samples were placed in an extraction vial with 15 ml of cyclohexane and sonicated for 20 min. A total of 8 ml of the suspension was filtered through a glass intertube G4 (Alihn) and collected in a pre-weighed 10 ml vial. After evaporation of the organic solvent under nitrogen and subsequently 2 h drying at 40°C , the organic soluble residue (cyclohexane soluble matter, CSM) was weighed by means of a microbalance. After evaporation of the organic solvent from the extraction vial at 20°C under nitrogen, the filter or VDU wipe was consecutively extracted with dichloromethane and methanol according to the same procedure as described for cyclohexane and collected in the same 10 ml vial. Mass of the dry residue after cyclohexane extraction was used to calculate CSM and mass of the combined dry extracts was used to estimate total soluble matter (TSM) of the TSPM and surface wipe samples. Dry extracts were stored at -20°C until further analyses.

2.4.2. Mutagenicity testing

Extracts of TSPM and surface contamination samples were tested for mutagenic activity with

S. typhimurium YG1041 in the Ames mutagenicity assay [20]. The *S. typhimurium* strain YG1041, with elevated nitroreductase and *O*-acetyltransferase activity, is extremely sensitive for the presence of mutagenic nitroarenes and/or aromatic amines. Dry extracts were dissolved in 2.5 ml of dimethylsulfoxide (DMSO) (Merck) and assayed at five different dose levels in triplicate for mutagenic activity in the presence of S9-mix derived from aroclor 1254 induced rat livers (mutagenicity of references, spontaneous 144 ± 19 rev/plate; positive control (2-aminopyrene, $0.1 \mu\text{g}/\text{plate}$) 2584 ± 232 rev/plate).

For determination of the mutagenic activity, the arithmetic mean of the triplicate tested dose level was calculated. Data acquired at different dose levels were used to construct a dose-response curve and the slope of the linear component was used as an estimate of the mutagenic potency [21]. Samples were considered mutagenic if explained variation in revertants exceeded 90% ($r > 0.95$) and the observed number of revertants was higher than the limit of detection (LOD) for at least two dose levels. LOD was calculated at three standard deviations above the mean blank (DMSO, $n = 10$). Mutagenicity of TSPM and surface wipe samples was expressed as number of revertants per cubic meter (rev/m³) and as revertants per square centimeter (rev/cm²), respectively. Samples that were not mutagenic were arbitrarily assigned 2/3 of the mutagenic activity of the sample with the lowest detectable mutagenicity level (19 and 17 rev/cm² for TSPM and surface contamination samples, respectively).

2.5. Statistical analyses

Pearson correlation coefficients were used to investigate the relation between exposure indices (TSPM, TSM, CSM), mutagenic surface contamination and mutagenicity of TSPM samples. To calculate the Pearson correlation coefficient between TSPM mutagenic activity and surface mutagenicity levels, the mutagenic activity of the TSPM and surface wipe samples were averaged per department/company combination ($n = 48$) and subsequently compared. Associations between company, production function, exposure indices and mutagenic exposure conditions were further studied with linear regression models using continuous variables (TSPM, TSM and CSM exposure) and dummy variables (company and production function).

All statistical analyses were performed using SAS version 6.12 software [22].

3. Results

In total 145 repeated TSPM samples were collected at 83 different sampling sites within nine rubber companies. Total suspended particulate matter exposure and mutagenicity of TSPM and contaminated surfaces are presented in Tables 1 and 2 stratified for each sampling site by company and production function, respectively. Overall a low median TSPM concentration (0.17 mg/m^3) was observed. Mutagenic activity measured with *S. typhimurium* YG1041 with metabolic activation was detected in 76 of the 83 pooled TSPM samples (95%). A large difference in median mutagenic TSPM exposure was observed between companies (range 49–1056 rev/m^3), with the highest median mutagenic TSPM exposures found in companies 3 and 4, both of which produced large quantities of technical rubber goods. No systematic difference in median mutagenic TSPM exposure was, however, observed between rubber tire, technical rubber goods and retreading companies. Median mutagenic TSPM exposure varied only by

a factor of 3 between production functions (range 129–402 rev/m^3). The production function curing revealed overall the highest mutagenic exposure levels (median 402 rev/m^3) followed by the production functions finishing and moulding (median 262 and 252 rev/m^3 , respectively).

Forty-one percent of the 104 collected surface wipe samples had detectable mutagenic activity levels with a range of 26 to 665 rev/cm^2 for the Ames-positive samples. Wipe samples with no detectable mutagenic activity were found for all companies and in all production functions. Therefore, variation in mutagenic surface contamination levels was large within production functions and companies. Variability in median mutagenic surface contamination levels between companies was again larger than between production functions although less pronounced as for mutagenic TSPM exposure. Interestingly, the production function mixing revealed the largest proportion of positive samples and consequently the highest median mutagenic surface contamination level (median 39 rev/cm^2).

No correlation was observed between TSPM exposure and the mutagenicity of TSPM samples ($r = 0.07$, $P = 0.56$) (Table 3). Extractable mass (TSM and CSM) showed an overall good correlation with the observed TSPM mutagenicity, $r = 0.75$ and $r = 0.71$,

Table 1
Total suspended particulate matter (TSPM) exposure and mutagenicity of TSPM and contact surfaces stratified by company

Company (SBI-code) ^a	TSPM (mg/m^3)				Mutagenicity (<i>S. typhimurium</i> strain YG1041)									
	N ^b	AM ^c	Median	Range ^d	TSPM (rev/m^3)				Surface contamination (rev/cm^2)					
					n (%) ^e	AM	Median	Range	N	n (%)	AM	Median	Range	
1 (3112)	7	0.20	0.17	0.12–0.40	7 (100)	99	99	41–169	10	4 (40)	64	17	17–243	
2 (3112)	7	0.14	0.13	0.09–0.27	7 (100)	207	153	126–366	10	2 (20)	32	17	17–123	
3 (3112)	6	0.29	0.23	0.16–0.61	6 (100)	675	753	383–895	4	3 (75)	111	103	17–221	
4 (3112)	5	0.29	0.25	0.14–0.55	5 (100)	1043	1056	861–1263	8	4 (50)	42	33	17–90	
5 (3112)	11	0.12	0.11	0.04–0.31	8 (73)	64	49	19–221	12	2 (17)	56	17	17–449	
6 (3111)	14	0.65	0.24	0.05–2.75	13 (93)	340	238	19–1178	14	2 (14)	25	17	17–105	
7 (3111)	12	0.30	0.16	0.03–1.45	12 (100)	296	231	28–752	17	5 (29)	37	17	17–198	
8 (3111)	11	0.20	0.13	0.03–0.49	10 (91)	406	452	19–658	19	14 (74)	142	52	17–665	
9 (3121)	10	0.18	0.16	0.10–0.32	8 (80)	270	299	19–494	10	7 (70)	172	129	17–574	
All	83	0.29	0.17	0.03–2.75	76 (95)	333	225	19–1263	104	43 (41)	65	17	17–665	

^a Dutch standard industrial classification: 3111 rubber tire; 3112 general rubber goods, 3121 retreading.

^b Number of samples.

^c Arithmetic Mean.

^d Minimum and maximum value.

^e Number and proportion of samples with detectable mutagenicity levels between parenthesis.

Table 2

Total suspended particulate matter (TSPM) exposure and mutagenicity of TSPM and contact surfaces stratified by production function

Production function	TSPM (mg/m ³)				Mutagenicity (<i>S. typhimurium</i> strain YG1041)								
	N ^a	AM ^b	Median	Range ^c	TSPM (rev/m ³)				Surface contamination (rev/cm ²)				
					<i>n</i> (%) ^d	AM	Median	Range	<i>N</i>	<i>n</i> (%)	AM	Median	Range
Mixing	15	0.33	0.17	0.06–1.45	11 (73)	182	139	19–636	24	15 (63)	69	39	17–304
Pre-treating	9	0.14	0.12	0.05–0.30	8 (89)	276	129	19–1104	14	7 (50)	87	33	17–285
Moulding	19	0.29	0.14	0.03–1.56	18 (95)	319	252	19–1056	28	8 (29)	39	17	17–293
Curing	25	0.29	0.18	0.08–1.92	24 (96)	514	402	19–1263	16	7 (44)	128	17	17–665
Finishing	8	0.49	0.16	0.09–2.75	8 (100)	267	262	60–627	12	3 (25)	40	17	17–167
Shipping	4	0.20	0.21	0.14–0.26	4 (100)	170	173	122–212	3	1 (33)	203	17	17–574
Engineering service	2	0.20	0.20	0.08–0.32	2 (100)	246	246	55–437	6	2 (33)	91	17	17–449
Laboratory	1	0.03	0.03	–	1 (100)	157	157	–	1	0 (0)	17	17	–
All	83	0.29	0.17	0.03–2.75	76 (95)	333	225	19–1263	104	43 (41)	65	17	17–665

^a Number of samples.^b Arithmetic Mean.^c Minimum and maximum value.^d Number and proportion of samples with detectable mutagenicity levels between parenthesis.

Table 3

Pearson correlation coefficients (and *P*-values) between several exposure indices (TSPM, CSM and TSM) and mutagenicity of total suspended particulate matter (*n* = 83)

Exposure index	Mutagenicity TSPM (rev/m ³)	TSM	CSM
Total suspended particulate matter (mg/m ³)	0.07 (0.56)	0.07 (0.55)	0.05 (0.62)
Cyclohexane soluble matter (μg/m ³)	0.71 (0.0001)	0.96 (0.0001)	
Total soluble matter (μg/m ³)	0.75 (0.0001)		

respectively. Both exposure indices were also strongly correlated with each other ($r = 0.96$, $P = 0.0001$). Correlation between TSPM and surface mutagenicity, aggregated per department/company combination, showed only a weak correlation ($r = 0.28$, $P = 0.05$).

Variability in mutagenicity of TSPM samples was to a great extent explained by company ($r^2 = 0.59$), TSM exposure ($r^2 = 0.56$) and CSM exposure ($r^2 = 0.50$) (Table 4). Combination of all determinants in one multivariate regression model, excluding CSM exposure due to collinearity with TSM exposure, explained 79% of the total variance in TSPM mutagenicity levels. Surface mutagenic contamination was associated with company and production function, however, the explained variance was considerably lower at 18 and 10%, respectively. The interaction term between company and production function was statistically significant for surface mutagenicity levels ($P = 0.0001$). This indicates that surface mutagenic

contamination levels were determined by specific conditions in each production function in each company. Combination of company, production function, TSM exposure and the interaction term in one multivariate regression model explained 81% of the total variance in surface mutagenicity levels (Table 4).

4. Discussion

Exposure to mutagenic compounds in the rubber manufacturing industry may occur by inhalation, ingestion, or dermal absorption [11–13]. Skin contact with contaminated surfaces and deposition of rubber particles on the skin have been identified as important exposure determinants of dermal CSM exposure in the rubber manufacturing industry [5,14]. Therefore, assessment of mutagenic activity of possible contact surfaces and rubber dust and fume exposure could

Table 4
Univariate and multivariate regression models for mutagenicity of total suspended particulate matter and mutagenic surface contamination

	Univariate regression model		Multivariate regression model	
	<i>P</i> -value ^a	<i>r</i> ² ^b	<i>P</i> -value ^c	<i>r</i> ² ^d
Mutagenicity of total suspended particulate matter (rev/m ³)				
Production function	0.04	0.17	0.15	0.79
Company	0.0001	0.59	0.0001	
TSPM (mg/m ³)	0.56	0.01	0.83	
TSM (μg/m ³)	0.0001	0.56	0.0001	
CSM (μg/m ³)	0.0001	0.50	–	
Mutagenicity of surface contamination (rev/cm ²)				
Production function	0.16	0.10	0.0001	0.81 ^e
Company	0.01	0.18	0.0001	
Production function × company	0.0001	0.79	0.0001	
TSM (μg/cm ²)	0.27	0.01	0.011	
CSM (μg/cm ²)	0.51	0.01	–	

^a *P*-value derived from the univariate regression analyses.

^b Explained proportion of variance per individual determinant.

^c *P*-value derived from the multivariate regression analyses.

^d Total proportion of variance explained by all factors in a multivariate regression model (exclusion of CSM exposure due to collinearity with TSM exposure).

^e With inclusion of the interaction term between production function and company in the multivariate regression model (without interaction term $r^2 = 0.31$).

yield an estimate of potential exposure to mutagenic compounds through the dermal and inhalation route. The validity of surface wipe samples as an indicator of dermal exposure depends greatly on the level of contamination and the frequency and duration of skin contact with the contaminated surfaces. In a previous study a relation was observed between dermal CSM exposure and CSM contamination of the same contact surfaces as were tested for mutagenicity in this study [14]. Therefore, it was assumed that the detected mutagenicity in the surface wipe samples was indicative for dermal exposure to mutagenic compounds.

We studied the mutagenic exposure conditions in several rubber companies in The Netherlands ($n = 9$) by measuring mutagenicity of TSPM and surface contamination samples. A large variation in mutagenic activity of TSPM and surface contamination samples was observed between companies and to a lesser extent between production functions. Modeling of the mutagenic activity of TSPM and surface contamination samples confirmed the importance of a company effect on the observed mutagenic exposure levels. The existence of substantial differences in mutagenic exposure levels between companies was also observed in a previous study, in which a significant difference

in mutagenic rubber dust and fume exposure was found between two apparently comparable rubber tire companies [19]. The influence of production function on TSPM and surface contamination mutagenicity levels was less pronounced. However, a significant interaction term was observed between company and production function in relation to mutagenic surface contamination ($P = 0.0001$), indicating that surface mutagenicity levels were determined by specific conditions in each production function within each company. The fact that mutagenic exposure levels are largely determined by company specific conditions and not so much by production function points towards the importance of company specific exposure determinants such as rubber chemicals used, production volume and overall level of control measures. Company 3 and 4 had the highest production volume of technical rubber goods and consisted mainly out of one large curing area. As the production function curing showed overall the highest mutagenic exposure levels it is not surprising that these two companies revealed the highest TSPM mutagenicity levels. The same was observed for the rubber tire companies included in this study, where the rubber tire company with the largest mixing and production volume

capacity (company 8) showed also the highest mutagenic TSPM exposure levels. If production volume is indeed an important determinant than the detected mutagenic compounds must be commonly used or generated. Only a few rubber chemicals were used in all companies of which 2,2-dibenzothiazyl disulphide (MBTS) and 2-(morpholinothio) benzothiazole (MBS) have been found to constitute some potency in short-term genotoxicity tests [23]. However, natural and synthetic rubber, fillers and process oils, with known and partly unknown constituents are also generally used.

TSPM and surface mutagenicity levels were only weakly correlated. The production function 'curing' revealed the highest TSPM mutagenicity levels, but less than half of the surface wipe samples was considered mutagenic. Inhalable exposure in the curing departments consist mainly out of rubber fumes and gases, which are not likely to deposit on contact surfaces. Mutagenicity in wipe samples originating from the curing department were mostly detected in wipe samples from recently (warm) cured rubber tires or from surfaces, which were in frequent contact with these products (data not shown). For the production function 'mixing' almost the opposite was observed with relatively low levels of TSPM mutagenicity but with the largest percentage of positive surface wipe samples. Most likely the mutagenic contamination on the tested surfaces was caused by other exposure pathways than deposition of particles like for instance spills and splashes and ejection of large particles.

The mutagenic activity of TSPM was significantly correlated with total soluble matter ($r = 0.75$, $P < 0.0001$). Interestingly, cyclohexane soluble matter revealed only a marginally lower correlation with TSPM mutagenicity levels ($r = 0.71$, $P < 0.0001$). CSM is used in the regulation of rubber dust and fume exposure in the UK since 1987, and serves as a surrogate for the complex mixture of rubber curing fumes [24]. The results of this study suggest that because CSM exposure had an overall better correlation with TSPM mutagenicity levels than the mass of the particulate matter itself, assessment of CSM exposure in the rubber industry might be a better indicator for biological activity than measurement of particulate mass.

In conclusion, mutagenic activity in TSPM and surface wipe samples was found for all production functions and companies and was certainly not restricted

to the mixing and curing departments of the surveyed companies. Although specific exposure determinants remained unclear, company related factors seemed to be of great importance. Furthermore, detection of substantial mutagenic activity on possible contact surfaces in the rubber manufacturing industry is of importance for the estimation of genotoxic exposure due to multiple exposure routes in this particular industry. These results support the evidence presented in earlier reports suggesting the potential importance of the dermal route in the uptake of genotoxic compounds for workers in the rubber manufacturing industry [5,11–13].

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