Genotoxic exposure and biological effects in the rubber manufacturing industry

Relevance of the dermal route

Roel Vermeulen

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Genotoxic exposure and biological effects in the rubber manufacturing industry

Relevance of the dermal route

Blootstelling aan genotoxische verbindingen en biologische effecten in de rubberverwerkende industrie; relevantie van de dermale blootstellingsroute (met een samenvatting in het Nederlands)

Proefschrift

Proefschrift ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de Rector Magnificus, Prof. Dr. H.O. Voorma, ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen op donderdag 18 januari 2001 des middags te 12.45 uur door

Roel Vermeulen

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'…the health of the people employed in rubber factories, coupled with verbal questioning of many hands, male and female, and likewise of foreman and others connected with such works for months and years, in several instances 20 years and upwards, has led me to the conclusion that the occupation is productive of no definitive disease, nor of lasting inconveniences. This is the concurrent testimony of all the employed, as well as of the employers.'

Dr J.T. Arlidge in a report to the Chief Inspector of Factories in 1894 [1]

Contents

Chapter 1
General introduction

Chapter 1 outlines the background and main research objectives of the study described in this thesis. Epidemiological evidence for genotoxic risk in relation to exposure in the rubber manufacturing industry is briefly discussed and a strategy for the evaluation of exposure to complex mixtures is presented. Subsequently, the study design and research methods are described.

General introduction

Background

The rubber industry has been associated with cancer risks for more than 5 decades. When in 1949, β-naphthylamine was found as an impurity in some of the rubber antioxidants it was possible to establish with reasonable certainty a direct causal link between that usage and the excess of urinary bladder cancer cases observed by Case and Hosker [2] in the British rubber industry. Although β-naphthylamine was subsequently phased out in the following years it became apparent that other less evident cancer risks were present in this industry [3-6]. In 1982 the International Agency for Research on Cancer (IARC) reviewed the evidence for carcinogenic risk in the rubber industry. It concluded that there was sufficient evidence for excess mortality from cancer of the urinary bladder, lung, stomach and leukemia and limited evidence for excess mortality from cancer of the skin, colon, prostate and lymphoma [7]. Epidemiological evidence of cancer risk in the rubber industry published after this initial IARC evaluation was reviewed recently by Kogevinas *et al.* [8]. Their aim was to examine whether health and safety measures taken since 1982 had resulted in a decrease in previously observed cancer risks. Although the magnitude of the observed risks varied considerably between studies, the findings still supported an excess mortality from cancer of the lung, urinary bladder, and leukemia. An excess risk of stomach cancer among rubber workers was no longer found, but now an excess risk of laryngeal cancer was found to be consistent between studies that was not seen in the initial evaluation by IARC [7]. It was concluded that preventive measures taken in the rubber industry might have decreased risks, but that this had not yet been picked up in epidemiological studies so far. Due to the long latency time between exposure to a carcinogen and the clinical manifestation of solid tumors, at least 10 to 20 years, the observed excess risk for urinary bladder, laryngeal and lung cancer are likely related to exposure conditions encountered in the remote past. These exposure circumstances would still have included among others β-naphthylamine exposure. It remains therefore uncertain if the observed cancer risks in recent epidemiological studies are related to exposures from the past or, from exposures that still exist today.

Although 33 cohort and 15 industry specific nested case control studies [7; 8] have been conducted in the rubber industry since 1972, it has generally not been possible to identify chemicals which might be responsible for the increase in malignant neoplasms in the rubber manufacturing industry. The exceptions are urinary bladder cancer associated with the exposure to aromatic amines, especially β-naphthylamine and benzidine and leukemia associated with exposure to solvents in particular benzene [7]. However, the exclusive link of urinary bladder cancer to β-naphthylamine exposure was questioned by recent data indicating an excess of urinary bladder cancer among workers with no recorded exposure to β-naphthylamine [9]. Unfortunately, an absence of detailed exposure

data in epidemiological studies in this particular industry has prohibited the linkage of certain chemicals to specific cancer sites. The primary reason for this deficiency is the considerable amount of chemical additives (several hundreds) that are being used in the rubber industry. Moreover, due to high pressures and temperatures during curing more (unknown) chemical substances are being formed and released. This complex exposure profile does not only differ between rubber factories but also in time due to variations in production and control technology, process requirements for different products and variation in work practices [7; 10].

Exposure in the rubber manufacturing industry

Some general studies of environmental contamination in the rubber manufacturing industry have been carried out. Most of these surveys were conducted in the late seventies and early eighties in the USA and UK and focussed on exposure to airborne particulate matter and solvents [11-13]. Later, more specific studies were conducted of exposure to nitrosamines and several polycyclic aromatic hydrocarbons [14-16]. Although these studies yielded a wide range in average exposure levels, some general exposure patterns could be identified. Exposure to airborne particulate matter occurs mostly in the beginning of the production process during the handling of raw materials, weighing and mixing, and decreases further down the production process. Solvents are mainly used during assembling of rubber goods and in lubricants sprayed on rubber goods before curing. Curing itself generates rubber fumes and gases occasionally containing benzene, polycyclic aromatic hydrocarbons and nitrosamines. During inspection, finishing and storage of rubber goods, products still off-gas contaminants like nitrosamines into the workplace atmosphere [7].

Little attention has been paid to dermal exposure in this particular industry. Falck *et al.* [17] and Kilpikari [18] already suggested in the early eighties that dermal absorption of chemical compounds could play an important role in the rubber industry. Direct evidence for this hypothesis was found in a study by Bos *et al.* [19] in an aircraft tire retreading company where a direct relation was found between dermal exposure to cyclohexane soluble matter (CSM) and urinary mutagenicity while no relation was found between urinary mutagenicity and rubber particulates and fumes in air. Later, Kromhout *et al.* [10] studied dermal exposure to CSM in the rubber manufacturing industry on a large scale. Calculations suggested that the amount of cyclohexane soluble compounds on the skin of hands and wrists was potentially higher than the inhaled amount. Depending on the specific situation in a factory and the use of protective devices for skin contamination, the amount available for uptake through the skin could be up to a tenfold higher than the amount inhaled [10].

Evaluation of exposure to complex mixtures

In the evaluation of exposure of complex mixtures of potentially carcinogenic chemicals several approaches can be followed. If the chemical composition of the complex mixture is well characterized and detailed toxicological as well as epidemiological data on the mixture are available, compound specific exposure indicators of external, internal, and/or biological effective dose would suffice. However, selection of appropriate biomarker(s), as an index of exposure to carcinogens is difficult as complex mixtures frequently contain more than one carcinogen and in addition the concentration of the marker in the mixture often varies within workplaces and time. In most instances, the exact composition of the complex mixture is unknown. In such exposure situations non-specific exposure variables have to be used. In the evaluation of exposure to complex mixtures of potentially carcinogenic chemicals, these estimates are usually derived from techniques that make use of the notion that one step in carcinogenesis is damage to or mutation in DNA. To further unravel what proportion of the total genotoxic effect is attributable to specific components in the mixture, a step-wise evaluation has been proposed [20]. First, external exposure is characterized as completely as possible (Figure 1-1). This provides an estimate of the level and pattern of exposure both to the mixture and if practical to its individual constituents. The next step is to analyze the relation between external exposure and relevant (early) biological effect markers. Identification and quantification of early genotoxic effects consequently evolves from non-specific effect indicators (chromosome aberrations, sister chromatide exchanges, gene mutations, oncogene activation etc.) through specific effect indicators (DNA adducts). Ultimately this scheme would result in the identification of specific constituent(s) responsible for the observed cancer risk.

In the assessment of external exposure additional 'non-specific' exposure indicators can be used to estimate the toxic properties of the exposure. An example of such a 'non-specific' technique for the estimation of mutagenicity is the *S. typhimurium* mutagenicity assay [21], which has been used in several studies to evaluate life-style and occupational exposure to mutagens and potential carcinogens [22; 23]. Although the bioassay does not discriminate between specific mutagens, it has the advantage of detecting the integrated genotoxic potency of these components, without the need for analytical methods to identify each mutagenic compound separately [24].

In several studies in the rubber manufacturing industry, exposure to mutagenic compounds was established with this assay either by measuring mutagenicity of airborne particulates and fumes or by measuring mutagenicity in urine of workers [14; 17; 19; 25; 26]. Although, these studies showed occupational exposure to mutagens, components responsible for the mutagenic activity were hardly identified [27].

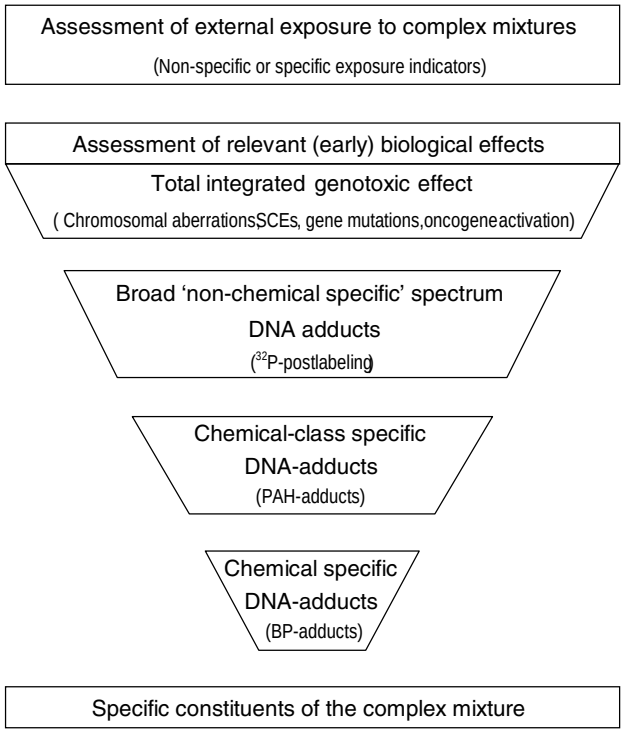

Figure 1-1 Stepwise approach for assessment of potential carcinogenic risk of complex mixtures (Based on Perera et al.[20])

Measurement of exposure or effects of contaminants and/or their metabolites in human body fluids and tissues are likely influenced by gene-environment interactions, including biotransformation polymorphisms. It appears that many of the carcinogen metabolizing enzymes are inducible by xenobiotics [28]. Furthermore, many of the encoding genes are polymorphic and multiple allelic variants relevant for the phenotype exist in human populations [29]. Cytochrome P450 (CYP), glutathione *S*-transferase (GST) and *N*-acetyltransferase (NAT) gene families play an important role in the human variability to metabolize carcinogens. In the metabolism of aromatic and heterocyclic amines and hydrazines of which some are used in occupational processes (e.g. rubber and dye industry) NAT capacity plays a significant role [30]. In humans, two genes, NAT1 and NAT2 (both polymorphic) are responsible for *N*-acetyltransferase activity.
Slow NAT2 acetylation status has been associated with increased levels of urinary mutagenicity [31-33], DNA adducts [34-37], increased urinary bladder cancer risk [38] and several other cancer risks [39]. In contrast rapid acetylators were suggested to be at higher risk of developing cancer of the colon [40]. Although the increase in cancer risk due to specific biotransformation polymorphisms in most cases is small they are widespread in the general population and of importance for individuals with a particular environmental exposure [41].

Research objectives

The objectives of the studies described in this thesis were:

- To evaluate current inhalable particulate and dermal exposure levels in the rubber manufacturing industry in The Netherlands (Sections 3.1 & 3.2)
- To characterize external mutagenic exposure conditions in the rubber manufacturing industry in The Netherlands (Sections 4.2 & 4.3)
- To study the relation between inhalable particulate matter, dermal exposure levels, urinary mutagenicity and DNA adducts in exfoliated urothelial cells (Sections 4.4 & 4.5)

As a decrease in the barrier function of the skin could lead to increased dermal uptake [42; 43], workers with a damaged skin possibly reveal higher internal doses. Therefore, a considerable effort was made to ascertain adverse skin conditions and possible workplace related and personal factors (Sections 2.1 & 2.2). As non-chemical specific exposure indicators were used to evaluate genotoxic exposure conditions, special attention was paid to control for the possible influence of other sources of genotoxic exposures such as active and passive smoking (Section 4.1).

Study description

The results presented in this thesis stem from two studies. A pilot study was conducted in 1995 in two rubber tire companies located in The Netherlands and Sweden. The main study was conducted in 1997 in 9 different rubber manufacturing companies. The selected companies formed a representative cross-section of the rubber manufacturing industry in The Netherlands (3 rubber tire (including the pilot company from The Netherlands), 5 general rubber goods and 1 retreading company). Seven of the selected companies already participated in an earlier industry-wide survey conducted in 1988 [10]. This enabled direct assessment of trends in inhalable particulate matter and dermal exposure levels and subsequently the effectiveness of control measures implemented over a nine-year period.

In total 225 subjects took part in the industry-wide survey. Subjects were asked to complete a self-administered questionnaire prior to the survey on, among others demographics, lifestyle factors, skin symptoms and known risk factors of skin diseases. Evaluation of current skin condition was performed on the last day of the exposure survey by a dermatologist. During the one-week exposure survey per company ambient and personal inhalable particulate matter and dermal exposure levels were quantified. Subsequently, ambient measurements were used to estimate the genotoxic properties of the samples collected in the rubber manufacturing industry. Based on these results the study population was divided in four categories of different inhalation and/or dermal mutagenic exposure levels. Subsequently, 116 subjects, both smokers (n=45) and non-smokers (n=71) were randomly selected from the a-priori defined exposure groups to

unravel the relative contribution of the dermal exposure route. Spot urine samples of these selected subjects, collected during the exposure survey were analyzed for urinary mutagenicity and cotinine levels. Additionally, subjects were phenotyped for CYP1A2 and NAT2 biotransformation polymorphism. Finally, 60 out of the 71 non-smokers, were selected to investigate the possible relation between genotoxic exposures and DNA adduct formation in exfoliated urothelial cells of workers in the rubber manufacturing industry.

Chapter 2
Skin aberrations in the rubber manufacturing industry

In chapter 2 some methodological considerations regarding the ascertainment of hand dermatitis in an industrial population are described and the differences in perception of skin diseases between different (occupational) populations are addressed (Section 2.1). In section 2.2 prevalence of hand dermatitis among rubber workers and possible work-related risk factors such as dermal exposure and hand washing practices are investigated.

Section 2.1
Ascertainment of hand dermatitis using a symptom-based questionnaire; Applicability in an industrial population

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In this study, the applicability of a symptom-based questionnaire on hand dermatitis was assessed in a population of rubber workers. The questionnaire was previously validated in a study among nurses. 224 subjects employed in 9 different companies completed a questionnaire on skin complaints. Subsequently, 202 workers attended the physical examination of the skin by a dermatologist.

The ascertainment of skin complaints according to the questionnaire was compared to the medical evaluation. The 2 different diagnostic tools used for assessing dermatitis resulted in dissimilar estimates of the prevalence of active hand dermatitis, ranging from 6.9% to 38.1% of all workers. Using the medical evaluation as 'gold standard' we observed a moderate sensitivity and specificity (respectively 71.4%; 95% CI: 47.7-95.1 and 76.1%; 95% CI: 70.0 – 82.2), a low positive predictive value (18.2%; 95% CI: 8.0 –28.4) and a high negative predictive value (97.3%; 95% CI: 94.7 –99.9) for the classification based on the self-administered questionnaire. When evaluated against 'first symptoms of dermatitis' the sensitivity decreased, while the specificity remained almost the same.

The deviant findings between the present and the original validation study of the same questionnaire among nurses hamper its applicability in populations with different occupations. Therefore, if questionnaires are to be used, validity studies have to be carried out to evaluate differences in perception of skin diseases between different (occupational) populations.

Contact dermatitis, 2000, 42, 202 - 206

Introduction

Skin diseases such as contact dermatitis are common occupational illnesses and are estimated to account for 9-35% of all occupational diseases [44-47]. However, because these estimates are based on several different diagnostic methods, such as medical records, questionnaires and physical examinations, it is difficult to draw comparisons between occupational groups and the general population. It is generally believed that the use of medical records leads to an underestimation of the prevalence of contact dermatitis as only relatively few people with skin complaints seek medical assistance [48]. Other studies have used medical evaluations by trained physicians. However, the use of such a labor-intensive diagnostic tool in large epidemiological studies is often not feasible. As a consequence, questionnaires are most often used as a diagnostic tool for estimating the prevalence of skin diseases or as a primary screening method. Their validity, however, has been addressed in only a few studies, in which questionnaire-based diagnoses of hand

dermatitis were compared to an objective assessment of hand dermatitis by trained physicians [48-52]. The results of these validation studies range from high specificity (100%) and lower sensitivity (60%) to a high sensitivity (100%) but lower specificity (64%) [48; 52].

In this study a symptom-based questionnaire on hand dermatitis, previously validated among nurses, and a physical examination were used to identify skin diseases in a population of rubber workers. The aim of the study was to assess the applicability of such a symptom-based questionnaire in an industrial population.

Materials and methods

Study population

The study was conducted from January 1997 through July 1997 among 225 subjects employed in the rubber manufacturing industry in The Netherlands. Subjects were part of a large exposure survey in the rubber manufacturing industry investigating the relative contribution of dermal exposure to the total genotoxic dose. Subjects were employed in 9 companies and were selected on the basis of their production function [53]. Subjects were asked to complete a self-administered medical questionnaire prior to the exposure survey (Monday). 224 (99.6%) Subjects successfully completed the questionnaire; of these, 202 (90.2%) workers attended the medical evaluation at the end of the week (Friday).

Hand dermatitis

Hand dermatitis was assessed using a symptom-based questionnaire and a medical evaluation. Questions concerning hand dermatitis were adapted from a validated questionnaire developed by Smit *et al.* [48]. In total 4, different criteria were used for the diagnosis of hand dermatitis on the basis of the questionnaire.

Hand dermatitis was defined as a positive answer to at least 1 of the following: 'Have you had one of these symptoms at your hands or fingers in the past 12 months: (a) 'red swollen hands or fingers', (b) 'red hands or fingers and fissures', (c) 'vesicles on the hands or at the sides of the fingers', (d) 'scaling hands or fingers with fissures', (e) 'itching hands or fingers with fissures'. Moreover, subjects had to give a positive answer to either of these 2 questions: 'did one or more of these symptoms last for more than three weeks?'; 'did one or more of these symptoms occur more than once ?' (Criteria I). A more lenient additional classification based on 1 or more symptoms but without the requirement of long-lasting or recurrent symptoms (Criteria II) and a more stringent classification based on the occurrence of 2 or more symptoms with the addition of long-lasting or recurrent symptoms were also used (Criteria III). The question 'do you have a skin disease at this moment?' was used to estimate the point prevalence of current skin diseases (Criteria IV).

The medical evaluation of current skin conditions was conducted by 2 dermatologists. Objective skin symptoms were used for the classification: 'active hand dermatitis' ('major' dermatitis), and 'the first symptoms of dermatitis' ('minor' dermatitis). 'Major' dermatitis was defined as: erythema, papules, vesicles and fissures, comprising a clear eczematous picture. 'Minor' dermatitis was exhibited as erythema, slight chapping and scaling of the skin [51; 54]. No distinction was made between irritant and allergic dermatitis, as morphologic characteristics of these skin disorders are similar. Neither the dermatologist nor the worker was informed about the results of the questionnaire on hand dermatitis prior to the medical evaluation.

Data analysis

The questionnaire's applicability was studied by computing its sensitivity (% of individuals with the disease, who are classified as having the disease), specificity (% of individuals without the disease who are classified as not having the disease), positive predictive value (PPV) (% of subjects with a positive test who have the disease) and negative predictive value (NPV) (% of subjects with a negative test who do not have the disease). The medical diagnosis served as 'gold standard' in these calculations. For the statistical procedures regarding 'minor' dermatitis, subjects with 'major' dermatitis were excluded from the analysis. 95% confidence intervals were calculated assuming prevalence and validity statistics as a proportion (ρ) drawn from a normal distribution ($\rho \pm 1.96 \sqrt{\rho (1- \rho)/n}$).

Results

The prevalence of hand dermatitis as estimated by the symptom-based questionnaire and medical evaluation is presented in Table 2.1-1. The different classifications and diagnostic tools resulted in dissimilar estimates of the prevalence of hand dermatitis. The prevalence of workers with hand dermatitis based on the symptom-based questionnaire ranged from 14.9 % to 38.1% depending on the criteria used. 35 subjects (17.3%) had skin complaints at the time of the survey. Although 70 subjects (34.6%) were diagnosed with skin diseases of some kind, only 14 cases (6.9%) of 'major' hand dermatitis were identified according to the medical evaluation.

Both sensitivity and specificity for 'major' hand dermatitis were moderate (respectively 71.4% and 76.1%), the positive predictive value was low (18.2%) and the negative predictive value high (97.3%) (Table 2.1-2). Estimates of these statistics for 'minor' dermatitis resulted in a considerable decrease in sensitivity (39.3%) while the specificity remained almost unaffected (82.6%). Application of the more lenient classification (Criteria II) of hand dermatitis based on the symptom-based questionnaire resulted in a higher sensitivity for 'major' and 'minor' dermatitis, respectively 85.7% and 57.1%. However, the specificity decreased slightly. For the more stringent categorization (Criteria III) the opposite was observed, as the sensitivity decreased and the specificity

Table 2.1-1 Prevalence of hand dermatitis as estimated by a symptom-based questionnaire and medical evaluation (95% confidence interval between parentheses).

Criteria	Type of diagnosis of hand dermatitis	Prevalence		
		N	% [c]	(95% CI)
	Questionnaire			
I	One or more symptoms and recurrent or lasted more than 3 weeks[a]	55	27.2	(21.1 - 33.3)
II	One or more symptoms [a]	77	38.1	(31.4 – 44.8)
III	Two or more symptoms and recurrent or lasted more than 3 weeks[a]	30	14.9	(10.0 – 19.8)
IV	Complaints at time of survey [b]	35	17.3	(12.0 – 22.5)
	Medical evaluation			
	'Major' Dermatitis	14	6.9	(3.4 – 10.4)
	'Minor' Dermatitis	56	27.7	(21.5 – 33.9)

a) Period prevalence (last 12 months)
b) Point prevalence
c) N=202 subjects

Table 2.1-2 Validity statistics of self-reported hand dermatitis compared to physical examination of hand dermatitis (95% confidence intervals between parentheses).

Criteria		'Major' Dermatitis		'Minor' Dermatitis		Dermatitis [c]	
		%	(95% CI)	%	(95% CI)	%	(95% CI)
I	**Symptom based diagnosis: ≥ 1 symptoms and recurrent or lasted more than 3 weeks**						
	Sensitivity	71.4	(47.7 – 95.1)	39.3	(26.5 – 52.1)	45.7	(34.0 – 57.4)
	Specificity	76.1	(70.0 – 82.2)	82.6	(76.1 – 89.1)	82.6	(76.1 – 89.1)
	PPV [a]	18.2	(8.0 – 28.4)	48.9	(34.3 – 63.5)	58.2	(45.2 – 71.2)
	NPV [b]	97.3	(94.7 – 99.9)	76.2	(69.2 – 83.2)	74.1	(67.0 – 81.2)
II	**Symptom based diagnosis: ≥ 1 symptoms**						
	Sensitivity	85.7	(67.4 – 100)	57.1	(44.1 – 70.1)	62.9	(51.6 – 74.2)
	Specificity	65.4	(58.6 – 72.2)	75.0	(67.6 – 82.4)	75.0	(67.6 – 82.4)
	PPV [a]	15.6	(7.5 – 23.7)	49.2	(37.0 – 61.4)	57.1	(46.0 – 68.2)
	NPV [b]	98.4	(96.2 – 100)	80.5	(73.5 – 87.5)	79.2	(72.1 – 86.3)
III	**Symptom based diagnosis: ≥ 2 symptoms and recurrent or lasted more than 3 weeks**						
	Sensitivity	28.6	(4.9 – 52.3)	21.4	(10.7 – 32.1)	22.9	(13.1 – 32.7)
	Specificity	86.2	(81.3 – 91.1)	89.4	(84.1 – 94.7)	89.4	(84.1 – 94.7)
	PPV [a]	13.3	(1.1 – 25.5)	46.2	(27.0 – 65.4)	53.3	(35.4 – 71.1)
	NPV [b]	94.2	(90.7 – 97.7)	72.8	(65.9 – 79.7)	68.6	(61.7 – 75.5)
IV	**Skin complaints at time of medical evaluation**						
	Sensitivity	57.1	(31.2 – 83.0)	12.5	(3.8 – 21.2)	21.4	(11.8 – 31.0)
	Specificity	85.6	(80.6 – 90.6)	84.8	(78.7 – 90.9)	84.8	(78.7 – 90.9)
	PPV [a]	22.9	(9.0 – 36.8)	25.9	(9.4 – 42.4)	42.9	(26.5 – 59.3)
	NPV [b]	96.4	(93.6 – 99.2)	69.6	(62.5 – 76.7)	67.1	(60.0 – 74.2)

a) Positive Predictive Value
b) Negative Predictive Value
c) Summation of 'major' and 'minor' dermatitis

increased. Remarkably, the sensitivity for 'major' and 'minor' dermatitis both dropped when only skin complaints at time of the study were considered, although the specificity improved slightly.

Discussion

Validation of the questionnaire revealed an overall moderate sensitivity and specificity, indicating moderate agreement. Overestimation of the prevalence of 'major' hand dermatitis based on the questionnaire was clearly illustrated by the very low positive predictive value (18.2%; 95% CI: 8.0 –28.4). More stringent criteria for identifying dermatitis applied at the medical evaluation probably resulted in the observed difference. Hence, when less severe skin diseases like 'minor' dermatitis were included, the positive predictive value increased, indicating that self-reported complaints were related to these skin diseases. Overestimation of the prevalence could also have been influenced by the different time frame the medical evaluation and questionnaire covered, respectively a point prevalence vs. a 12-month period prevalence. However, the validity calculated for reported complaints at the time of the study was even poorer than for the criteria based on self-reported symptoms, indicating that the different time frames reflected are probably not the most important factors for the observed difference. Although overreporting of hand dermatitis was apparent in this study, only a moderate sensitivity was observed, indicating that a number of workers (29%) did not report pronounced symptoms of hand dermatitis as confirmed by the medical examination.

In a validation study of the same questionnaire among nurses, a high sensitivity (100%) and negative predictive value (100%) was observed, while the specificity (64%) and positive predictive value (38%) were relatively low [48]. The difference in sensitivity found between these 2 validation studies can likely be explained by a different perception of skin diseases. Susitaival *et al.* [52] validated their questionnaire in a population sample of Finnish farmers and found also a low sensitivity, probably, indicating that workers with a more physical workload have got used to having skin diseases. Although medical evaluations by trained physicians were used as a 'gold standard' in all of these validity studies, the observed differences could have been influenced by inter-expert variability. However, among dermatologists a clear consensus on the morphological signs of hand dermatitis is present.

Questionnaires are not only used for estimating the prevalence of skin diseases but also as a primary screening method. The high negative predictive value (97.3%; 95% CI: 94.7-99.9) found for 'major' hand dermatitis in this study suggests that the questionnaire could be used as a diagnostic tool for primary screening. However, due to the low prevalence of hand dermatitis (6.9%), the NPV is severely influenced by the large amount of healthy subjects. Hence, if the questionnaire had been used for this purpose, 29% of the cases of hand dermatitis would still have remained undetected.

The more lenient classification without the requirement of long-lasting or recurrent symptoms is to be preferred in this case, as the sensitivity was considerably higher. Therefore, workers reporting even the mildest symptoms of possible hand dermatitis should be included in a medical screening. However, none of the classifications used resulted in a good diagnostic tool for estimating the prevalence of hand dermatitis, as the overall sensitivity and specificity remained moderate for all criteria used.

The 'true' prevalence (6.9%; 95% CI: 3.4 – 10.4) of 'major' hand dermatitis as assessed by the dermatologist in this study, was similar to findings in previously reported studies in the rubber manufacturing industry using the same methodology [55; 56]. Reported prevalences of hand dermatitis in the general population are nevertheless similar (2-10%) [57-62]. However, these estimates are mainly based on information gathered from questionnaires and therefore are likely an overestimation of the true prevalence. The apparent differences between the several diagnostic tools for identifying hand dermatitis complicate comparisons of prevalences between occupational groups and the general population. Furthermore, it was concluded that the applicability of a questionnaire for identifying skin diseases, and in particular hand dermatitis, is limited, as large differences were found in validity when applying a standardized questionnaire to different populations. Moreover, if questionnaires are to be used, validity studies must be carried out to evaluate perception of skin diseases in specific occupational populations or subgroups of the general population.

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Section 2.2
Dermal exposure, hand washing and hand dermatitis in the rubber manufacturing industry

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Skin diseases, such as hand dermatitis are thought to be a common problem in the rubber manufacturing industry as workers are exposed to a wide range of chemicals with known irritant and sensitizing potential. We conducted a cross-sectional survey of a representative sample of rubber manufacturing workers (n=202), selected from 9 different rubber companies. Prevalence of hand dermatitis ('major'- and 'minor' dermatitis) and skin injuries was assessed based on a diagnosis by a dermatologist. The possible relations between actual skin exposure, hand washing practices and hand dermatitis were investigated. Prevalence of 'major' hand dermatitis (7%) was comparable with that in the general population, 'minor' signs of dermatitis, however, were more common among the surveyed population (28%) as were traumata's of the skin (17%). Dermal exposure to cyclohexane soluble agents during work was related to the occurrence of 'major' hand dermatitis, but not to the occurrence of 'minor' hand dermatitis. Moderate and frequent hand washing especially with industrial surfactants containing scrubbing particles were found to be strongly associated with the occurrence of 'minor' dermatitis OR=4.27 (95% C.I. 0.90 – 20.27) and OR=6.38 (95% C.I. 1.33 – 30.17), respectively.

Epidemiology, in press

Introduction

Skin diseases are estimated to account for 9-35% of all occupational diseases [44-46; 63], of which allergic and irritant contact dermatitis comprise the majority of occupational cases [64]. Frequent causes of occupational allergic contact dermatitis (ACD) are rubber chemicals, chromates, and epoxy resins. Detergents, cutting fluids, organic solvents, and environmental factors such as humidity, occlusion and mechanical friction have been shown to elicit irritant contact dermatitis (ICD) [64-66]. Several of these stimuli are present in the rubber manufacturing industry and as a result the rubber industry has often been considered as a high-risk industry in relation to skin diseases [67; 68]. Most of the data, however, stem from routinely collected governmental statistics or clinical series [18; 64]. Epidemiological investigations identifying particular occupational processes are scarce, and have focused on working conditions encountered in large rubber tire companies [55; 69; 70]. Rubber manufacturing in The Netherlands, is predominantly a small-scale industry producing mainly custom-made technical rubber goods.

The focus of most of these industry-based epidemiological studies has been on the occurrence of ACD due to exposure to chemical substances with great sensitizing potential, such as thiuram-, carbamate-, paraphenylene- and mercapto-compounds [71; 72]. A recent study has indicated that not only are rubber additives an important risk factor

for allergic reactions but so is natural rubber, as allergenic natural rubber latex (NRL) proteins have been detected in extracts from rubber products [73]. On the other hand, scant data are available on ICD and skin trauma related to working conditions in the rubber manufacturing industry. Furthermore, none of the epidemiological studies controlled for potential confounders or effect modifiers such as past or present atopic dermatitis, hand washing practices and domestic exposures.

The aim of this study was to investigate the prevalence of skin disorders and the possible relation between dermal exposure and hand dermatitis in a cross-sectional study of rubber manufacturing workers. Detailed information on actual skin exposure, hand washing practices, personal characteristics and domestic exposures facilitated the identification of specific risk factors associated with increased likelihood of work-related skin disorders.

Subjects and methods

Study population

The study was conducted from January 1997 through July 1997 as part of a large cross-industry survey to evaluate the possible role of dermal exposure on the total genotoxic dose of workers in the rubber manufacturing industry in The Netherlands. Subjects were employed in 9 companies (3 rubber tire, 5 general rubber goods and 1 retreading company) and were randomly selected based on their production function (e.g. compounding and mixing, pre-treating, moulding, curing, finishing, shipping, engineering service and laboratory) [74] to cover a variety of production processes and exposures. General characteristics of the companies and production functions studied are presented in Table 2.2-1. Total workforce in the surveyed companies equaled 1355 subjects, of which 225 subjects were selected. 202 (90.2%) subjects took part in the medical evaluation and exposure survey and successfully completed a self-administered questionnaire. The self-administered questionnaire included detailed questions concerning demographics (age, ethnicity, etc.), known risk factors of skin complaints and diseases, atopic dermatitis, absenteeism and medical consultation due to skin complaints. All 202 subjects were male, between 19 and 60 years of age, with a mean age of 37.6 (sd. 9.1) years.

Hand dermatitis

Two dermatologists conducted a medical evaluation of current skin condition. Objective skin symptoms were used for the classification: active hand dermatitis ('major' dermatitis), the first symptoms of dermatitis ('minor' dermatitis) and skin injuries (traumata). 'Major' dermatitis was defined as: erythema, papules, vesicles and fissures, comprising a clear eczematous picture. 'Minor' dermatitis was exhibited as erythema,

Table 2.2-1 General characteristics of the surveyed plants, production functions and prevalence of 'major' and 'minor' dermatitis and traumata at the hands of workers in the rubber manufacturing industry per company and production function.

Factory (SBI-code) [a]	No. of workers [b]	No. of subjects [c]	Production	'Major' Dermatitis	'Minor' Dermatitis	Traumata
1 (3112)	25	19 (76%)	Mould and extruding articles, rubber foils	0 (0%)	10 (53%)	4 (21%)
2 (3112)	35	17 (49%)	Mould and extruding articles, roller covering, metal to rubber bonded articles	1 (6%)	3 (18%)	3 (18%)
3 (3112)	40	16 (40%)	Mould articles	4 (25%)	0 (0%)	1 (6%)
4 (3112)	50	18 (36%)	Mould and extruding articles, metal to rubber bonded articles	0 (0%)	2 (11%)	7 (39%)
5 (3112)	150	15 (10%)	Mould and extruding articles, metal to rubber bonded articles	1 (7%)	4 (27%)	2 (13%)
6 (3111)	190	34 (17%)	Bicycle and moped tires	4 (12%)	7 (21%)	5 (15%)
7 (3111)	150	32 (21%)	Belting, hose	1 (3%)	10 (31%)	6 (19%)
8 (3111)	660	31 (5%)	Industrial and passenger car tires	1 (3%)	10 (32%)	3 (10%)
9 (3121)	55	20 (36%)	Retreading truck and industrial tires	2 (10%)	10 (50%)	3 (15%)
Production function						
Compounding and mixing	125	19 (15%)	Raw material handling, weighing, mixing and milling	2 (11%)	6 (32%)	2 (11%)
Pre-treating	160	29 (18%)	Degreasing, spraying and repair buffing	2 (7%)	10 (35%)	4 (14%)
Moulding	380	52 (14%)	Extruding and calendering, component assembly and building	3 (6%)	18 (35%)	10 (19%)
Curing	295	48 (16%)	Curing	3 (6%)	7 (15%)	10 (21%)
Finishing	155	18 (12%)	Inspection and finishing	2 (11%)	4 (22%)	2 (11%)
Shipping	85	14 (16%)	Storage and dispatch	1 (7%)	5 (36%)	2 (14%)
Engineering	135	14 (10%)	Engineering services	1 (6%)	6 (33%)	4 (22%)
Laboratory	20	4 (20%)	Laboratory	0 (0%)	0 (0%)	0 (0%)
All	1355	202 (15%)		14 (7%)	56 (28%)	34 (17%)

a) Dutch Standard Industrial Classification: 3111 rubber tire; 3112 general rubber goods, 3121 retreading
b) Number of workers directly involved in production
c) Number of selected subjects participating in the study

slight chapping and scaling of the skin. Traumata of the skin comprised cuts and burns. No distinction was made between irritant and allergic dermatitis, as morphologic characteristics of these skin disorders are similar [54; 75].

Hand washing

Information regarding hand washing frequency and type of surfactant used during the workday was gathered as part of the medical survey based on standardized questions. Contents of identified surfactants were subsequently verified and categorized as mild surfactants (normal household soaps) and industrial surfactants (soaps containing scrubbing particles with or without the addition of an organic solvent).

Dermal occupational exposure

Personal dermal exposure to cyclohexane soluble matter (CSM) was measured with a dermal pad sampler on three consecutive days (Tues., Wed., and Thurs.). The pad sampler consisted of 24 layers of cotton surgical gauze with a surface of 9 cm^2, worn on the volar forearm (wrist) of the hand of preference throughout the 8-h working period [10; 76]. CSM content of the pad sampler was determined by means of the NIOSH P+CAM 217 method [77].

Natural rubber latex (NRL) allergy

Plasma of all subjects was analyzed for anti-NRL IgE by enzyme immunosorbent assay (EIA) [78]. Flat-bottom 96-wells polystyrene microtiter plates with high binding capacity were coated overnight at 4°C with latex allergen dilution (10 µg/ml) (*Hevea Brasiliensis*, ALK Benelux, Houten, The Netherlands). Sera diluted 1/10 in phosphate-buffered saline (PBS) containing 0.5% (w/v) Tween were added to the wells, and incubated for 2h at 37°C. Bound IgE was measured with a four-step procedure, consisting of three 1h incubations at 37°C with monoclonal mouse anti-human-IgE, biotinylated rabbit anti-mouse immunoglobulins, avidin-peroxidase and finally an incubation for 30 minutes with o-phenylenediamine (OPD) containing 0.015% (v/v) H_2O_2. The reaction was stopped after 30 minutes by the addition of 50 µl 2N HCl and the absorption was read at 492 nm. All sera were tested in duplicate wells on the same microtiter plate. Each plate included a positive control serum tested in duplicate, and two reagent blanks (no-serum controls). Sera were considered positive if the mean OD_{492} was 0.05 units higher than the reagent blanks.

Calculations and statistical analyses

Mean individual dermal exposure levels were calculated from the repeated individual measurements. The median of the aggregated exposure distribution was used as cut-off point to classify exposure dichotomous as low/high. Use of protective gloves was evaluated based on the actual use of gloves during the exposure survey. If gloves were worn during more than 50% of the measurement days, subjects were classified as

'frequent' glove users. Information about domestic activities and hobbies, such as gardening, car maintenance etc., was evaluated by two of the authors (RV & DB), blind to outcome, on known skin aggravating potency resulting in a dichotomous classification (yes/no).

Possible relations between personal and work related determinants and hand dermatitis were initially studied by computing crude prevalence odds ratios (OR) from univariate logistic regression analyses. Identified risk factors were further investigated by means of a multiple logistic regression analysis. In the regression analyses subjects with a particular skin condition (e.g. 'major' and 'minor' dermatitis) were compared to subjects without any adverse skin condition (n=113). In evaluating 'major' hand dermatitis, the regression analyses were adjusted for reported past or present atopic dermatitis. All statistical analyses were performed with Statistical Analysis System (SAS) V6.12 packages [79].

Results

Among the 202 subjects, 14 subjects were diagnosed with 'major' hand dermatitis. 28% showed symptoms of 'minor' hand dermatitis and 17% were diagnosed with traumatized skin at time of the study (Table 2.2-1). The overall clinical picture of 'major' hand dermatitis was characterized by erythema and papules with vesicles and sometimes fissures on the palms and the palmar sides of the fingers and fingertips. The clinical manifestation of 'minor' dermatitis was characterized by almost exclusively erythema with occasionally scaling and chapping of the hands. Large differences in prevalence of skin disorders between the different companies were observed. Variation in prevalence among different production functions was, however, smaller than between companies (Table 2.2-1).

34.8% of the subjects with skin disorders attributed their skin condition to working conditions encountered in the rubber manufacturing industry. 41.6% claimed relief of skin problems while not working for several days. A clear trend was observed between the severity of skin disorders and the proportion of subjects who claimed beneficial effects from a few days off work; 64.3% of those with 'major' dermatitis, 42.9% of those with 'minor' dermatitis and 29.4% of those with traumata. Only 2 subjects reported having had an allergic reaction due to contact with rubber goods and/or chemical additives. None of the 202 workers showed a class II or higher positive anti-NRL IgE reaction. Although skin disorders were prevalent in this population, only in 7 instances did the occurrence of hand dermatitis result in a subject's absenteeism from work in the last 12 months.

Median (31.7 µg/cm^2) of the aggregated personal dermal CSM exposure distribution was used as cut-off point to classify dermal exposure as low or high. Subjects without any skin disorders (n=113) had a median dermal CSM exposure concentration of 31.2 µg/cm^2 (Q1-Q3= 19.1- 57.7 µg/cm^2), which was similar to subjects with major or minor hand dermatitis (median=31.3 µg/cm^2; Q1-Q3= 14.9- 70.5 µg/cm^2). In Table 2.2-2 crude

prevalence odds ratios and results of the multiple logistic regression analyses are presented. Crude odds ratios did not differ much from the odds ratios derived from the multiple logistic regression analyses, except for dermal CSM exposure and hand washing. High dermal exposure levels measured at the wrist was associated with 'major' hand dermatitis (OR=2.15, 95% C.I. 0.58 – 7.95). Other determinants under consideration suggested an overall protective effect in relation with 'major' hand dermatitis, especially for domestic activities with potentially skin aggravating potency (OR=0.33, 95% C.I. 0.10 -1.10). In contrast, 'minor' hand dermatitis was positively associated with the studied determinants except for glove use (OR=0.58 95% C.I. 0.27 – 1.23). Moderate and frequent hand washing during the workday showed a strong positive effect OR 3.09 (95% C.I. 1.16 – 8.21) and OR 2.27 (95% C.I. 0.92 – 5.56) after adjustment. The use of industrial surfactants seemed to augment the occurrence of 'minor' hand dermatitis (OR 1.92, 95% C.I. 0.91 – 4.02). Domestic activities with potentially skin aggravating potency were found to be one of the major risk factors for the occurrence of 'minor' dermatitis (OR 4.33, 95% C.I. 1.72 – 10.92). Little association was observed between age and 'major' or 'minor' dermatitis (data not shown).

Table 2.2-2 Associations between hand dermatitis and personal and work related determinants. Presented as prevalence odds ratios (OR) with 95% Confidence Intervals from multiple logistic regression analyses.

	Controls (N=113)	**'Major' Dermatitis** [a] (N=14)			**'Minor' Dermatitis** (N=56)		
	N [b]	N	Crude OR [c]	OR (95% C.I.)	N	Crude OR	OR (95% C.I.)
High dermal Exposure [d]	56	8	1.36	2.15 (0.58 - 7.95)	26	0.88	0.82 (0.40 - 1.69)
Hand washing [e] 5 – 9	33	3	0.72	0.53 (0.11 - 2.66)	18	1.68	3.09 (1.16 - 8.21)
> 10	40	6	1.20	1.18 (0.30 - 4.62)	25	1.92	2.27 (0.92 - 5.56)
Industrial surfactant use	60	7	0.88	0.64 (0.19 - 2.21)	35	1.47	1.92 (0.91 - 4.02)
Glove use	51	5	0.68	0.61 (0.18 - 2.11)	19	0.62	0.58 (0.27 - 1.23)
Domestic activities [f]	71	6	0.44	0.33 (0.10 - 1.10)	46	3.40	4.33 (1.72 - 10.92)

a) Regression analyses adjusted for atopic dermatitis (OR=6.6, p=0.07)
b) Number of subjects with particular determinant
c) Odds ratio derived from the univariate logistic regression analyses
d) Tested against low dermal exposure to cyclohexane soluble matter
e) Tested against low hand washing frequency (# 0 – 4)
f) Domestic activities with potentially skin aggravating potency

The relation between hand washing frequency, surfactant use and 'minor' hand dermatitis was further investigated in a stratified analysis according to the type of surfactant used. A clear dose-response relation was found between the frequency of industrial surfactant use and the prevalence of 'minor' dermatitis (Table 2.2-3). This trend between hand washing frequency and 'minor' dermatitis was not observed among the subjects using only mild surfactants during the workday.

Table 2.2-3 Relation between 'minor' hand dermatitis and hand washing frequency stratified for industrial and mild surfactant use [a]. Presented as prevalence odds ratios (OR) with 95% Confidence Intervals.

Hand washing frequency	All detergents (N=167)	Industrial surfactant (N= 76) [b]	Mild surfactant (N=71) [c]
5 – 9	3.09 (1.16 - 8.21)	4.27 (0.90 – 20.27)	2.38 (0.52 - 10.95)
> 10	2.27 (0.92 - 5.56)	6.38 (1.35 – 30.17)	1.17 (0.28 - 4.80)

a) Analyses adjusted for personal dermal exposure, glove use and domestic exposures (model table 2)
b) Exclusive use of industrial surfactants
c) Exclusive use of mild surfactants

Discussion

As the study was not primarily designed to study the occurrence of skin disorders in this particular industry, selection bias is not likely to have occurred. As the relative sample size per production function and company was not equal, however, the prevalence of skin disorders could have been biased by the relative over-representation of a particular production function or company. Adjusted prevalences were similar when compared with crude prevalences for 'major'- and 'minor' dermatitis and traumata of the skin (respectively 7.2% vs. 6.9%, 29.0% vs. 27.7% and 17.8% vs. 16.8%). Therefore, the observed prevalence of skin disorders and associated risk factors is representative for the rubber manufacturing industry in The Netherlands. Although some authors [80; 81] have argued for the use of prevalence ratios, the standard effect measure in prevalence studies is the prevalence odds ratio [82; 83] since, in a stable population, this provides an estimate of the ratio of the products of disease incidence and average disease duration in the two populations being compared. Thus, if an exposure does not affect disease duration, then the prevalence odds ratio directly estimates the incidence rate ratio [84].

Prevalence of 'major' hand dermatitis (7%) was similar to previous reports from the rubber manufacturing industry [55; 56; 62]. Although 27% of the subjects with 'major' hand dermatitis attributed their skin condition to the working conditions encountered in the rubber industry, the reported prevalences of hand dermatitis in the general population are nevertheless comparable (2-10%) [59]. Minor signs of dermatitis, however, were more common in the rubber workers (28%). Although symptoms were mild, irritated, damaged skin is a precursor to eczema [54]. The overall absence of self-reported allergic reactions due to contact with rubber goods or chemical additives suggests that irritant contact dermatitis is the predominant form of hand dermatitis observed in this industry. Nevertheless, as morphologic characteristics of allergic and irritant contact dermatitis are similar [54; 75], only application of diagnostic patch tests could have ruled out with certainty the presence of allergic contact factors.

The presented results could have been prone to selection bias in the form of a healthy worker effect owing to the cross-sectional character of the survey. As not many people seek employment elsewhere because of skin disorders (except in the case of proven ACD) this phenomenon is not very likely to have occurred [85].

A large difference in prevalences of all skin disorders between companies and production functions was observed. As variation in prevalences between production functions was lower than between companies, company specific production characteristics and/or working conditions (including hand washing) probably play an important role in the occurrence of skin disorders. Nevertheless, no consistent picture was observed between rubber tire, general rubber goods and retreading companies. Investigation of possible underlying risk factors for 'major' hand dermatitis showed an overall protective effect with the exception of dermal CSM exposure. Damage to the skin can be repaired, at least in part, when the interval between individual damaging processes is sufficiently long and when the damage is not too extensive [86]. This rehabilitation was demonstrated in our study as 42% of all subjects with diagnosed skin disorders claimed relief of their complaints while not working for several days. Thus, it is likely that subjects with diagnosed 'major' hand dermatitis consciously try to avoid skin damaging activities leading to the observed reversed relations with e.g. industrial surfactant use and domestic activities. Since symptoms for 'minor' dermatitis are mild, they are seldom considered as an adverse health effect by the subjects themselves [75]. Consequently, subjects do not change their working habits and domestic activities accordingly.

Hand washing practices (frequency and soap use) were found to be an important risk factor for 'minor' hand dermatitis. Even though the mechanisms of skin irritation due to surfactants are not fully understood, it is well documented that repetitive hand washing can lead to irritant contact dermatitis [87; 88]. The use of industrial surfactants, containing scrubbing particles and often organic solvents, elicited the observed relation, due to the fact that the chronically irritated skin is more vulnerable to other chemical exposures and is less able to withstand mechanical stress [89]. Industrial surfactants were available in all companies and although differences in average use per day between companies were observed (range 0.9 – 7.1) this did not account fully for the observed differences in prevalence of 'minor' dermatitis between these companies. Therefore, additional unidentified company related risk factors may be associated with the occurrence of contact dermatitis in this industry.

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Chapter 3
Inhalable dust and dermal exposure in the rubber manufacturing industry

In chapter 3 personal inhalable and dermal exposure levels in the rubber manufacturing industry in The Netherlands are evaluated. Long-term trends in exposure to inhalable particulate and dermal contamination and effectiveness of implemented control measures in the last decade are discussed in section 3.1. In section 3.2 relevant exposure pathways involved in the process of dermal contamination are considered based on a conceptual model for dermal exposure assessment.

Section 3.1
Trends in exposure to inhalable particulate and dermal contamination in the rubber manufacturing industry; Effectiveness of control measures implemented over a nine-year period

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Exposure to inhalable particulates and dermal exposure to cyclohexane soluble matter (CSM) were evaluated in seven rubber manufacturing companies in 1988 and 1997. The identified exposure trends were used to study the effectiveness of control measures implemented over a nine-year period.

Sampling and analytical methodologies were identical for both surveys. Inhalable particulate exposure was measured with a PAS6 sampling head. Dermal exposure was assessed by means of a dermal pad sampler worn at the lower wrist of the hand of preference. Changes in working organization and control measures taken after 1988 were identified based on discussions with management representatives and two walk-through surveys performed in 1994 and 1997. Exposure data were aggregated for comparison between years both at company and production function level. The mixed effect statistical procedure was used to evaluate the influence of control measures and seniority on current exposure levels.

Comparison of the exposure levels between 1988 and 1997 revealed a reduction rate of 5.7% and 6.7% per year for inhalable particulate and dermal exposure, respectively. Companies and production functions with the highest exposure levels in 1988 and workers with seniority (more experience) showed a steeper decline in exposure levels. Fifty-seven control measures, mostly designed to control the levels of inhalable exposure were identified. Elimination of sources significantly reduced the inhalable particulate and dermal exposure by two-thirds of the level of 1988. Reduction of emission did not show a significant overall decrease in exposure concentrations. Control measures designed to control the levels of contaminants showed a significant reduction for both inhalable and dermal exposure, respectively 34 and 49% of the exposure level of 1988.

These result indicate that efforts taken to improve work conditions in the rubber manufacturing industry in The Netherlands over this decade have been successful in reducing both inhalable particulate and dermal contamination.

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Introduction

Epidemiological studies among workers employed in the rubber manufacturing industry have indicated a significant excess cancer risk in a variety of sites [7;8]. Although, several associations between exposures and observed cancer risks have been hypothesized, it has in general not been possible to identify specific agents for the majority of observed cancer risks.

General reviews of environmental contamination, conducted in the late seventies and early eighties in the USA and UK, focused on inhalable particulate exposure to rubber dust and fumes and solvents [11-13]. Later on, more specific surveys were conducted for exposure to nitrosamines and several polycyclic aromatic hydrocarbons [14-16]. These industry-wide cross-sectional surveys described the exposure levels throughout the industry and the influence of control measures on the inhalable particulate exposure levels. Little attention has been paid to dermal exposure in this particular industry, although dermal exposure was found to be an important contributor to the total genotoxic dose [19]. Only one large-scale exposure survey systematically studied dermal exposure to cyclohexane soluble matter (CSM) in the rubber industry [90]. This study showed that the amount of CSM on the skin of hands and wrists was higher than the amount inhaled. Depending on the specific situation in a factory and the use of protective devices for skin contamination, the amount available for uptake through the skin could be up to a tenfold higher than through inhalation [10].

In 1988 a large project for workplace improvement in the rubber manufacturing industry in The Netherlands was carried out. Based on empirical modeling of the inhalable particulate and dermal exposure, exposure affecting factors were identified for which working conditions could be improved [10]. These results were consequently used in a covenant for improvement of working conditions in the Dutch rubber manufacturing industry entered into by the Ministry of Social affairs and the social partners [91]. Five years after the initial survey the implemented control measures were evaluated. The greatest effort had been taken in reducing and eliminating dust hazards. However, almost no attempts were made to control exposure to curing fumes and exposure to dermal contaminants [92]. Whether these control measures were effective or not could not be further substantiated as no quantitative exposure data were available.

This study was performed to evaluate current exposure to inhalable particulates and dermal exposure to cyclohexane soluble matter (CSM) in seven out of ten companies that were originally surveyed. The paper describes exposure trends in inhalable particulate and dermal exposure and the effectiveness of control measures taken in the last decade. All measures and changes in the production process a-priori assumed to result in reduced exposure levels (inhalable particulate and dermal CSM contamination) were regarded as control measures. The effectiveness of the control measures was studied with a simple conditional hierarchical linear mixed effect model allowing estimation of fixed and random effects simultaneously.

Material and methods

Exposure information collected in two industry wide surveys in the rubber manufacturing industry in The Netherlands was used in this study. The initial survey was completed in 1988 [10; 90; 93] and the second survey in 1997.

Study population

Companies involved in the initial exposure survey formed a representative cross-section of the rubber manufacturing industry in The Netherlands [10]. The companies (two rubber tire, two retreading and six general rubber goods companies) were approached to participate in the second survey of which seven agreed to do so. Of the three companies that did not take part in the second survey; two companies had merged and subsequently moved to another location and one company refused to co-operate as a result of a major re-organization. The general characteristics of the plants studied are presented in Table 3.1-1.

Table 3.1-1 General characteristics of the surveyed companies.

Factory (SBI-code) [a]	No. of workers [b]		Production
	1988	1997	
A (3111)	270	195	Bicycle and moped tires
B (3111)	135	150	Belting, hose
C (3112)	40	25	Mould and extruding articles, rubber foils
D (3112)	35	35	Mould and extruding articles, roller covering, metal to rubber bonded articles
E (3112)	50	40	Mould articles
F (3112)	40	50	Mould and extruding articles, metal to rubber bonded articles
G (3112)	180	150	Mould and extruding articles, metal to rubber bonded articles
All	750	645	

a) Dutch Standard Industrial Classification: 3111 rubber tyre; 3112 general rubber goods
b) Number of workers directly involved in production

A random sample of the total workforce in each company was selected, stratified by production function and job in both surveys. Subjects present in both surveys and who still worked in the same production function were identified as more experienced workers (seniority).

Exposure measurements

In 1988 measurements were carried out on random days during the course of a one week period (Tues.- Fri.), resulting in two to three repeated inhalable particulate and dermal exposure measurements per subject. A slightly different exposure strategy was employed in 1997 where three repeated personal exposure measurements per subject were collected on three consecutive days within one week (Tues., Wed., and Thurs.). Measurements were taken in the same period of the year (± 1 week) to minimize the effect of differences in meteorological conditions.

Sampling and analytical methodologies were kept the same for both surveys as previously described by Kromhout *et al.* [10]. Personal inhalable particulates were measured

with a PAS6 sampling head mounted near the breathing zone of the worker [94; 95]. Personal dermal exposure was measured by means of a dermal pad sampler. The pad sampler consisted of 24 layers of cotton (3x3 cm) and was worn on the lower part of the wrist of the hand of preference [10; 76]. Cyclohexane soluble matter (CSM) on the pad sampler was determined by means of the NIOSH P+CAM 217 method [77]. The pad was placed into cyclohexane and sonificated for 30 minutes after sampling. The suspension was consequently filtered through a glassintertube G4 (Schott, Germany) and collected in a pre-weighed 10 ml vial. After evaporation of cyclohexane under nitrogen and subsequently 2h drying at 40°C the residue was weighed by means of a microbalance.

Evaluation of control measures

Changes in working organization and control measures taken after 1988 and a-priori assumed to result in reduced inhalable particulate and dermal exposure levels were inventoried in 1994 and 1997. On both occasions changes were discussed with management representatives and a walk-through survey in each company was carried out, using company-specific reports of the initial survey as a basis. Control measures were categorized by two different classification schemes; one related to the production process, and another one related to the type of control measures [92]. For the production process the design analysis was used, dividing a production process into its key activities: production function, production principle and production form. Production function is analogous to the unit of operation and divides the production process into its core activities (for example weighing, curing, etc.). The general principle by which the production function is achieved is described by the production principle (for example manual or mechanical driven operations). The production form relates to the actual design of the installation or machine (materials, tools and machines used) [53]. The type of control measures was divided into source-oriented and exposure-oriented control measures, using the division of elimination and reduction of emission (source-oriented) and control of level of exposure and personal protective equipment (exposure-oriented).

Statistics

Exposure data were aggregated for comparisons between years at both company and production function level. Exposure concentrations were successively averaged for each worker (arithmetic mean of repeated individual measurements), for each production function within each company (median of individual means) and eventually for each production function or company (median of production function or company medians) (Figure 3.1-1).

Within- and between-worker components of exposure variance were estimated from the log-transformed exposure concentrations employing a one-way nested random-effects ANOVA model. Variance components were estimated for each company production function combination.

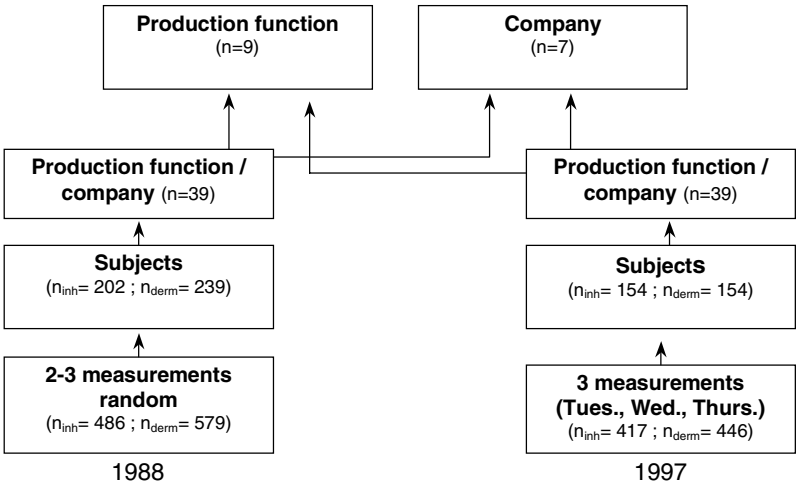

Figure 3.1-1 Aggregation of exposure data for comparison between years at both company and production function level. Exposure concentrations were successively averaged for each worker (arithmetic mean of repeated individual measurements), for each production function within each company (median of individual means) and eventually for each production function or company (median of production function or company medians). Number of measurements and subjects available in each survey between parenthesis for both inhalable particulate (inh) and dermal exposure (derm).

The mixed effect procedure of SAS systems for Windows (version 6.12) [96] was used to evaluate the effectiveness of the control measures and to quantify the influence of seniority on current exposure levels. As workers were nested within a production function and company a conditional hierarchical linear model was used [97]. The model used to study the fixed and random effects is described by the following expression:

$$Y_{gh(ij)} = \mu_{gh} + \alpha_{g(h)} + \beta_1 + \ldots. + \beta_n + \delta + \chi_{gh(I)} + \varepsilon_{gh(ij)}$$

$Y_{gh(ij)}$ is the logarithm of the exposure concentration measured at the j^{th} day ($j=1,2\ldots n_{gh(I)}$) of the i^{th} worker ($i=1,2\ldots k_{gh}$) in the g^{th} production function ($g=1$ to 8) of the h^{th} company ($h=1$ to 7); μ_{gh} is the true underlying mean of log-transformed exposure averaged over all companies and production functions; $\alpha_{g(h)}$ is the fixed effect of the g^{th} production function in the h^{th} company; $\beta_1,\ldots, \beta_n$ is the fixed effects of the control measures taken [fixed effect per control measure, for example β_1 is local exhaust ventilation, β_2 is automation (control measure indicator 0/1)]; δ is the fixed effect of seniority (seniority indicator 0/1); $\chi_{gh(I)}$ is the random effect of the i^{th} worker; $\varepsilon_{gh(ij)}$ is the random within-worker variation.

To estimate the fixed effect of the separate or categorized control measures dummy variables were used in the above model for each measure taken ($\beta_1,\ldots \beta_n$). As the 1988 survey served as a 'base-line' the control measures were only assigned, if appropriate, to the individual observations of the 1997 survey. As the pad sampler was directly attached to the lower wrist, the use of gloves and towels in both the 1988 and 1997 surveys could possibly have affected dermal exposure measurements. Therefore, the use of dermal

protection was included in the mixed effect model. Workers were assigned a unique id-code for each of the surveys. Seniority was assigned to the observations in 1997 when a subject had participated in the previous survey.

Variance components derived from the mixed effect models were estimated by the Restricted Maximum Likelihood (REML) method. It is assumed that $\chi_{gh(I)}$ and $\varepsilon_{gh(ij)}$ are normally distributed with zero mean and variance $s^2_{B,gh}$ and $s^2_{W,gh}$ ($\chi_{gh(I)} \sim N(0, s^2_{B,gh})$; $\varepsilon_{gh(ij)} \sim N(0, s^2_{W,gh})$) and that $s^2_{B,gh}$ and $s^2_{W,gh}$ are mutually independent.

Explained variance of the used models was estimated using the mixed effect model without the fixed effect variables for the implemented control measures and seniority but with inclusion of a dummy variable for sampling period as random effect as the reference. Explanation of the period variance component by the studied fixed effects is consequently treated as explained variance of the observed changes in inhalable particulate and dermal exposure.

Results

Rubber manufacturing in The Netherlands has always been a relative small industry with a main activity in custom-made technical rubber goods. In 1988 employment totaled about 7000 workers, which decreased over the years to about 4000 workers in 1997. In contrast to the overall industry statistics the companies in the presented survey showed reasonable stable employment figures (Table 3.1-1). The bicycle and moped tire company formed an exception as it had moved a large part of its production to low income countries resulting in a 28% reduction of the workforce over the nine-year period. Of the 750 production workers, 240 subjects (32%) were monitored in 1988 while in 1997, 24% (154 subjects) of the workforce in the companies were studied.

In total 57 control measures for inhalable particulate and dermal exposure were identified (Table 3.1-2). Most of these control measures were implemented in the production functions ‘compounding and mixing’, ‘moulding’ and ‘curing’ and were mainly directed towards the control of exposure. Furthermore, measures were mainly taken at the production form level (79%), followed by control measures that interfered with the production function (16%). Control measures at the production principle level, mainly related to the distance between worker and source, were only applied in three instances.

The use of personal protective devices as a control measure was evaluated based on the information gathered at time of measurements (Table 3.1-3). In both surveys approximately 5% of the workers wore some kind of respiratory protective device during the exposure measurements. Even at production function level the use of respiratory protection did not differ much, except for the engineering services where the use of welding masks had increased. The same overall view was observed for the use of dermal

protective devices, which were worn during approximately 45% of the dermal exposure measurements. The only noteworthy difference was the decreased usage of towels as dermal protection particularly in the curing departments.

Table 3.1-2 Control measures taken since 1988 for inhalable particulate and dermal exposure by production function categorized by two classification schemes, respectively related to the key activities and to type of control measures. Number of companies with particular control measure between parentheses.

		Type of control measures		
Production function	**Key activities**	**Elimination of sources**	**Reduction of emission**	**Control of exposure**
Compounding / mixing	Production function	Closing department (2) [a]	Stock blender (1) [c] Masterbatches (1) [b]	
	Production form	Liquid anti-tacking(1) [b]	Empty bag compactor (2)[b] Dust free chemicals (7) [b]	Improved LEV (6) [b]
Pre-treating	Production function	No anti-tacking usage (1)[b]	Cold feed extruder (1) [c]	
	Production form	Plasma degreasing (1) [c] Anti-tacking foil (1) [c]		Improved LEV (2) [b] General ventilation (1) [b]
Moulding	Production function		Cold feed extruder (1) [c]	
	Production principle		Automation (2) [c]	Mechanizing transport routes (1) [c]
	Production form	Liquid anti-tacking(1) [b]		Improved LEV (6) [b] General ventilation (1) [b] Enclosure talc drum (1) [b]
Curing	Production function		Cold feed extruder (2) [c]	
	Production form			Improved LEV (7) [b] General ventilation (3) [b]
Finishing	Production form		Removing anti-tacking (1)[b]	General ventilation (2) [b]
Engineering service	Production form			Improved LEV (1) [b] Vacuum cleaning (1) [b]

a) Control measures a-priori assumed relevant for exposure to particulates and dermal contamination
b) Control measures a-priori assumed relevant for exposure to particulates
c) Control measures a-priori assumed relevant for exposure to dermal contamination

Table 3.1-3 Use of personal protective devices during the 1988 and 1997 exposure survey.

Production function	Respiratory protective devices [a]		Dermal protective devices [b]	
	$\%_{88}$	$\%_{97}$	$\%_{88}$	$\%_{97}$
Compounding / mixing	28.8	26.3	57.3	72.9
Pre-treating	1.8	8.6 (2.9) [c]	50.0	29.3 (0.8) [d]
Moulding	10.7	3.1 (1.0) [c]	35.4	27.1
Curing	0	0.9	59.7 (18.0) [d]	72.1 (4.1) [d]
Finishing	0	0	16.9	16.7
Engineering service	2.4	0 (15.4)	37.5	16.3
Laboratory	0	0	40.0	55.6
Shipping	4.3	0	8.0	3.7
Total	5.0 (0.8) [c]	4.8 (1.8) [c]	43.0 (5.3) [d]	40.0 (1.2) [d]

a) Percentage of face pieces used for respiratory protection
b) Percentage of gloves used as dermal protection
c) Percentage of other devices used for respiratory protection in parentheses
d) Percentage of towels used as dermal protection in parentheses

For comparison of the inhalable particulate exposure levels between the two surveys 903 repeated measurements were used (n_{88}=486, n_{97}=417). Exposure concentrations were averaged by worker (k_{88}=202, k_{97}=154) and consequently grouped in 39 production function / company combinations per surveyed period. The median inhalable particulate exposure ranged from 0.56 to 1.85 mg/m^3 when aggregated per company in 1988. In 1997 the exposure range had diminished to 0.49 to 0.90 mg/m^3. All companies, except one showed a decrease in exposure levels ranging from 0.07 to 0.95 mg/m^3 over the nine-year period (Table 3.1-4).

Table 3.1-4 Median exposure to inhalable particulates per company for the 1988 and 1997 exposure surveys.

Company	Measurements		Subjects						
	N_{88} [a]	N_{97} [b]	K_{88} [a]	K_{97} [b]	N [c]	$Median_{88}$ (mg/m^3)	$Median_{97}$ (mg/m^3)	Difference (mg/m^3)	Percentage difference
A	102	85	42	32	5	0.75	0.56	-0.19	25.3
B	107	82	49	34	6	0.56	0.49	-0.07	12.5
C	58	47	23	16	6	1.85	0.90	-0.95	51.4
D	48	43	20	15	6	1.15	0.66	-0.49	42.6
E	32	49	14	18	5	0.84	0.49	-0.35	41.6
F	50	53	20	18	5	1.02	0.75	-0.27	26.5
G	89	58	34	21	6	0.66	0.85	+0.19	28.8
Total	486	417	202	154	39	1.00	0.59	-0.41	41.0

a) Number of observations available for computation from the 1988 survey
b) Number of observations available for computation from the 1997 survey
c) Number of production functions within a particular company available in both surveys

At production function level the exposure range diminished from 0.50 to 3.71 mg/m^3 in 1988 to 0.22 to 1.47 mg/m^3 in 1997. This resulted in an overall decrease in inhalable particulate exposure levels ranging from 0.02 mg/m^3 at the 'curing' department to 2.24 mg/m^3 at the 'compounding and mixing' department (Table 3.1-5).

Table 3.1-5 Median exposure to inhalable particulates per production function for the 1988 and 1997 exposure surveys.

Production function	N [a]	Median$_{88}$ (mg/m^3)	Median$_{97}$ (mg/m^3)	Difference (mg/m^3)	Percentage difference
Compounding / mixing	6	3.71	1.47	-2.24	60.4
Pre-treating	5	1.00	0.85	-0.15	15.0
Moulding	6	1.71	0.50	-1.21	70.8
Curing	7	0.58	0.56	-0.02	3.4
Finishing	5	0.69	0.54	-0.15	21.7
Engineering	7	1.02	0.94	-0.08	7.8
Laboratory	1	0.50	0.22	-0.28	56.0
Shipping	2	1.13	0.56	-0.57	50.4

a) Number of companies within a particular production function available in both surveys

In total 1025 (n_{88}=579, n_{97}=446) dermal exposure measurements gathered from 393 workers (k_{88}=239, k_{97}=154) workers were used in the comparison of the two surveys. Median dermal exposure to CSM ranged from 41 to 128 µg/cm^2 per 8h in 1988 to 38 to 77 µg/cm^2 per 8h in 1997 across companies. Five out of seven companies showed a decrease in dermal exposure levels ranging from 18 to 72 µg/cm^2 per 8h. The two remaining companies showed a small increase of respectively 3 and 14 µg/cm^2 per 8h, respectively (Table 3.1-6). Across production functions a small increase in exposure range was observed between 1988 and 1997, respectively 32 - 161 µg/cm^2 per 8h and 22 - 184 µg/cm^2 per 8h (Table 3.1-7). While a decrease in dermal exposure levels was found for the production functions 'compounding and mixing', 'pre-treating', 'moulding', 'curing' and 'finishing' varying from 24 to 109 µg/cm^2 per 8h, an increase was found for 'engineering', 'laboratory' and 'shipping' ranging from 5 to 46 µg/cm^2 per 8h.

The reduction in mean inhalable particulate and dermal exposure levels had a considerable effect on the variability in exposure concentrations of workers grouped by company and production function combination. For inhalable particulate exposure a decrease in both the between- and within-worker variance was observed. While for dermal exposure an increase in both variance components was observed (Table 3.1-8).

Table 3.1-6 Median dermal exposure levels to cyclohexane soluble matter per company for the 1988 and 1997 exposure surveys.

Company	Measurements		Subjects						
	N_{88} [a]	N_{97} [b]	N_{88}	N_{97}	N [c]	$Median_{88}$ (µg/cm^2.8h)	$Median_{97}$ (µg/cm^2.8h)	Difference (µg/cm^2.8h)	Percentage difference
A	133	91	55	32	5	82	64	-18	22.0
B	99	96	42	34	6	110	53	-57	51.8
C	58	45	24	16	6	110	38	-72	65.5
D	62	45	24	15	6	128	56	-72	56.3
E	48	53	21	18	5	41	44	+3	7.3
F	67	54	26	18	5	63	77	+14	22.2
G	112	62	47	21	6	105	40	-65	64.4
Total	579	446	239	154	39	101	54	-47	46.5

a) Number of observations available for computation from the 1988 survey
b) Number of observations available for computation from the 1997 survey
c) Number of production functions within a particular company available in both surveys

Table 3.1-7 Median dermal exposure levels to cyclohexane soluble matter per production function for the 1988 and 1997 exposure surveys.

Production function	N [a]	$Median_{88}$ (µg/cm^2.8h)	$Median_{97}$ (µg/cm^2.8h)	Difference (µg/cm^2.8h)	Percentage difference
Compounding / mixing	6	161	52	-109	67.7
Pre-treating	5	60	22	-38	63.3
Moulding	6	137	73	-64	46.7
Curing	7	68	44	-24	35.3
Finishing	5	64	37	-27	42.2
Engineering	7	138	184	+46	33.3
Laboratory	1	32	76	+44	137.5
Shipping	2	70	65	+5	7.1

a) Number of companies within a particular production function available in both surveys

Table 3.1-8 Between- and within-worker variability of exposure to particulates and dermal exposure for the two exposure surveys.

	1988 exposure survey		1997 exposure survey	
	S^2_{bw} [a]	S^2_{ww} [b]	S^2_{bw}	S^2_{ww}
Inhalable particulate exposure [c]	0.14 (0-12.06)	0.30 (0.02 – 6.66)	0.08 (0 – 5.45)	0.14 (0 – 2.31)
Dermal exposure [c]	0.10 (0 – 3.89)	0.54 (0.01 – 5.01)	0.14 (0 – 2.99)	0.77 (0 – 7.70)

a) S^2bw= Between-worker variance
b) S^2ww= Within-worker variance
c) Median of variance components grouped by company and production function combination, range of variance components in parentheses

Table 3.1-9 Influence of control measures and seniority as fixed effects on inhalable particulate and dermal exposure levels (960 observations, random effects shown below table [a]): #p<0.10, * p<0.05, ** p<0.01, ***p<0.001.

	Inhalable particulate exposure			Dermal exposure		
	N [b]	Relative exposure	95% CI	N [b]	Relative exposure	95% CI
Model 1 [c]						
Type of control measures						
Elimination	36	0.33**	(0.15 - 0.71)	38	0.34**	(0.17 – 0.67)
Reduction	97	0.86	(0.53 - 1.40)	105	0.89	(0.58 – 1.37)
Control	233	0.66*	(0.47 - 0.93)	249	0.51***	(0.38 – 0.69)
Seniority	134	0.71#	(0.50 – 1.02)	147	1.01	(0.74 – 1.37)
Model 2 [d]						
Control measures						
General ventilation	63	0.93	(0.54 – 1.65)	62	0.74	(0.43 – 1.27)
Local exhaust ventilation	194	0.89	(0.62 – 1.26)	206	0.68*	(0.48 – 0.96)
Anti-tacking	40	0.57#	(0.29 – 1.11)	44	0.34***	(0.19 – 0.63)
Dust free chemicals	25	0.78	(0.36 - 2.39)	27	0.66	(0.29 – 1.53)
Closing department	8	0.28#	(0.07 – 1.18)	8	0.28*	(0.08 – 0.99)
Empty bag compactor	10	0.36#	(0.11 – 1.14)	10	0.26*	(0.09 – 0.75)
Automation	47	0.22***	(0.12 – 0.39)	55	0.83	(1.39 – 0.49)
Cold feed extruder	27	1.82	(0.84 – 3.90)	27	0.54#	(0.27 – 1.08)
Other	22	0.52	(0.20 – 1.41)	24	0.56	(0.24 – 1.27)
Seniority	134	0.72#	0.50 – 1.03	147	0.98	(0.72 – 1.34)

a) Results of the random effect part of the applied mixed effect models: Model 1 inhalable particulate exposure, S^2bw=0.83, S^2ww=0.50; Model 2 inhalable particulate exposure, S^2bw=0.78, S^2ww=0.50; Model 1 dermal exposure, S^2bw=0.45, S^2ww=0.87; Model 2 dermal exposure, S^2bw=0.44, S^2ww=0.87
b) Number of observations with a particular control measure present
c) Model explained, respectively 83 and 29% of the observed reduction in inhalable particulate and dermal exposure
d) Model explained, respectively 80% and 40% of the observed reduction in inhalable particulate and dermal exposure

In Table 3.1-9 the influence of control measures on inhalable particulate and dermal exposure levels are shown. Model 1, based on the classification of type of control measures, explained respectively 83% and 29% of the observed reduction in inhalable particulate and dermal exposure. Another model (model 2), in which all control measures were included as dummy variables explained slightly less of the reduction in inhalable particulate exposure (80%) but explained more of the observed decline in dermal exposure levels (40%).

The estimated coefficient (β) for the control measures derived from the mixed effect model yields a factor for the relative exposure (*RE*) in a situation with a particular

measure present. Elimination of a source was found to be the most effective control measure efficiently reducing both inhalable particulate and dermal exposure levels by 67 and 66%, respectively. Measures to control the level of exposure reduced the inhalable particulate and dermal exposure levels by 34 and 49%, respectively. Reduction of the emission did not reveal a statistical significant decrease in exposure levels.

Investigation of specific control measures (model 2) showed a significant reduction in inhalable particulate exposure levels as a result of automation of the production process (*RE*=0.22, p<0.0001), closing of department (*RE*=0.28, p<0.10), installing empty bag compactors (*RE*=0.36, p<0.10) and the reduced used of anti-tacking agents (*RE*=0.57, p<0.10). Dermal exposure to CSM was successfully reduced by installment of empty bag compactors (*RE*=0.26, p<0.05), closing of department (*RE*=0.28, p<0.05), reduced use of anti-tacking agents (*RE*=0.34, p<0.0001), installment of local exhaust ventilation (*RE*=0.68, p<0.05) and use of cold feed extruders instead of warm up mills (*RE*=0.54,p<0.10).

Inclusion of an interaction term for control measures and company production function combination showed statistically significant effects for elimination of the source and control of exposure, automation, empty bag compactors and the reduced use of anti-tacking agents in relation to inhalable particulate exposure. For dermal exposure, statistically significant interaction terms for control measures and company production function combination were found for elimination of the source and control of exposure, local exhaust ventilation and reduced use of anti-tacking agents (results not shown).

In the mixed effect model, used to study the influence of control measures on dermal contamination, the use of dermal protection equipment (e.g. gloves) was included in the model as these devices could have affected dermal exposure measurements. Overall the use of gloves and towels showed a significant decrease in dermal exposure levels (*RE*=0.80, p<0.10) for both the 1988 and 1997 survey. However, inclusion of an interaction term for dermal protective equipment and company production function combination was found to be statistically significant. Investigation of the underlying estimates revealed both decrement and increment of dermal exposure levels in combination with the use of dermal protective equipment.

Besides the influence of control measures on current exposure levels in the rubber manufacturing industry the possible influence of seniority was studied. Of the 154 subjects participating in the 1997 survey, 49 workers had also participated in 1988. Investigation of the age of these workers at the time of the 1997 survey, showed a significant increased age of approximately 9 years between workers with or without assigned seniority (t-test, p<0.0001). Assigned seniority was found borderline statistically significant negatively associated with the level of inhalable particulate exposure (p=0.06), indicating that workers with more work experience were more successful in reducing their inhalable particulate exposure than their colleagues with less familiarity with the production process. Examination of the actual tasks performed by these subjects in both surveys revealed that subjects with seniority had reduced the

number of tasks which were statistically associated with high inhalable exposure levels in the 1988 survey [10] with about 8%. The association between seniority and reduced exposure levels was, however, not confirmed for dermal exposure to CSM.

Discussion

It is generally believed that exposures to airborne contaminants have decreased over the last decades. This long believed paradigm in occupational hygiene was recently confirmed by Symanski *et al.* [98; 99] in a large comprehensive evaluation of long-term trends in occupational exposures. Estimates of reduction rates for airborne exposures showed typically downwards trends at rates between 4% to 14% a year, with a median value of 8% for datasets with a significant downward trend.

Unfortunately, not only the levels of exposure differ over the years but also the methodologies used to quantifying the exposures, hampering the analyses of time trends in occupational exposures. Moreover, information about changes in the work environment and process conditions is often lacking. As a result observed time trends are most likely affected by factors other than the actual decrease in exposure levels [98].

This paper describes exposure conditions in the rubber manufacturing industry in The Netherlands encountered in the last decade. The study consisted of two cross-sector surveys performed in 1988 and 1997. Companies, analytical and sampling methodologies were kept identical in both surveys. This enabled direct comparison of the exposure situations in both years. Only one minor difference in sampling strategy between the two surveys was present. In the 1988 exposure survey, all measurements were performed on random days while the exposure survey in 1997 was carried out on fixed days of the week. However, as no significant day-effect was observed in the 1988 data [10], this will probably have had little to no effect on the comparison of the observed exposure levels.

As exposures in the rubber manufacturing industry are classified as exposure circumstances that are carcinogenic to humans [7] the ALARA principle in exposure management should be employed. Therefore, the obligatory effort to reduce the exposure to levels that are as low as reasonable achievable automatically implies that every reduction in exposure levels is relevant at all times. Furthermore as no relevant occupational exposure limits exist for the particular inhalable and dermal exposure conditions under study, at least in The Netherlands, exposure levels are only compared between the two surveys and not relative to an OEL.

Comparison of the exposure levels between 1988 and 1997 revealed a significant drop of 41% in inhalable particulate exposure levels over all companies and production functions. Assuming a constant relative decrease of exposure over the years in between the exposure surveys the rate of reduction is approximately 6% per year. This observed decline rate is comparable to the rates found in the study of Symanski *et al.* [98], although a decline rate of 6% is on the lower side of the decline rate ranges, especially when one

takes into account that the data were collected in Western Europe, in manufacturing, for aerosol contaminants and after 1972. These were all identified as factors that caused a more rapid decline in particulate exposure [99]. Whether the lower decline rate is a consequence of specific circumstances within the rubber manufacturing industry in The Netherlands, or the described decline rates in the comprehensive evaluation are an overestimation of the true reduction rates is unknown. As Symanski *et al.* [99] indicated, changing sampling strategies from worst-case to a more random approach would indeed yield an overestimation of the decline rate [100; 101]. Remarkably, an almost identical drop of 47% in dermal exposure levels was found over the nine-year follow-up period, resulting in a decline rate of approximately 7% per year. As no other data are available on time trends in dermal exposures, at least to our knowledge, no comparison with the literature can be made. The results are extremely interesting as it is often hypothesized that because of declining airborne exposure levels the dermal exposure route is becoming increasingly important [102; 103]. The presented results of this study do not support this hypothesis.

Companies with the highest inhalable exposure levels in 1988 were found to be most effective in reducing the exposure levels. As a result the range of inhalable particulate exposure levels between companies was significantly reduced compared with the initial survey. The same phenomenon was observed when inhalable exposure levels were aggregated at production function level. The largest reductions in exposure levels were observed in front processing. As a result the large differences in airborne particulates formerly observed between workers in front and back processing are apparently something from the past [12]. Consequently the reduction in inhalable exposure levels resulted in a significant drop in the between- and within-worker variance component when grouped by company and production function. This indicates that especially workers with formerly high inhalable particulate exposure levels were able to effectively reduce their personal exposure to airborne contaminants. This drop in the between- and within-worker variance component was not observed for dermal exposure, instead an increase was observed. These result imply that although an overall reduction in dermal exposure levels was achieved exposure conditions were not steadily controlled which would have resulted in a decrease in the variance components.

Fifty-six per cent of the control measures taken since the initial survey in 1988 focused on the control of exposure, whereas engineering controls interfering with the emission of contaminants were less common. Control measures designed to eliminate the source of contamination were especially sparse (12%). An investigation by the Labor Inspectorate in The Netherlands revealed that 76% of the control measures taken in industry were designed to control the level of exposure; the remaining measures were taken at the source of emission (e.g. elimination and reduction) [104]. The figures derived from the present survey indicate that within the rubber manufacturing industry relatively more effort has been paid to reduce or eliminate sources of emission than on average in industry (respectively 44 and 24%, respectively).

However, almost 80% of the control measures were taken at the production form level. These control measures are predominantly the 'add-on' type of measures or 'retrofit' solutions (e.g. changes or alterations to existing equipment), which are believed to have very limited effect in controlling the exposure to particulates, mainly due to inadequate construction and maintenance schemes [53]. Furthermore, virtually all control measures taken were directed towards the exposure to inhalable particulates. Control measures especially designed to reduce dermal contamination were scarce (7 out of 57). The absence of dermal occupational exposure limits [102; 105] and the limited evidence of the relevance of dermal uptake in the rubber manufacturing industry [10; 19] apparently did not encourage the industry to take adequate control measures in this period. Nevertheless, a reduction of approximately 47% in dermal exposure concentrations was achieved.

Evaluation of the effectiveness of implemented control measures showed that all control measures resulted in a decline in both inhalable particulate and dermal exposure levels. Exceptions to this rule were automation, which only reduced the inhalable exposure, and the introduction of cold feed extruders, which only reduced dermal contamination but increased inhalable exposure levels. Automation separates workers from the source and as a result reduces the emission of contaminants in the direct surroundings of the worker. However, manual handling of the materials and products still occur at the start and end of the production process. Empirical modeling of the chemical exposures in the 1988 survey revealed that tasks with frequent contact with warm compound resulted in high dermal exposure [10]. The introduction of cold feed extruders instead of heating mills followed by extrusion would therefore diminish the contact with warm rubber and as a result cause a decline in dermal contamination. However, the pre-mixed rubber compounds used for the cold feed extruders are treated with anti-tacking agents, which are in turn associated with higher inhalable particulate exposures.

Elimination of the source was found to be the most effective type of control measure, with closing down the mixing department as the most prominent underlying control, resulting in a 72% drop in inhalable particulate and dermal exposure levels. Conversely, on a more global scale the reduction in exposure levels by elimination of departments is questionable, as it is merely a transfer of high exposure situations to another facility or country. Whether the concentration of mixing activities will result in a decrease in exposure levels and workers exposed remains to be seen.

Introduction of dust free chemicals was believed to be an important measure to reduce inhalable particulate concentrations in the mixing departments. Although a relative exposure of less than one was observed for this particular control measure no statistical significant reduction in exposure levels was observed. Kromhout *et al.* [10] postulated that the absence of large differences in exposure levels between front and back processing workers in the 1988 survey was due to the replacement of chemicals in the form of powders by chemicals in other forms. Although, this replacement continued after 1988 the main effect of introducing non-powder forms of chemicals had probably occurred before the initial survey. A third important measure taken was the phasing-out of

powdered anti-tacking agents by either anti-tacking foils or liquid anti-tacking agents. That this control measure also effectively reduced the dermal exposure levels to cyclohexane soluble matter is remarkable. However, anti-tacking agents like talc, zinc stearate and chalk have been shown to contribute to higher CSM levels in exposure samples containing vast amounts of these anti-tacking agents [106]. The reduced use of these agents could therefore have reduced the dermal exposure level to CSM.

Identification of significant interaction terms between several control measures and company production function combinations indicate that the effectiveness of a certain control measure depends on the actual situation and design of the workplace. Therefore, results regarding the effectiveness of certain control measures can give guidance but can not be used as a rule of thumb.

Seniority has been shown to influence exposure levels both in the upward and downward direction [107; 108]. In this study seniority showed a possible association with reduced inhalabe particulate exposure levels but no association was found between work experience and dermal exposure. The criteria used for seniority in this study was not based on actual information about the work history but were assigned to subjects present in both surveys and still working in the same production function. Therefore, misclassification of workers who did not participate in the 1988 survey but who were selected in the 1997 survey could have occurred. However, as most companies were relatively small a fair amount of the total population that worked in those companies was sampled in both 1988 and 1997. As a result misclassification due to the fact that subjects were initially not selected seems unlikely. Furthermore, a nine-year difference in mean age was observed between subjects with and without seniority, while for the total population no differences in mean age between the two surveys was found. This indicates that indeed these subjects were the eldest workers in the company with at least nine years of workpractice. The observation that seniority at the same time did not show a significant association with declining dermal exposure levels indicates that workers were able to consciously reduce their inhalable exposure but not their dermal contamination.

The remarkable similar drop in inhalable particulate and dermal exposure levels concurrently with comparable results regarding the effectiveness of control measures indicates a relation between inhalable and dermal contamination levels in the rubber manufacturing industry. Dermal exposure can occur through direct contact with sources or contaminated surfaces and deposition of particulates on the skin [109]. Therefore, reduction of inhalable exposure concentrations will directly reduce dermal exposure levels as a result of reduced deposition of contaminants on the skin. However, direct contact with warm rubber products and contaminated surfaces is thought to be the primary route in the rubber industry [10]. Nevertheless, it is plausible that a reduction in inhalable particulate exposure will result in a decline in surface contamination, which will indirectly reduce the contamination of the skin as a result of contact with less contaminated surfaces.

The industrial agreement, signed by the Ministry of State and the social partners [91] emphasized the necessity of a source-oriented approach to control occupational hazards. For some particular hazards the convenant ordered specific control measures and solutions such as replacement of dusty chemicals. Based on this convenant and the result of the study for improvement of working conditions in the rubber manufacturing industry, many companies have implemented control measures to reduce occupational hazards. Modeling the effectiveness of these control measures showed that these measures explained almost entirely the observed drop in inhalable exposure levels but only partly the drop in dermal exposure levels. To a certain extent this could be explained by the fact that seniority explained about 14% of the reduction in inhalable exposure levels while it did not affect the decline in dermal exposure. Other factors such as type of rubber used, process conditions and individual work practices could not be evaluated but may also have influenced the decline in dermal contamination.

In conclusion, the result of this study indicate that efforts taken to improve work conditions in the rubber manufacturing industry in The Netherlands over the last decade have been successful in reducing both inhalable particulate and dermal exposure. Whether the observed decline in exposure levels reduces the observed genotoxic risks in this particular industry remains to be seen.

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Section 3.2
Identification of dermal exposure pathways in the rubber manufacturing industry

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Current existing dermal exposure assessment strategies are predominantly based on regulatory protocols. In order to develop effective and efficient strategies more data driven approaches are needed. In a recently developed conceptual model for dermal exposure, compartments, barriers and mass transport processes relevant for dermal exposure were described. We systematically applied this conceptual model to the rubber manufacturing industry to assess dermal exposure to cyclohexane soluble matter (CSM) and used quantitative data to design an exposure assessment strategy.

Identification of the spatial distribution of the dermal contamination showed high CSM surface concentrations for the upper body. Moreover, because of the high correlation between dermal exposure at the wrist and calculated total body exposure ($r=0.89$, $p<0.01$) an exposure assessment strategy based on only one pad sampler was employed to estimate CSM surface concentrations in the skin contaminant layer.

Qualitative and quantitative evaluation of the relevant compartments and related mass transport processes demonstrated the importance of deposition of airborne contaminants and direct transfer of contaminants from sources and surfaces to the skin contaminant layer. Interestingly, the importance of the different exposure pathways varied considerably between production functions.

The use of a model driven exposure assessment strategy in the rubber manufacturing industry revealed relevant skin regions, compartments and mass transport processes and enabled development of an effective and efficient strategy for dermal exposure assessment and hazard control in this particular occupational setting.

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Introduction

Well-designed exposure assessment strategies comprise the selection of both appropriate sampling techniques and sampling strategies. For inhalable exposure such strategies have been developed over the years and are now commonly used in occupational and epidemiological studies to evaluate the exposure by inhalation [110-112]. For dermal exposure to chemicals such standardized exposure assessment strategies are lacking. Partly, this is because of insufficient attention given to dermal absorption in environmental and occupational hygiene [113] but more importantly because of the alleged problems concerning quantification and interpretation of dermal exposure data. Nevertheless, several authors have proposed dermal exposure sampling strategies which address issues as sampling method, representativeness and sample duration [102; 114; 115]. They concluded that the assessment of dermal exposure should in any case be based on a

sampling strategy that takes into account the distribution of the contaminant on the body, the temporal variation of the exposure, the duration of exposure as well as the degree of skin protection afforded by clothing [113]. Furthermore, the sampling strategy design should be based on a recognition that exposures are likely to vary widely over time and between workers [102; 110].

In a recently developed conceptual model for dermal exposure, compartments, barriers and mass transport processes relevant for dermal exposure were described [109]. This simple model focussed on transport of contaminant mass from the source of the hazardous substance to the surface of the skin. It was argued that systematic application of such a structured model for selecting relevant dermal exposure parameters could form a basis for a dermal exposure assessment strategy. This, together with quantitative information regarding spatial distribution across the body and dermal exposure pathways could enable a more effective and efficient exposure assessment strategy as well as more efficient hazard control.

We studied dermal exposure to cyclohexane soluble matter (CSM) in the rubber manufacturing industry using this conceptual model. In the first survey, the conceptual model was used to identify compartments and exposure pathways involved in the process of skin contamination. As these parameters almost certainly vary for the different exposed body areas, parameters were assessed separately for different anatomical locations. In a second survey, exposure pathways that resulted in skin contamination of the lower wrist were further investigated and quantified.

Material and methods

Identification and quantification of exposure pathways and body distribution of cyclohexane soluble matter (CSM) exposure in the rubber manufacturing industry was studied in two surveys. The first survey focussed on subjective assessment of the major exposure pathways and quantification of CSM exposure distribution across the body. The second survey focussed on the quantification of compartment mass and consequently the identification of the exposure pathways relevant to the exposure at the lower part of the wrist of the hand of preference.

Study population

The first survey was conducted in a mixing department of a rubber tire company including both production workers (n=5) and technical engineers (n=4). The second survey was part of a large industry-wide exposure survey of the rubber manufacturing industry in The Netherlands. In total 225 subjects employed in nine different companies were selected based on their production function [75].

Identification of exposure pathways

In the first survey, workers were observed three times a day for 15 minutes while executing their specific tasks. Based on the observations of workers' tasks the compartments (air, surface contaminant layer, outer and inner clothing contaminant layer) and mass transport processes (emission, deposition, transfer, redistribution) leading to transport of contaminant mass to the surface of the skin was qualitatively assessed for six skin regions: neck / jaw, shoulder, upper arm, wrist, groin and ankle (Figure 3.2-1).

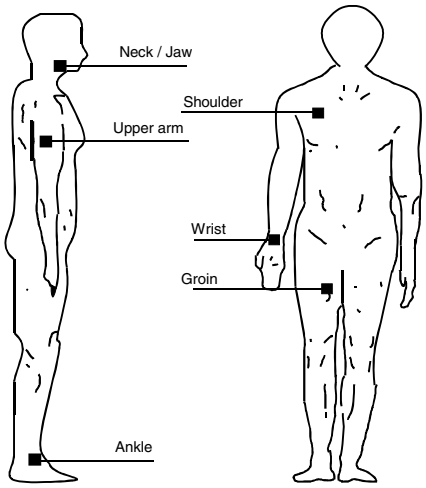


Figure 3.2-1 Body location of individual pad samplers (figure adapted from Rooij van et al. [118]).

In the second survey, CSM (surface) concentration in the relevant compartments (air, surface and skin) was quantified. 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 cm^2.

Surface contaminant layer

Surface CSM contamination was determined by obtaining wipe samples of potential contact surfaces. Samples were taken by a modification of the OSHA wipe sampling procedures [116]. In the modified procedure, a surface area of 10x10 cm of a potential contact surface was chosen as sampling region. The region was wiped three times consecutively with Clean cylce™ wet VDU wipes (Inmac, Wargave, UK) 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 experiment. Wipe pattern consisted of wiping once around the edge of the sampling region in an anti-clockwise direction and then wiping across the sampling area five times in parallel followed by nine consecutive circles. Repeated samples (n=3) were pooled and stored at –20°C before CSM analysis.

Air compartment

The air compartment was divided into two interlinked compartments e.g. near-field and far-field [117]. Airborne exposure measurements at fixed locations were used to quantify the far-field CSM exposure. Personal inhalable exposure measurements were used to estimate near-field CSM exposure. Ambient total suspended matter (TSPM) exposure was measured on random days by means of a high-volume sampler [94]. On average two samples were collected per department within a company, which were consequently pooled for CSM analysis. Personal inhalable exposure was measured on three consecutive days by means of a PAS6 sampling head mounted near the breathing zone of the worker [94; 95]. Filters were stored at –20°C until CSM analysis.

Skin contaminant layer

Total dermal body exposure to CSM was measured by placing six exposure pad samplers on different spots of the skin of a worker: neck/jaw, shoulder, upper arm, wrist, groin and ankle (Figure 3.2-1) [118]. In the second survey, repeated personal dermal exposure to CSM was measured by means of a dermal pad sampler worn at the lower part of the wrist of the hand of preference on the same days as the personal inhalable exposure measurements. In both surveys, pad samplers consisted of 24 layers of cotton (3x3 cm) and were worn throughout the workday [10; 76]. Immediate after sampling pad samplers were removed and stored at –20°C until CSM analysis.

Cyclohexane soluble matter analysis

Cyclohexane soluble matter was determined by means of the NIOSH PCAM 217 method [77; 95]. Dermal, surface, ambient and personal airborne particulate samples were placed in cyclohexane, respectively 10, 15, 15, and 3ml and sonificated for 20 minutes. Eight ml (3ml for the personal airborne particulate samples) of the suspension was filtered through a glassintertube G4 (Schott, Germany) and collected in a pre-weighed 10 ml vial. After evaporation of cylcohexane under nitrogen and subsequently 2h drying at 40°C the residue was weighed by means of a microbalance. All samples were conditioned at least 24h before weighing in a conditioned weighing room at a temperature of 20 ±2°C and 50 ±5% relative humidity.

Calculations and statistics

In both surveys dermal exposure surface concentrations (µg/cm^2) were transformed to an 8-h time weighted average exposure concentration (µg/cm^2/8h). In the first survey, total dermal body contamination (mg/8h) was calculated by multiplying the CSM TWA surface concentration of the individual pad sampler with the anatomical dimensions described by Popendorf and Leffingwell [119]. In the calculation it is assumed that each of the six pad samplers represent a skin region with a certain surface area (Table 3.2-1).

Table 3.2-1 Assumed percentage of total body area represented by each pad sampler.

Skin site	Skin region	% of total body area [a]
Neck/Jaw	Head	6.8
	Neck	
Shoulder	Shoulder	22.8
	Back	
	Chest	
Upper arm	Upper arms	9.7
Wrist	Forearms	12.3
	Hands	
Groin	Hips	27.1
	Thighs	
Ankle	Calves	19.9
	Feet	

a) According to van Rooij *et al.* [118]

In the second survey mean personal inhalable and dermal CSM TWA exposures were determined by calculating the averages of the repeated exposure measurements per person. Surface contamination was calculated by averaging the CSM contamination levels of relevant contact surfaces for each subject. In these calculations, every surface wipe sample was weighted equally regardless of the frequency and duration of contact with the particular surface. Mean ambient CSM concentrations were calculated based on the pooled TSPM samples according to the work areas the subject was employed in.

Pearson correlation coefficients were used to investigate the relation between dermal exposure at individual skin regions and total dermal body exposure and to study the correlation between CSM contamination levels in different compartments acknowledged in the conceptual model described by Schneider *et al.* [109]. In these analyses natural logarithms of the exposure values were used.

Results

Identification of exposure pathways and body distribution (survey 1)

Relevant compartments and mass transport processes for skin contamination based on the task observations are presented in Table 3.2-2. Identified compartments and transport processes were found to be almost identical for production workers and technical engineers, with the exception of the contribution of the inner clothing compartment to the skin contamination of the neck/jaw skin region of the technical engineers because of the frequent use of coveralls with hood. Moreover, a clear distinction in relevant compartments and processes could be made by comparing covered with uncovered skin areas. For the covered skin regions (shoulder, upper arm, groin and ankle) only the inner clothing compartment was found relevant. As for the uncovered neck/jaw region, deposition of contaminants from the air, redistribution and transfer of contaminants from hands (skin contaminant layer) and outer clothing compartment were found to be relevant. For the wrists, direct contact with surfaces and consequently the transfer of contaminants from the surface to the skin contaminant layer was identified as the most relevant compartment and mass transport process, followed by direct contact with the source, deposition of contaminants from the air and transfer of contaminants from the inside of the gloves to the skin.

Table 3.2-2 Identification of compartments and mass transport processes involved in skin contamination of production workers and technical engineers of a mixing department in a rubber tire company. Compartments and corresponding processes are given in descending order of estimated importance per skin site.

	Production workers		**Technical engineers**	
Skin site	Compartment	Process	Compartment	Process
Neck/Jaw	Air Skin Outer clothing	Deposition Redistribution Transfer	Air Skin Outer clothing Inner clothing	Deposition Redistribution Transfer Transfer
Shoulder	Inner clothing	Transfer	Inner clothing	Transfer
Upper arm	Inner clothing	Transfer	Inner clothing	Transfer
Wrist	Surface Source Air Inner clothing	Transfer Emission Deposition Transfer	Surface Source Air Inner clothing	Transfer Emission Deposition Transfer
Groin	Inner clothing	Transfer	Inner clothing	Transfer
Ankle	Inner clothing	Transfer	Inner clothing	Transfer

Mean cyclohexane soluble matter (CSM) surface concentrations varied considerably across the body surface (range 12.6 – 113.5 µg/cm²/8h) (Table 3.2-3). Highest surface concentrations were found at the wrist of both production workers and technical engineers, respectively 113.5 and 108.7 µg/cm²/8h. Overall lower CSM surface concentrations were found for the lower body compared to the upper body. Calculated total body contamination (mg/8h) showed similar variation across the body with relative high contribution of the shoulder and wrist skin region and only minor contribution of the ankle and neck/jaw skin region for both production workers and technical engineers. The groin area of the production workers had also a moderate contribution to the total body exposure, which was less pronounced for the technical engineers.

Table 3.2-3 Cyclohexane soluble matter surface concentration (µg/cm²/8h) and calculated total exposure (mg/8h) per skin site and for the total body by production function.

		CSM surface concentration (µg/cm²/8h)			Total exposure (mg/8h)			
Skin region	**N**	**AM**	**GM**	**GSD**	**AM**	**GM**	**GSD**	**%** [a]
Production workers								
Neck/Jaw	5	41.6	35.1	1.80	54	45	1.80	5.0
Shoulder	5	77.4	48.1	2.70	335	208	2.70	31.0
Upper arm	5	68.9	37.5	2.91	127	69	2.91	11.8
Wrist	5	113.5	76.9	3.23	265	180	3.23	24.6
Groin	5	42.2	34.3	2.14	217	176	2.14	20.1
Ankle	5	21.4	19.3	1.65	81	73	1.65	7.5
Total					1079	928	1.82	100
Engineering service								
Neck/Jaw	4	35.1	31.8	1.65	45	41	1.65	6.2
Shoulder	4	57.4	41.6	2.78	249	180	2.78	34.1
Upper arms	4	22.6	19.1	1.99	42	35	1.99	5.7
Wrist	4	108.7	65.2	3.82	254	152	3.82	34.7
Groin	4	18.2	16.4	1.67	93	85	1.67	12.7
Ankle	4	12.6	12.2	1.31	48	46	1.31	6.6
Total					731	624	2.10	100

a) Percentage of total body exposure

Total CSM mass at the wrist showed a high correlation with total CSM body exposure ($r=0.89$, $p<0.01$) (Table 3.2-4). This relation was found both for production workers ($r=0.87$, $p<0.10$) and technical engineers ($r=0.95$, $p<0.05$). The skin regions shoulder

and upper arm were also significantly correlated with total body exposure, respectively r=0.71 (p<0.05) and r=0.69 (p<0.05). However, correlation coefficients for the two production functions were lower and non-significant. Correlation between contaminant mass at individual sites were overall low except between the shoulder and upper arm (r=0.83, p<0.01), between the groin and ankle (r=0.63, p<0.10) and interestingly, the wrist was overall moderate correlated with both upper and lower body areas (range 0.33 to 0.59) but not with the neck/jaw skin area (r=0.06).

Table 3.2-4 Pearson correlation coefficients (r) between total cyclohexane soluble matter mass (mg/8h) at individual skin regions and total body contamination: # p<0.10 * p<0.05, ** p<0.01.

Skin region	All	Production workers	Technical engineers
	(n=9)	(n=5)	(n=4)
Neck / Jaw	0.06	-0.36	0.53
Shoulder	0.71*	0.58	0.89
Upper arm	0.69*	0.80	0.49
Wrist	0.89**	0.87#	0.95*
Groin	0.47	0.30	0.53
Ankle	0.50	0.53	0.27

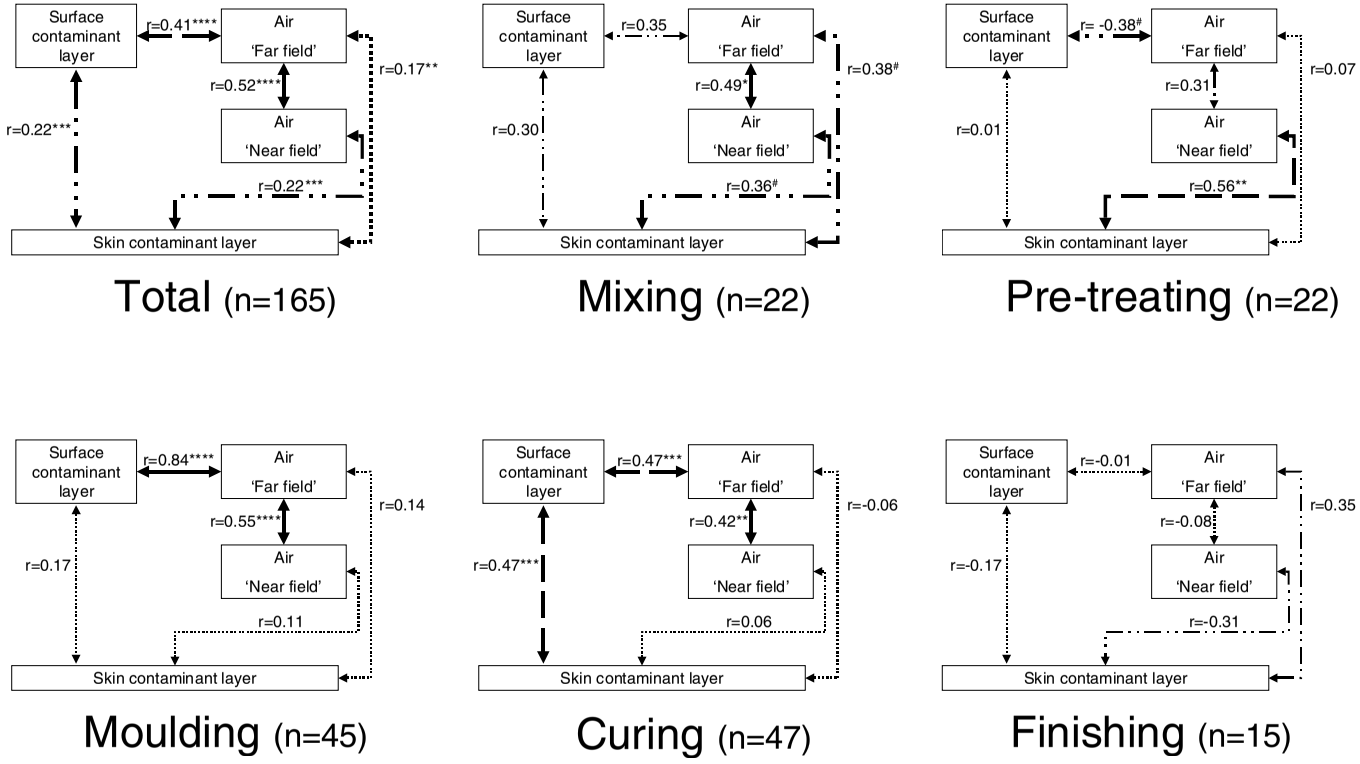
Quantification of exposure pathways (survey 2)

For subjects employed as technical engineer (n=19) no reconstruction of the exposure pathways could be made as the work practice was too diverse to enable identification of relevant contact surfaces. Forty-one observations were omitted from the analysis, because of missing data for one of the compartments. Most (91.5%) of the remaining subjects were employed in either the production function mixing, pre-treating, moulding, curing or finishing.

Results of the correlation analysis between CSM (surface) concentrations in the different compartments are presented in Figure 3.2-2 for each production function and the total workforce. Between the far- and near-field air compartment and surface contamination layer a moderate to good correlation was observed for the total workforce and the production functions mixing, moulding and curing. Pre-treating showed a moderate negative correlation between far-field exposure and the surface contamination layer. No correlation was found between the far- and near-field air compartments and the surface contamination layer for the production function finishing.

Correlation analysis between the skin contaminant layer and air and surface compartments for the total workforce revealed significant but low correlation coefficients. Correlation patterns observed for the three exposure pathways differed among the individual production functions. Mixing and pre-treating showed significant moderate

Figure 3.2-2 Correlation patterns of cyclohexane soluble matter contamination in different compartments for the total workforce and major production functions. Correlation patterns are graphically depicted by arrows according to the strength of the Pearson correlation coefficient (r) and statistical significance (bold when significant); $r < 0.2$ ◄·····►; $0.2 \leq r < 0.5$ ◄- - -► ; $r > 0.5$ ◄——►. # p<0.10, * p<0.05, ** p<0.01, *** p<0.001, **** P<0.0001.



correlation coefficients between the air compartment and skin contaminant layer. However, for pre-treating this relation was only observed between the near-field air compartment and the skin contaminant layer. Curing revealed a moderate significant correlation between the surface contaminant layer and skin contaminant layer. No significant correlation coefficients were found between the compartments for the production functions moulding and finishing.

Discussion

Relevance of dermal exposure in occupational hygiene and health is widely recognized and several techniques for the direct and indirect assessment of dermal contamination have been developed [102; 113]. Furthermore, concepts for deriving quantitative dermal occupational exposure limits have been developed and elaborated for a few chemicals [105; 120]. However, exposure assessment strategies are almost non-existent and in most cases the applied strategies are based on legally required regulatory procedures [121] and not on actual information of the exposure scenario.

In a recent developed conceptual model for dermal exposure, essential variables for dermal and surface contamination were described [109]. Consistent use of such a model ensures that the most relevant variables are taken into consideration in any given situation. Therefore, the model has the potential to be used as a starting point for the development of a sampling strategy. Inherent qualitative and quantitative assessment of dermal exposure pathways could consequently be used to design a more efficient and effective measurement strategy for a specific occupational setting.

Workers in the rubber manufacturing industry are exposed to a wide range of different potentially hazardous chemicals and reaction products that are known to constitute genotoxic activity [14; 19; 122]. It is generally believed that most of the genotoxic activity is attributable to the organic fraction of the complex mixture. Therefore, the use of an organic solvent soluble matter (e.g. benzene soluble matter, cyclohexane soluble matter) is often used as marker for exposure to these complex mixtures [123]. Since 1987 cyclohexane soluble matter (CSM) has been used in the regulation of dust and fume exposure in rubber factories in the United Kingdom [106]. Consequently, CSM exposure has been used in the rubber manufacturing industry to quantify both inhalable and dermal exposure to rubber dusts and fumes [10].

In the first survey, qualitative assessment of the most relevant compartments and mass transport processes in a mixing department of a rubber tire company showed a clear distinction between covered and uncovered skin areas. For the skin areas covered by clothing, the inner clothing compartment was the only directly important compartment for the skin contamination layer. Whereas for the uncovered skin, both deposition of contaminants and direct contact with surfaces and sources were thought to be of importance for both production workers and technical engineers. During mixing of rubber compounds high dust levels are generated as a result of weighing and emptying

bags of chemicals. Furthermore, because of the general low level of automation manual contact with raw chemicals and rubber compounds occurs frequently. These factors have previously been identified as important dermal exposure determinants [10] and are in concordance with the task observations made with the aid of the conceptual model. However, the task observations did not enable a relative ranking of exposed individuals, as it is was impossible to estimate dermal exposure accurately solely on observed tasks. Therefore, it seems more promising to a-priori identify exposure-affecting factors, which can consequently be more objectively assessed.

Spatial distribution of dermal contamination was investigated by means of dermal exposure pad samplers placed on six anatomical regions of the body. An underlying assumption in this assessment is that the exposure is uniformly distributed within the discreet anatomical region. Whether this criterion was met in this particular setting could not be further substantiated, as application of visualization techniques was not feasible because no fluorescent tracers could be added to the raw materials [124]. Production workers and technical engineers showed high dermal contamination levels for the upper body areas especially the wrist and shoulder skin region. The uncovered skin area neck/jaw had only a minor contribution to the total body contamination (5.0 - 6.2%) because of the low CSM surface concentration and the relative small contribution to the total body area (6.8%). In contrast, the uncovered skin area of the wrist was found to be highly exposed. Therefore, direct contact with surfaces and sources is probably the most important exposure pathway assuming a similar effect of deposition to uncovered skin for both the wrist and neck/jaw skin region.

Total body exposure showed high statistically significant correlation coefficients with the upper body skin regions, especially with the wrist. Exposure classification based solely on the dermal exposure surface concentration measured at the lower wrist would therefore not result in a different exposure classification than based on calculated total body exposure utilizing all six pad samplers. However, it has been shown that differences in permeability of the skin could exist between anatomical regions [125]. Therefore the above described relation between CSM contamination at the lower wrist and total body contamination does not necessarily have to be relevant for total dermal uptake. Interestingly, the wrist area was the only skin area showing moderate correlation with both the upper- and lower body areas with exception of the jaw/neck area.

In the second survey CSM mass in the far- and near field air compartment, surface contamination layer and skin contamination layer were assessed in a cross-industry survey in the rubber manufacturing industry. CSM contamination in the clothing compartment was not taken into account, as clothing did normally not cover the lowest part of the wrist and hands. However the use of gloves could have influenced the interrelations between the several compartments and the skin contaminant layer. However, stratified analysis of the correlation patterns by glove use revealed overall comparable results (results not shown).

Correlation patterns between compartments for the total workforce revealed overall moderate correlation coefficients illustrating the importance of both deposition of particles on the skin and transfer of contaminants from surfaces to the skin contamination layer by direct contact. It is noteworthy that the moderate to high correlation between surface contamination and the CSM concentration in the air, indicates the importance of deposition of air contaminants on surfaces at the workplace, which in return could contribute to dermal contamination. Correlation patterns for the major production functions within the industry revealed different underlying exposure pathways. Mass transport of contaminants from the air compartment to the skin contamination layer by deposition was found relevant for the production functions 'mixing' and 'pre-treating'. However, for 'pre-treating' this relation was only found between near-field air exposure and the skin contaminant layer suggesting localized sources of CSM contamination. Transfer of CSM mass from contaminated surfaces to the skin compartment layer was found relevant for the production function 'curing' only. Although, contact transfer was previously identified as the most important exposure pathway in the rubber industry the correlation analyses revealed only low to moderate correlation coefficients. Contaminant mass in the surface contaminant layer was assessed by averaging the CSM contamination levels of relevant contact surfaces for each subject regardless of the frequency and duration of contact with the particular surface. Depending on the number of relevant contact surfaces and variability in duration and frequency of contact considerable exposure misclassification might have occurred obscuring the possible relation between the surface and skin contaminant layer.

The production functions 'moulding' and 'finishing' revealed no specific exposure pathway. Kromhout *et al.* [10] identified several exposure affecting factors related to high dermal exposure levels within the rubber manufacturing industry. For the production function mixing the identified factors were predominantly associated with both high dust and dermal exposure levels, supporting the importance of the deposition exposure pathway found in this survey. Identified dermal exposure affecting factors for the production function curing were all related to the handling of warm rubber [10] confirming the importance of direct transfer of contaminants. Contribution of mass transport due to splashing, spilling and ejection of particles and contact with contaminated handheld tools could not be adequately evaluated by the used approach. These mass transport processes could, however, have had a significant contribution to dermal contamination for people working with raw chemicals (mixing), solvents (pre-treating) and handheld tools (technical engineers).

Application of a structured model to assess dermal exposure was found useful for the identification of relevant compartments and mass transport processes. However, in order to be an effective tool for qualitative assessment of dermal exposure a more objective tool has to be developed which focuses on the exposure affecting factors and not on visual contamination moments.

Identification of spatial distribution of dermal exposure and the relation with calculated total body exposure enabled a more effective and efficient sampling strategy. Hence, in this particular occupational setting the number of pad samplers used per worker could be reduced from six to only one sampler, reducing the total number of pads to be analyzed dramatically for a population of 225 workers. Furthermore, identification of the relation between mass in several compartments was found useful in understanding the importance of different exposure pathways for different production functions and facilitates implementation of control measures to minimize dermal exposure levels within the rubber manufacturing industry.

Acknowledgements

This work was facilitated by the Dermal Exposure Network, supported by European Commission Contract SMT4-4CT96-7502 (DG12-RSMT). The authors are indebted to the employers and employees in the rubber manufacturing industry for their close co-operation in this study. We also would like to acknowledge Hillion Wegh and Jeroen de Hartog for their support in the chemical analyses and technical assistance.

Chapter 4
Genotoxic exposure conditions in the rubber manufacturing industry

In chapter 4 genotoxic exposure conditions encountered in the present-day rubber manufacturing industry are described. In section 4.1 the possible confounding effect of weekly patterns in smoking habits on urinary mutagenicity is addressed. Mutagenic exposure conditions and related exposure determinants are described in sections 4.2 and 4.3. Consequently, possible relations between external mutagenic exposure, urinary mutagenicity and urothelial DNA adducts are described in sections 4.4 and 4.5. Potential influence of skin aberrations on dermal absorption and possible effect modification by CYP1A2 and NAT2 biotransformation polymorphisms on biological exposure and effect markers is addressed as well.

Section 4.1
Weekly patterns in smoking habits and influence on urinary cotinine and mutagenicity levels; Confounding effect of non-smoking policies in the workplace

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Lifestyle factors such as smoking have been shown to influence urinary mutagenicity. Therefore, these factors have to be considered carefully when evaluating occupational genotoxic exposures. We investigated day-to-day variability in active and passive tobacco smoke exposure by studying urinary cotinine levels and determined their influence on observed urinary mutagenicity. Urinary cotinine was assessed for 105 subjects employed in the rubber manufacturing industry in The Netherlands on Sunday, Wednesday and Thursday. Urinary mutagenicity was measured by the *S. typhimurium* strain YG1041 with metabolic activation for the Sunday and a pooled weekday urine sample. A sharp decrease in urinary cotinine concentration was observed during the week compared to Sunday urine sample for smokers (39%, $p<0.01$) and non-smokers (23%). Different smoking habits on Sunday resulted in higher regression-coefficients for categorical proxies for smoking habits and urinary mutagenicity levels. However, regression-coefficients for urinary cotinine and urinary mutagenicity were similar for the Sunday and weekday urine samples, (β=0.29 and β=0.28, respectively). Consequently, these estimates were used to adjust urinary mutagenicity for tobacco smoke intake. Cotinine adjusted urinary mutagenicity levels were comparable between smokers and non-smokers and a similar increase in urinary mutagenicity of respectively 39% and 34% was observed for both smokers and non-smokers due to occupational genotoxic exposures or other changes in lifestyle factors.

These results indicate that the introduction of non-smoking policies in the workplace has reduced exposure to mainstream and environmental tobacco smoke resulting in temporal variation in lifestyle related mutagenicity. Therefore, adequate adjustment for daily tobacco smoke exposure is a necessity when using the urinary mutagenicity assay to evaluate possible genotoxic exposures in the workplace.

Cancer Epidemiology Biomarkers & Prevention, in press

Introduction

Urinary mutagenicity is often used as a biomarker of exposure to study the genotoxic effect of occupational exposures [126-129]. Because of the non-selective character of the urinary mutagenicity assay, the assay has been found particularly useful in occupational settings with exposure to complex mixtures [130-134]. However, the non-selective character of the assay makes it prone to confounding factors such as smoking and diet [135-138]. Therefore, these factors have to be considered carefully, especially when genotoxic

exposures are low [135]. Because of the influence of lifestyle factors on urinary mutagenicity, large variations in background mutagenicity levels can be expected between subjects. Therefore, when comparing occupationally exposed and non-exposed groups large samples are needed to ensure random distribution of confounding lifestyle factors. Another approach that has been used to control for variation in mutagenicity due to lifestyle factors is the use of the subject as their own internal control. In this approach, the increment in urinary mutagenicity between urine samples collected before and after suspected mutagenic exposure is studied [19]. The underlying assumption in this approach is that confounding lifestyle factors do not significantly change within subjects over time. However, differences exist in activity patterns during the weekend and weekdays like more frequent visits to restaurants and bars and increased numbers of active smokers in the personal environment. These factors have been shown to influence the exposure to mainstream smoke (MS) and environmental tobacco smoke (ETS) [139; 140].

The aim of the present investigation was to study the day-to-day variability in active and passive smoking by studying urinary cotinine levels and to determine their influence on observed urinary mutagenicity. Urinary mutagenicity was measured by the *S. typhimurium* strain YG1041 with metabolic activation. S9-mix from aroclor induced rat livers was used as metabolic activation system to detect indirect mutagenic compounds or metabolites in urine.

Material and methods

Subjects

After completion of a survey, subjects (n=116), were randomly selected, based on their reported smoking habits and external genotoxic exposure profile from a group of 224 male subjects participating on voluntary bases in a large exposure survey among 9 companies in the rubber manufacturing industry in The Netherlands [75]. Subjects were employed full-time. All companies but one had a strict non-smoking policy at the workplace allowing, smoking only during breaks in designated areas in the company.

Spot urine samples were collected on Sunday, Wednesday and Thursday at approximately the same time of day (around 4 PM), stored in polyethylene containers, and kept at -20°C until use. Information regarding smoking status (yes/no) and average number of cigarettes smoked per day (0, 1-10, >10) was obtained by self-administered questionnaire completed before the survey and by assessing urinary cotinine levels.

Cotinine analysis

Cotinine in urine was quantified by high performance liquid chromatography according to the method of Barlow *et al.* [141], using the modifications described by Parviainen and Barlow [142]. Urine samples with undetectable cotinine levels (limit of detection, 25 nmol/l) were arbitrarily assigned a value of one-half the detection limit. Creatinine levels were

used to estimate urinary dilution, using a colometric test, based on the Jaffé reaction between creatinine and sodium picrate. Cotinine levels were expressed in µg/g creatinine.

Analysis of mutagenic activity

Urine samples collected on Wednesday and Thursday were pooled for each subject before mutagenicity analysis. A volume, corresponding to 0.5 mmol of creatinine of the Wednesday and Thursday urine sample was pooled resulting in a volume corresponding to 1 mmol of creatinine. Urine aliquots corresponding to 1.0 mmol of creatinine from Sunday urine sample and a pooled week urine sample were neutralized to pH 7.0 and extracted with XAD-2 resin (6-cm^3 bed volume). After the urine was passed through the resin, the column was washed with distilled water and the adsorbed material was eluted with 10 ml of acetone. After evaporation at 40°C under nitrogen, the residue was dissolved in 2.5 ml of DMSO [19]. Urine extracts were assayed for mutagenic activity with the *S. typhimurium* bacteria strain YG1041 with S9-mix of aroclor induced rat liver [21; 143]. Mutagenic activity was calculated based on the dose- response curves acquired at different dose levels. The slope of the linear component was used as an estimate of the mutagenic potency [144]. Urinary mutagenic activity levels were expressed in revertants/g creatinine.

Statistical methods

Urinary cotinine and mutagenicity levels for both smokers and non-smokers were lognormally distributed. Therefore, the natural logarithm of the cotinine concentration and mutagenic activity was used in all statistical procedures. Intra- and interindividual variance components of urinary cotinine based on the Wednesday and Thursday urine samples were estimated using a one-way nested random-effects ANOVA model. The influence of smoking habits and urinary cotinine levels on the mutagenic activity in urine was studied in separate linear regression models. Mean urinary cotinine concentration was calculated for the pooled weekday urine sample based on the cotinine levels of the Wednesday and Thursday urine sample. All statistical analyses were performed using SAS version 6.12 software [79].

Results

Of the 116 subjects, 105 subjects (90.5%) had complete data concerning smoking habits, urinary cotinine (Sunday, Wednesday and Thursday urine samples) and mutagenic activity levels (Sunday urine sample and a pooled weekday urine sample). No systematic differences in smoking habits, urinary cotinine and mutagenic activity levels were observed for subjects with incomplete data (n=11) or those with complete data (n=105). Consequently, the results presented in this study are based on these 105 subjects. Subjects were all male with a mean age of 37.9 (sd. 9.0) year.

A large statistical significant difference (*t*-test, p<0.0001*)* in mean urinary cotinine concentration between smokers and non-smokers was observed for Sunday and weekday urine samples (Table 4.1-1, Figure 4.1-1). Furthermore, a clear dose-response relationship was found between the average number of cigarettes smoked per day and urinary cotinine levels for smokers on Sunday. However, this dose-response relationship was not found for the weekday samples, in which almost no difference in mean urinary cotinine levels was observed for the different categories of average number of cigarettes smoked per day.

Table 4.1-1 Mean urinary cotinine concentration (µg/g creatinine) and intra- and interindividual cotinine variability by smoking status and average number of cigarettes smoked per day.

	Sunday			**Weekday samples** (Wednesday, Thursday)					
	N [a]	**GM** [b]	**GSD** [c]	**K** [d]	**GM**	**GSD**	**GSD_{intra}** [e]	**GSD_{inter}** [f]	**λ** [g]
Non-smokers	67	4.4	4.33	134	3.4	3.95	2.30	2.98	0.60
Smokers	38	1096.7	2.06	76	665.4	1.91	1.35	1.78	0.27
1-10/day	17	933.9	2.06	34	683.2	2.10	1.40	1.95	0.25
>10/day	21	1249.1	2.04	42	651.3	1.77	1.30	1.67	0.26

a) Number of subjects
b) Geometric mean
c) Geometric standard deviation
d) Number of samples
e) Estimated geometric standard deviation of intraindividual distribution of the log-transformed cotinine concentrations
f) Estimated standard deviation of interindividual distribution of the log-transformed cotinine concentrations
g) Variance ratio of the intra- and interindividual variance components

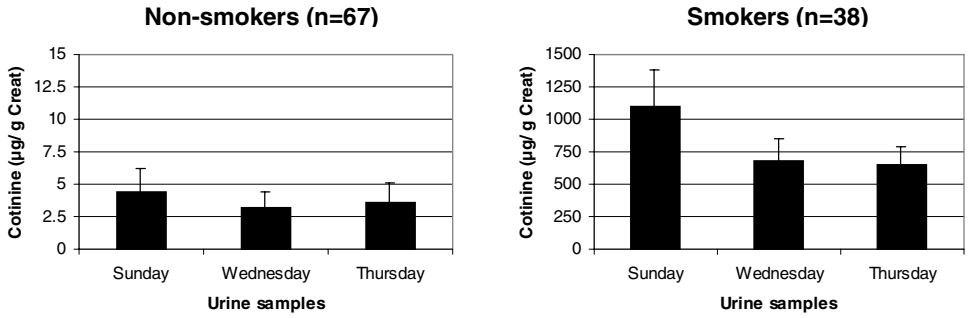

Figure 4.1-1 Geometric mean (GM) and 95% upper confidence limit of urinary cotinine concentration (µg/g creatinine) by smoking status and sampling day.

Analyses of the inter- and intraindividual variability in urinary cotinine levels of Wednesday and Thursday samples revealed an overall higher interindividual variability. Hence, the ratio (λ) of the intra- and interindividual variability for all smoking

categories was well below 1 indicating that the observed cotinine levels were predominantly influenced by the individual. It is noteworthy that this phenomenon was more pronounced for smokers than for non-smokers.

Smoking status (yes/no), categories of average number of cigarettes smoked per day, and urinary cotinine levels were clearly associated with urinary mutagenicity, with only minor differences in the explained proportion of the total variance for the different proxies used for smoking habits in these models (Table 4.1-2). However, an overall larger proportion of the total variability in urinary mutagenicity was explained by the proxies of smoking habits for weekday urine samples than for Sunday urine samples. Observed regression-coefficients for smoking status and categories of average number of cigarettes smoked per day were higher for Sunday urine samples than for pooled weekday urine samples, with the exception of light smoking (1-10 cigarettes/day). Regression-coefficients observed for the relation between urinary cotinine levels and urinary mutagenicity for the Sunday and pooled weekday urine samples were practically identical, (β=0.29 and β=0.28, respectively).

Table 4.1-2 Relation between smoking status, average number of cigarettes smoked per day and the natural logarithm of urinary cotinine levels and urinary mutagenicity levels on Sunday and weekdays.

		Urinary mutagenicity			
		Sunday		Weekdays	
		β(SE) [a]	R^2 [b]	β(SE)	R^2
Model 1	Smoking [c]	1.63 (0.29) [d]	0.23	1.51 (0.23) [d]	0.29
Model 2	1 – 10 [c]	1.24 (0.39) [d]	0.25	1.31 (0.31) [d]	0.30
	>10 [c]	1.94 (0.36) [d]		1.67 (0.28) [d]	
Model 3	Urinary cotinine ln(µg/g creat.)	0.29 (0.05) [d]	0.26	0.28 (0.04) [d]	0.31

a) SE, standard error
b) Explained proportion of total variance
c) Tested against non-smoking; $\exp^{\beta}$ yields an estimate of the multiplicative effect of the corresponding factor on urinary mutagenicity
d) p-value < 0.01

In Table 4.1-3 mean urinary mutagenicity levels of the Sunday and pooled weekday samples are presented. Smokers had, on average, a fivefold higher urinary mutagenicity level than non-smokers. Furthermore, both smokers and non-smokers showed elevated levels of urinary mutagenicity when Sunday and pooled weekday urine samples were compared, with an increase of 28% for non-smokers and 14% for smokers. However, cotinine adjusted urinary mutagenicity levels (based on the observed relationship between urinary cotinine and urinary mutagenicity), revealed an almost identical

increment in urinary mutagenicity for non-smokers and smokers (34% and 39%, respectively). Hence, the observed levels of cotinine adjusted urinary mutagenicity were almost similar for smokers and non-smokers.

Table 4.1-3 Mean urinary mutagenicity unadjusted and adjusted for urinary cotinine concentrations by smoking status.

	Sunday urinary mutagenicity (Rev / g creatinine) Mean [a] ± GSD	**Week urinary mutagenicity** (Rev / g creatinine) Mean ± GSD	**Increase in mutagenicity** % [b]
Unadjusted			
Non-smokers	6752 ± 4.98	8641 ± 3.36	28%
Smokers	34436 ± 2.95	39148 ± 2.63	14%
Cotinine adjusted [c]			
Non-smokers	4416 ± 4.92	5913 ± 3.37	34%
Smokers	4664 ± 2.70	6470 ± 2.49	39%

a) Geometric mean ± geometric standard deviation
b) Percentage of difference between urinary mutagenicity levels on Sunday and Weekdays
c) Non-smokers: Ln (Cotinine adjusted Sunday urinary mutagenicity) = ln (Sunday urinary mutagenicity) – 0.29 x ln (Sunday urinary cotinine concentration); Smokers: Ln (Cotinine adjusted Week urinary mutagenicity) = ln (Week urinary mutagenicity) – 0.28 x ln (Week urinary cotinine concentration)

Discussion

Smoking is almost invariably the most important confounder or effect modifier in studies focussing on occupational and environmental genotoxic exposures. In order to control for tobacco smoke intake several exposure indices have been used, including for example, the number of cigarettes smoked and several biochemical tests for plasma or saliva thiocyanate, expired carbon monoxide, and carboxyhemoglobin [145; 146]. These exposure proxies have been found unsuitable because of lack in sensitivity and specificity [147]. Cotinine, one of the major metabolites of nicotine, has been considered as the most accurate biochemical indicator of exposure to MS and ETS [147; 148]. Accordingly, urinary cotinine has been used in several studies to quantify the influence of tobacco smoke on urinary mutagenicity [134; 135; 149]. However, other lifestyle factors such as diet have been shown to influence urinary mutagenicity as well [135]. Because, no biochemical indicators are available to control for all lifestyle factors, another approach has been advocated. In this approach the studied subjects are used as their own internal control. The underlying assumption in this approach is that mutagenic exposure from other sources (lifestyle related mutagenicity) does not vary temporarily [19]. However, due to the introduction of strict non-smoking policies at indoor workplaces in The Netherlands

in the 1990's, this underlying assumption could possibly be refuted with regard to MS and ETS exposure. All companies but one in the present study had strict non-smoking policies allowing smoking only at designated times and areas of the company.

We investigated the cotinine levels in urine collected on Sunday, Wednesday and Thursday and observed a statistically significant decrease (*t*-test; $p<0.01$) for smokers in both unadjusted and creatinine adjusted urinary cotinine levels during the week when compared to Sunday. Non-smokers showed a similar downward trend, but the decrease did not reach statistical significance. Nevertheless, both downward trends in urinary cotinine levels suggest a decrease in both active and passive intake of tobacco smoke during the week compared to the weekend. Interestingly, heavy smokers (>10 cigarettes/day) showed a larger decrease in cotinine concentrations than light smokers (1-10 cigarettes/day), resulting in comparable cotinine concentrations during the week. Heavy smokers were probably more affected by non-smoking policies in the workplace, resulting in a tobacco smoke intake comparable to that of light smokers during weekdays.

The low ratio of the intraindividual: interindividual variability among active and passive smokers indicated a high degree of interindividual variability in cotinine concentration. Other investigators have found similar results, which presumably represents intersubject differences in nicotine metabolism and inhalation patterns [150; 151]. However, because of the half-life of approximately 20h for cotinine, urinary cotinine levels of Wednesday and Thursday could potentially have been autocorrelated and thereby have resulted in an underestimation of the intraindividual variability [152].

Regression analysis between several qualitative estimates of MS exposure and urinary mutagenicity showed a clear relation between active smoking and urinary mutagenicity. However, the observed association between urinary cotinine and urinary mutagenicity also implicates a relationship between passive smoking and urinary mutagenicity. Stratified analyses for smokers and non-smokers revealed no significant difference in the observed regression coefficients (data not shown). Therefore, the overall observed relationship between urinary cotinine and urinary mutagenicity was used to adjust for MS and ETS exposure.

In the presented study, urinary mutagenicity on Sunday was used as an estimate of mutagenic exposure due to lifestyle factors, whereas urinary mutagenicity on weekdays was used as a measure of mutagenic exposure due to lifestyle factors and occupational exposure. The difference between these two measures would therefore yield an estimate of the mutagenic activity due to occupational genotoxic exposure. Adjustment of urinary mutagenicity levels for urinary cotinine concentrations revealed comparable background mutagenicity levels due to lifestyle factors other than tobacco smoke for smokers and non-smokers, indicating adequate adjustment for tobacco smoke intake. Increase in mutagenicity due to workplace exposure or other changes in lifestyle factors, such as diet were similar for smokers and non-smokers after adjustment for MS and ETS exposure. Without adjustment for MS and ETS exposure, a different conclusion would have been reached. It is worth noting that adjusted urinary mutagenicity levels for

smokers, although statistically non-significant, were still slightly elevated compared with those for non-smokers. This could have been caused either by inadequate adjustment of urinary mutagenicity for cotinine levels or by smoking induced enzyme systems leading to higher urinary mutagenicity levels due to mutagenic exposures from other sources [153-155].

These results indicate that the introduction of non-smoking policies in the workplace has reduced exposure to tobacco smoke by active and passive smoking resulting in a temporal variation in lifestyle related mutagenicity. Therefore, adequate adjustment for daily tobacco smoke exposure is a necessity when using the urinary mutagenicity assay to evaluate possible genotoxic exposures in the workplace.

Acknowledgements

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Section 4.2
Mutagenic profile of rubber dust and fume exposure in two rubber tire companies

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The aim of this study was to evaluate curring mutagenic activity of ambient rubber dust and fume exposure in the mixing and curing department of two rubber tire companies situated in The Netherlands and Sweden. *S. typhimurium* strains YG1021, YG1024 and YG1041 were used to study the possible presence of mutagenic nitroarenes and aromatic amines. A large difference in mutagenic activity was found between the two companies. While the rubber tire company situated in The Netherlands revealed overall high mutagenic activity of rubber dust and fumes in the mixing and curing departments, respectively 430 and 279 rev/m^3 (YG1041), the Swedish company showed almost no mutagenic activity, respectively 18 and 54 rev/m^3 (YG1041). Further identification of the mutagenic profile showed that mutagenic activity was exclusively observed in *S. typhimurium* strains with elevated levels of *O*-acetyltransferase activity (YG1041 and YG1024) in the presence of a metabolic active liver S9-fraction, possibly indicating the presence of indirect mutagenic aromatic amines.

These results show that although production processes and lay-out within rubber tire companies are comparable, differences in rubber chemicals used and overall level of control measures (e.g., good housekeeping, cleanliness) are likely to result in substantial differences in mutagenic exposure levels between companies.

Mutation Research 2000, 468, 165 - 171

Introduction

Examination of recent epidemiological evidence has shown an excess cancer risk among workers in the rubber industry with most consistent results for urinary bladder, laryngeal, and lung cancer and leukemia [8]. Unfortunately, these recent epidemiological studies did not provide information associating specific job-related exposures with observed cancer risks, mostly due to the absence of detailed exposure assessment.

Different process conditions and the large number of rubber chemicals (more than 200) involved in the production of rubber goods mainly caused the absence of detailed exposure information, as the chemical complexity of the exposure precludes detailed chemical analysis of individual genotoxic components. Therefore, non-selective short-term bioassays have been applied in several studies in the rubber manufacturing industry to study the exposure to mutagenic compounds, either by measuring mutagenicity of airborne particulates or in urine of workers [14; 17; 19; 25; 26]. Although, these studies showed occupational exposure to mutagens, components responsible for the mutagenic activity were hardly identified [27].

Since the 1950's, health and safety measures have been widely applied in the rubber industry by substituting some chemical agents and controlling exposure to others [53]. Two recent industry wide exposure surveys showed that these measures have led to a significant reduction in rubber dust and fume exposure in The Netherlands and the UK [74; 156]. However, whether the reduction in exposure levels has resulted in a decrease in mutagenic activity could not be further substantiated in these studies.

In order to investigate current occupational exposure to mutagens present in rubber dust and fumes, two rubber tire companies were subjects of study. The *S. typhimurium* strain YG1041 with elevated nitroreductase and *O*-acetyltransferase activity [143; 157] was used to screen the airborne particulate matter. Consequently, *S. typhimurium* strains YG1021 and YG1024 were used to discriminate between the possible presence of mutagenic nitroarenes and aromatic amines in ambient rubber dust and fumes.

Material and methods

Location

Two rubber tire companies, one situated in The Netherlands (company A) and one in Sweden (company B), were included in the present survey. Company A produced both passenger and agricultural tires. Company B produced special tires for agricultural, industrial and forestry equipment. Both rubber tire companies had a comparable layout of the production process, although the Swedish company had a higher production volume and degree of automation than the rubber tire company situated in The Netherlands.

Air monitoring

Ambient air sampling was conducted in the mixing and curing departments where worker's activities were concentrated. Ambient total suspended particulate matter (TSPM) exposure was determined using a high-volume sampler at a flow rate of 0.9 m^3/min [94] in combination with Whatman GF/A glass fiber filters with a diameter of 12.5 cm. Flow rate was measured before and after sampling and the accepted range was set between 0.8 and 1.0 m^3/min. All samples were conditioned at least 24h before weighing and analyzed gravimetrically in a conditioned weighing room at a temperature of 20 ±2°C and 50 ±5% relative humidity. After measurements, filters were stored at -20 °C.

In company A 24h TSPM samples were collected during 3 consecutive days (Tuesday through Thursday) at two locations within the mixing and curing department. In company B repeated 8h TSPM samples were taken within 1 week at eight different locations within the mixing (five locations) and curing (three locations) department.

Filter extraction

Samples were extracted consecutively with cyclohexane (Merck, Darmstadt, Germany), dichloromethane (Merck) and methanol (Merck). A fourth part of the filter was placed in an extraction vial with 15 ml of cyclohexane and sonificated for 30 minutes. A total of 8 ml of the suspension was filtered through a glass intertube G4 (Schott, Germany) and collected in a pre-weighed 10-ml vial. After evaporation of the organic solvent under nitrogen and subsequently 2h drying at 40°C, the organic soluble residue was weighed by means of a microbalance. After evaporation of the organic solvent from the extraction vial at 20°C under nitrogen, the filter was consequently extracted with dichloromethane and methanol according to the previously described procedure for cyclohexane and collected in the same 10-ml vial. Mass of the dry residue after cyclohexane extraction was used to calculate cyclohexane soluble matter (CSM) exposure. Consequently, the mass of the combined dry extracts was used to estimate total soluble matter (TSM) exposure. For further identification of the mutagenic profile of the ambient air samples of company A, another fourth of the filter material was extracted. In this case, suspensions were collected separately after extraction with cyclohexane, dichloromethane, and methanol, respectively. All dry extracts were stored at –20°C until further analysis.

Mutagenicity testing

Collected ambient airborne particulate samples were tested for mutagenic activity with the *S. typhimurium* YG1041 strain. Additionally, combined and separate filter extracts of the company situated in The Netherlands (Company A) were tested for their mutagenic activity in *S. typhimurium* strains YG1021 and YG1024. Rubber dust and fume samples of the Swedish company (Company B) were additionally assayed with the conventional *S. typhimurium* strain TA98.

Dry extracts were dissolved in 2 ml of dimethylsulfoxide (DMSO) (Merck) and assayed at five different dose levels in triplicate for mutagenic activity in the *S. typhimurium* mutagenicity assay with S9-mix derived from aroclor induced rat livers [21]. In Table 4.2-1, the spontaneous mutagenicity, mutagenicity of the organic solvent (DMSO) and the mutagenicity of the positive controls (2-aminofluorene and 2-nitrofluorene, Janssen, Geel, Belgium) are presented.

For determination of mutagenic activity, the arithmetic mean of the in triplicate tested dose level was calculated. Dose-response curves acquired at different dose levels were constructed and the slope of the linear component was used as an estimate of the mutagenic potency [144]. 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 3 standard deviations above the mean blank (DMSO, n=10). Samples that were not

mutagenic were arbitrarily assigned $^2/_3$ of the LOD for the highest tested dose level and consequently the mutagenic potency was estimated. Mutagenicity was expressed as number of revertants per cubic meter (rev/m^3).

Table 4.2-1 Mutagenicity of references (rev/plate).

	TA98	**YG1041**	**YG1024**	**YG1021**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Spontaneous	29 ± 7	164 ± 21	41 ± 13	51 ± 8
DMSO	33 ± 5	96 ± 21	70 ± 8	42 ± 13
2-AF [a] (1 µg / plate)	3394 ± 182 [c]	1382 ± 47.6	-	-
2-NF [b] (1 µg / plate)	-	-	181 ± 21	1735 ± 222

a) 2-aminofluorene
b) 2-nitrofluorene
c) Concentration 2- AF 10 µg/plate

Statistical analyses

Exposure indices and mutagenic activity were lognormally distributed. Therefore, the natural logarithm of the exposure indices and mutagenic activity were used to test differences in sample means (student's *t*-test). All statistical analyses were performed using SAS version 6.12 software [79].

Results

Air concentrations of TSPM, TSM and CSM are presented in Table 4.2-2. Statistically significant higher exposure concentrations were found in company A compared to company B for all exposure measures (TSPM, TSM and CSM) for both the mixing and curing departments (*t*-test, $p<0.05$). These concentration differences were larger for TSM and CSM exposure than for TSPM exposure due to a lower percentage of extractable mass of the airborne particulate matter of company B.

TSPM exposures in the mixing and curing department within the two companies were almost similar, whereas for TSM and CSM exposure, except for CSM exposure in company B, statistically significant higher exposure levels were observed in the curing department compared to the mixing department (*t*-test, $p<0.05$).

Mutagenic activity measured with *S. typhimurium* strain YG1041 was detected in 17 of the 28 airborne particulate samples (61%). Filter extracts revealing no mutagenic activity were all collected in the mixing (n=9) and curing (n=2) department of company B, which consequently revealed overall statistically significant lower mutagenic exposure levels than company A for both the mixing and curing departments (*t*-test, $p<0.05$)

Table 4.2-2 TSPM, TSM and CSM exposure and mutagenic activity in the mixing and curing departments of two rubber tire companies.

	N [a]	**TSPM** (mg/m^3)			**TSM** (µg/m^3)			**CSM** (µg/m^3)			**Mutagenicity** (rev/m^3) [b]		
		AM [c]	GM [d]	Range [e]	AM	GM	Range	AM	GM	Range	AM	GM	Range
Company A													
Mixing	3	0.27	0.26	0.20 – 0.30	130.2	127.2	101 – 170	72.4	67.9	45 – 108	465	430	308 –735
Curing	2	0.30	0.30	0.29 – 0.30	247.0	246.1	226 – 268	170.9	170.8	164 – 177	285	279	224 – 346
Company B													
Mixing	15	0.10	0.08	0.03 – 0.34	26.8	24.8	9 – 44	8.5	6.1	1 – 16	14	12	3 – 48
Curing	8	0.09	0.08	0.04 – 0.23	50.2	47.3	25 – 81	9.8	8.3	2 – 16	52 [f]	36	6 – 139

a) Number of samples
b) Based on the results obtained with *S. typhimurium* strain YG1041
c) Arithmetic mean
d) Geometric mean
e) Minimum and maximum value
f) Mutagenicity based on *S. typhimurium* strain TA98 revealed mutagenic activity in 2 samples (AM= 5 rev/m^3)

(Table 4.2-2). Mutagenic activity of filter extracts of company B could only be detected with the bacteria strain TA98 in 2 out of 23 TSPM samples with a mean mutagenic activity of 5 rev/m^3. Both positive samples stemmed from the curing department. These samples exhibited a mean mutagenic activity of 92 rev/m^3 using the strain YG1041. Remarkably, the observed range in exposure measures was small because of low variability in both particulate exposure levels and genotoxic potency within the mixing and curing departments of the two companies.

Table 4.2-3 Mutagenic activity (rev/m^3) towards *S. typhimurium* strains YG1041, YG1024 and YG1021 in the mixing and curing department of company A.

	N	YG1041	YG1024	YG1021
		GM [a] (Range) [b]	GM (Range)	GM (Range)
Mixing	3	430 (308 - 735)	853 (721 – 989)	NM [c]
Curing	2	279 (224 - 346)	940 (684 – 1292)	NM

a) Geometric mean
b) Minimum and maximum value
c) Non mutagenic

Combined and separate filter extracts of ambient rubber dust and fume samples of company A were additionally assayed with the *S. typhimurium* strains YG1021 and YG1024. Mutagenic activity was only detected in the OAT-overproducing strain YG1024. *S. typhimurium* strain YG1021, overproducing nitroreductase exhibited no mutagenic activity (Table 4.2-3). Overall, higher mutagenic exposure concentrations were found in the mixing department compared to the curing department based on the *S. typhimurium* strain YG1041, respectively, 430 and 279 rev/m^3. Although the amount of TSM was lower in the mixing department, the mutagenic potency was considerably higher than in the curing department, respectively, 3.5 and 1.1 rev/µg extracted mass. For the strain YG1024 similar exposure levels in the mixing and curing department were found, respectively 853 and 940 rev/m^3. Analyses of the separate organic solvent fractions showed similar patterns regarding differences in mutagenic response to the different *S. typhimurium* strains used (Figure 4.2-1). Furthermore, these analyses showed that almost all mutagenic activity of filter extracts from the mixing and curing department was allocated in the cyclohexane soluble fraction (approximately 35%) and dichloromethane soluble fraction (approximately 60%). Remarkably, the sum of mutagenic activity of the separate fractions was about a factor 1.5 to 2 higher than the mutagenic activity found in the combined filter extracts.

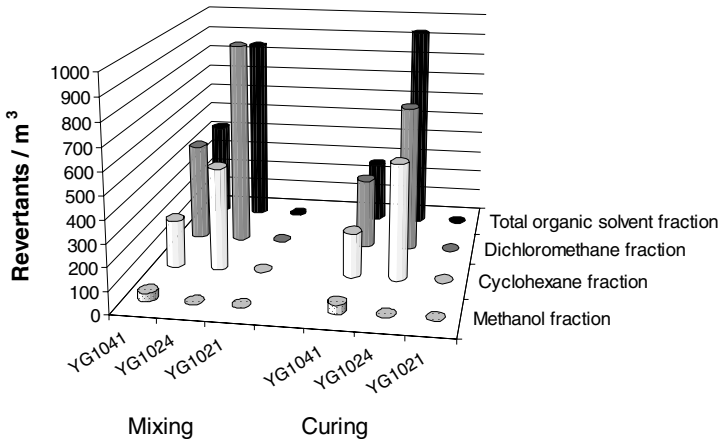

Figure 4.2-1 Mutagenic activity towards *S. typhimurium* YG1041, YG1024 and YG1021 for total organic and separate organic solvent fractions in the mixing and curing departments of company A.

Discussion

Several studies conducted in the 1980's have shown that workers in the rubber manufacturing industry are exposed to a complex mixture of chemical substances, many of which are potential genotoxic compounds [14; 158]. In quite a few of these studies, the *S. typhimurium* mutagenicity assay was used to study mutagenic activity of raw chemicals and ambient rubber dust and fume exposure. In addition, workers urine samples revealed an overall increase in mutagenic activity [17; 19; 25; 159-162]. Although several different base substitution and frameshift *S. typhimurium* strains (TA98, TA100, TA1537, TA1538) with or without metabolic activation were used, test conditions using *S. typhimurium* TA98 in the presence of metabolizing enzyme preparations resulted overall in the highest mutagenic activity [17; 25].

We used nitroarenes and aromatic amines sensitive derivatives of the conventional frameshift-type strain TA98 to study the current mutagenic activity of rubber dust and fume exposures in the mixing and curing department of two rubber tire companies. *S. typhimurium* strain YG1041, which overproduces both nitroreductase and acetyltransferase, with the addition of S9-mix was used to screen all collected ambient particulate samples. Additional assays with strains YG1021, which overproduces nitroreductase and YG1024, which overproduces *O*-acetyltransferase were carried out to elucidate the enzyme systems involved in the detected mutagenic activity. Furthermore, *S. typhimurium* TA98 was used to estimate the efficiency of the more sensitive *S. typhimurium* YG1041 strain in this particular occupational setting.

A large difference in mean mutagenic activity was found between the company situated in the Netherlands (company A) and Sweden (company B). The observed difference in

mutagenic activity could be attributed to differences in rubber dust and fume exposure, total solubility of TSPM and mutagenic potency of the exposure which were all found to be higher for company A compared to company B. Observed dust levels differed by a factor of three probably due to a higher degree of control measures implemented in the Swedish company (more automation throughout the whole production process). Above that differences in used chemicals and rubber compounds resulted in an additional difference in solubility of the dust samples (approximately with a factor of 1.5). Interestingly, only for the mixing department, a clear difference in mutagenic activity was observed while for the curing department, this difference was almost negligible.

Analyses of the filter extracts of company B with *S. typhimurium* TA98 revealed very low mutagenic activity in only two filter extracts (9%), whereas mutagenic activity was detected in 12 of the 23 samples (52%) when using the YG1041 tester strain. Based on the observed mutagenic activity, it was estimated that the strain YG1041 was about 5 - 30 times more sensitive in detecting mutagenic activity in the collected TSPM samples than the conventional strain TA98.

Analyses of filter extracts of company A with *S. typhimurium* YG1021, YG1024 and YG1041 revealed mutagenic activity in YG1024 and YG1041, but not in YG1021. These results indicate the presence of mutagenic *N*-hydroxyarylamines and/or aromatic amines, since detection of the mutagenicity of these chemicals depends on *O*-acetyltransferase activity but not on nitroreductase activity [143]. The absence of mutagenic activity in the strain YG1021, used for the greater efficacy in detecting some mutagenic nitroarenes, like nitropyrene are an indication that these chemicals are of minor importance in the rubber tire industry. However, since nitroarenes are direct acting mutagens, the addition of S9-mix could have resulted in partial metabolic deactivation as was observed in a recent study of Fracasso *et al.* [14].

The high mutagenic activity in *S. typhimurium* strains YG1041 and YG1024 together with the absence of mutagenic activity in YG1021 hints towards a substantial contribution of indirect acting mutagens and underline the importance of aromatic amine exposure in this particular industry. These findings are in concordance with recent epidemiological evidence that have shown an excess cancer risk among workers in the rubber industry with most consistent results, among others, for urinary bladder and lung cancer [8]. Aromatic amines have been identified as a possible causal group of chemical agents for these specific cancer sites [163; 164]. Furthermore, fractionation of mutagenic activity showed that most of the mutagenic activity of the samples was due to a-polar/neutral compounds much of which are possibly aromatic compounds. Summed mutagenic activity of the different organic fractions revealed a higher mutagenic activity than the total soluble fraction, which could have been caused by separation of toxic components from the mutagenic components.

The results of this study indicate that although production processes and lay-out within rubber tire companies are comparable, differences in rubber chemicals used and overall

level of control measures (e.g., good housekeeping, cleanliness) are likely to result in substantial differences in mutagenic exposure levels. Furthermore, the possible importance of aromatic amine exposure in the rubber tire industry is suggested.

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Section 4.3
Mutagenic exposure in the rubber manufacturing industry; An industry-wide survey

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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 *S. 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/m^3) and to a lesser extent between production functions (range 129 - 402 rev/m^3). 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 – 665 rev/cm^2. Mixing had the largest proportion of positive samples resulting in a median surface mutagenic contamination of 39 rev/cm^2. 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.

Mutation Research, in press

Introduction

Epidemiological studies among workers in the rubber industry have shown an excess cancer risk with most consistent results for urinary bladder, laryngeal and lung cancer and leukemia [8]. 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 [10-13]. More recently, compound(s) specific studies were conducted of exposure to nitrosamines and polycyclic aromatic hydrocarbons [14-16]. 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 [14; 17; 19; 25; 26]. 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 [10; 17; 18]. Direct evidence for the importance of the dermal exposure route was found in a study by Bos *et al.* [19] 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 analysis, 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 [165]. 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.

Material and methods

Location

The actual field study was conducted from January 1997 through July 1997 in 9 rubber manufacturing companies in The Netherlands (3 rubber tire, 5 general rubber goods and 1 retreading company). All production functions (e.g. compounding and mixing, pretreating, 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 [166; 167]. 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 one week per company.

Air monitoring

8h total suspended particulate matter (TSPM) exposure was measured on random days with a high-volume sampler at a flow rate of 0.9 m^3/min in combination with Whatman GF/A glass fiber filters with a diameter of 12.5 cm [94]. Flow rate was measured before and after sampling and the accepted range was set between 0.8 and 1.0 m^3/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 24h before weighing and analyzed gravimetrically in a conditioned weighing room at a temperature of 20 ±2°C and 50 ±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.

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 cm^2 (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 [116; 165]. In the modified procedure, a surface area of 100 cm^2 of a potential contact surface was chosen as sampling area. Each area was wiped 3 times consecutively with Clean cylce™ wet VDU wipes (Inmac, Wargave, UK) 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 analysis.

Extraction procedure

Samples (filters and VDU wipes) were extracted consecutively with cyclohexane, dichloromethane and methanol (Merck, Darmstadt, Germany) as described previously [168]. In short, samples were placed in an extraction vial with 15 ml of cyclohexane and sonificated for 20 minutes. A total of 8 ml of the suspension was filtered through a glass intertube G4 (Schott, Germany) and collected in a pre-weighed 10 ml vial. After evaporation of the organic solvent under nitrogen and subsequently 2h 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 analysis.

Mutagenicity testing

Extracts of TSPM and surface contamination samples were tested for mutagenic activity with *S. typhimurium* YG1041 in the Ames mutagenicity assay [21]. 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 µg/plate) 2584 ± 232 rev/plate).

For determination of the mutagenic activity, the arithmetic mean of the in 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 [144]. 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 3 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/m^3) and as revertants per square centimeter (rev/cm^2), 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 rev/m^3 and 17 rev/cm^2 for TSPM and surface contamination samples, respectively).

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 [79].

Table 4.3-1 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 (*S. typhimurium* strain YG1041) TSPM (rev/m^3)				Surface contamination (rev/cm^2)				
	N [b]	AM [c]	Median	Range [d]	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 4.3-2 Total suspended particulate matter (TSPM) exposure and mutagenicity of TSPM and contact surfaces stratified by production function.

Production function	TSPM (mg/m^3)				Mutagenicity (*S. typhimurium* strain YG1041)								
					TSPM (rev/m^3)				Surface contamination (rev/cm^2)				
	N [a]	AM [b]	Median	Range [c]	n (%) [d]	AM	Median	Range	N	n (%)	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

Results

In total 145 repeated TSPM samples were collected at 83 different sampling sites within 9 rubber companies. Total suspended particulate matter exposure and mutagenicity of TSPM and contaminated surfaces are presented in Table 4.3-1 and 4.3-2 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, which both 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).

Table 4.3-3 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 (µg/m^3)	CSM (µg/m^3)
Total suspended particulate matter (mg/m^3) (TSPM)	0.07 (0.56)	0.07 (0.55)	0.05 (0.62)
Cyclohexane soluble matter (µg/m^3) (CSM)	0.71 (0.0001)	0.96 (0.0001)	
Total soluble matter (µg/m^3) (TSM)	0.75 (0.0001)		

No correlation was observed between TSPM exposure and the mutagenicity of TSPM samples (r=0.07, p=0.56) (Table 4.3-3). Extractable mass (TSM and CSM) showed an overall good correlation with the observed TSPM mutagenicity, r=0.75 and r=0.71, 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).

Table 4.3-4 Univariate and multivariate regression models for mutagenicity of total suspended particulate matter and mutagenic surface contamination.

	Univariate regression model		**Multivariate regression model**	
	p-value [a]	r^{2} [b]	*p*-value [c]	r^{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 x 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 analysis
b) Explained proportion of variance per individual determinant
c) *P*-value derived from the multivariate regression analysis
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).

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 4.3-4). 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, production function and surface TSM exposure, however, the explained variance was considerably lower at, 18%, 10% and 1%, 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 4.3-4).

Discussion

Exposure to mutagenic compounds in the rubber manufacturing industry may occur by inhalation, ingestion, or dermal absorption [17-19]. 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 [10; 165]. Therefore, assessment of mutagenic activity of possible contact surfaces and rubber dust and fume exposure could 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. 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 [168]. 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 & 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 [169]. 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 departments were mostly detected in wipe samples from recently (warm) cured rubber tires or from surfaces that 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 United Kingdom since 1987, and serves as a surrogate for the complex mixture of rubber curing fumes [123]. 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 [10; 17-19].

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Section 4.4
Skin aberrations and slow-acetylation phenotype among rubber workers result in increased urinary mutagen levels associated with inhalable particulate and dermal exposure

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Airborne particulate and dermal mutagenic exposure levels and mutagens in urine of rubber workers (n=105) in 9 rubber manufacturing companies in The Netherlands were studied to determine the relevance of the inhalable and dermal exposure route. In addition, the potential influence of skin aberrations and biotransformation polymorphisms (NAT2 and CYP1A2) on urinary mutagenicity levels was addressed. Mutagenic activity of the total suspended particulate matter, surface contamination and Sunday and weekday urine samples was assessed with *S. typhimurium* YG1041 in the presence of a metabolic activation system. Furthermore, personal inhalable particulate, inhalable CSM and dermal CSM exposure was quantified. A significant increase of 1605 rev./g. creat. in urinary mutagenicity during the workweek relative to Sunday was observed for the total population (*t*-test, p=0.08) and was most pronounced among technical engineers (+4196 rev./g. creat.; *t*-test, p=0.08) and subjects with potential high TSPM and surface mutagenic exposure levels (+3206 rev./g. creat. ;*t*-test, p=0.12). Subsequent multivariate regression analyses revealed significant associations between the mutagenic activity of contact surfaces, inhalable particulate exposure, skin aberrations and slow acetylation phenotype and weekday urinary mutagenicity. Similar trends were observed with Sunday urinary mutagenicity levels except for slow-acetylation phenotype that only modulated the weekday urinary mutagenicity levels. Surface mutagenic exposure levels were estimated to increase weekday urinary mutagenicity with about 67%, while inhalable particulate exposure increased weekday urinary mutagenicity levels with about 23%. These results suggest that the dermal exposure route contributes more to urinary mutagens of rubber workers than the inhalable route. Especially since subjects with skin aberrations revealed an additional increase in weekday urinary mutagenicity of about 40%. In addition, levels of mutagens in urine were modulated by NAT2-dependent enzyme activity with slow acetylators having higher levels of mutagens in their urine. Prevalence rates of these unfavorable skin and biotransformation conditions are, however, high in this population, 40% and 60%, respectively. Therefore, increase in urinary mutagenicity due to these factors is of real importance.

Submitted for publication

Introduction

Mutagens have been found in raw rubber chemicals, airborne rubber dust and fume samples and in urine of exposed rubber workers [25]. Although, elevated urinary mutagenicity levels were detected, direct relations with external exposure levels were seldom identified [17; 19; 159; 170]. Only in a study by Bos et al. [19] a direct relationship was found among workers in an aircraft tire retreading company for urinary mutagenicity and dermal exposure to cyclohexane soluble matter (CSM). The suggestion that dermal exposure could be an important exposure route in the rubber industry was later strengthened by the observation that the amount available for uptake through the skin could be up to a tenfold higher than through inhalation, depending on the specific situation in a rubber company and use of personal protective devices [10].

Recently, skin contact with contaminated surfaces and deposition of rubber particles on the skin were identified as important exposure determinants of dermal CSM exposure in the rubber manufacturing industry [10; 165]. Moreover, substantial mutagenic activity was detected on these contact surfaces making dermal exposure to mutagenic compounds more than likely [171]. No information is, however, available on the actual dermal absorption of these compounds and therefore the relevance of the dermal exposure route in relation to the inhalable route remains unclear.

Dermal absorption of chemicals can be drastically increased when the skin barrier function is impaired [172-174]. Skin diseases, such as hand dermatitis and traumata of the skin are a common problem in the rubber manufacturing industry with prevalence rates for skin diseases up to 56% among rubber workers [167]. The high prevalence of skin impairments in this industry could therefore significantly add to the relative contribution of the dermal route.

We studied the presence of mutagens in urine of rubber workers, which were selected from a large cross-industry survey in The Netherlands [74; 167]. Subsequently, the possible relationship between inhalable and dermal exposure, mutagenicity of total suspended particulate matter (TSPM) and mutagenicity of contaminated surfaces and urinary mutagens was investigated. Special attention was given to the possible influence of CYP1A2 and NAT2 phenotype on urinary mutagenicity as in a recent study the importance of exposures to aromatic amines in this particular industry was suggested [168]. Both biotransformation phenotypes modulate the primary metabolic pathways of carcinogenic aromatic amines in humans [163].

Material and methods

Study population

Selection of the study population is described in detail elsewhere [74; 75]. In short, 225 subjects, employed in 9 rubber manufacturing companies (3 rubber tire, 5 general

rubber goods and 1 retreading company), were randomly selected based on their production function (e.g. compounding and mixing, pre-treating, moulding, curing, finishing, shipping, engineering service and laboratory). Sample and data collection was carried out within one week per company and comprised a medical survey, ambient and personal exposure measurements and collection of biological samples. Informed consent was obtained after the study was explained to the subjects.

After completion of the field phase of the survey and preliminary analysis, 100 subjects were selected from the original population based on their reported smoking status (yes/no) and ambient mutagenic exposure profile. The study population was divided into 4 a-priori exposure groups using median levels of total suspended particulate matter (TSPM) mutagenicity and surface mutagenic contamination levels. The exposure groups are characterized as follows:

[a] low TSPM mutagenicity and low surface mutagenic exposure levels (LL);

[b] high TSPM mutagenicity and low surface mutagenic exposure levels (HL);

[c] low TSPM mutagenicity and high surface mutagenic exposure levels (LH);

[d] high TSPM mutagenicity and high surface mutagenic exposure levels (HH).

No subjects matched the a-priori categorization of low TSPM mutagenicity and high surface mutagenic exposure levels (LH) leaving only three exposure groups (LL, HL, HH). From each of these exposure groups 20 non-smokers were selected. Additionally, 20 smokers were selected from both the LL and HH exposure group. For subjects employed as technical engineer (n=16) the work practice was too diverse to enable identification of relevant TSPM and surface wipe samples. However, technical engineers are potentially high exposed workers and were therefore included in the present survey as a separate category. All subjects were male (n=116) and were full-time employed.

Skin aberrations

Skin aberrations at time of the exposure survey were assessed by two dermatologists [75]. Objective skin symptoms were used for the classification: active hand dermatitis ('major' dermatitis), the first symptoms of dermatitis ('minor' dermatitis) and skin injuries (traumata) [51; 54].

Ambient exposure measures

Ambient exposure measurements were collected as described previously [165; 168]. Briefly, TSPM exposure was measured on random selected days (Tue. – Thurs.) by means of a high-volume sampler [94]. On average 1.8 samples were collected per department within a company; samples within a department were subsequently pooled for mutagenicity analysis. Surface contamination was estimated by obtaining wipe samples of potential contact surfaces. Samples were taken by a modification of the OSHA wipe sampling procedure [116; 165]. In the modified procedure, a surface area of 100 cm^2 of a potential

contact surface was chosen as sampling region. The region was wiped 3 times consecutively with Clean cylce™ wet VDU wipes (Inmac, Wargave, UK) containing 70% water and 30% isopropyl alcohol.

Personal exposure measures

Personal inhalable particulate exposure was measured on three consecutive days (Tue. – Thurs.) by means of a PAS6 sampling head mounted near the breathing zone of the worker [94; 95]. Personal dermal exposure to cyclohexane soluble matter (CSM) was measured by means of a dermal pad sampler worn at the lower part of the wrist of the hand of preference on the same days as the personal inhalable exposure measurements were taken [10; 76]. CSM contents of inhalable and dermal exposure samples were determined by means of the NIOSH PCAM 217 method [77; 95]. In short, samples were placed in cyclohexane (Merck, Darmstadt, Germany) and sonificated for 20 minutes. Suspension was filtered through a glassintertube G4 (Schott, Germany) and collected in a pre-weighed 10 ml-vial. After evaporation of cyclohexane under nitrogen and subsequently 2h drying at 40°C the residue was weighed by means of a microbalance [74].

Mutagenicity assays

TSPM and surface contamination samples were extracted consecutively with cyclohexane (Merck), dichloromethane (Merck) and methanol (Merck) as described previously [168]. After evaporation of organic solvents under nitrogen at 40°C, the residue was dissolved in 2.5 ml of dimethylsulfoxide (DMSO) (Merck) [19].

Spot urine samples were collected on Sunday, Wednesday and Thursday approximately at the same time of the day (± 4 PM). A volume, corresponding with 0.5 mmol of creatinine of the Wednesday and Thursday urine sample was pooled for each subject prior to mutagenicity analysis. Urine volumes of 1.0 mmol of creatinine of the Sunday and pooled weekday urine samples were neutralized to pH 7.0 and extracted with XAD-2 resin (6-cm^3 bed volume) and eluted with 10 ml of acetone. After evaporation of acetone under nitrogen at 40°C, the residue was dissolved in 2.5 ml of DMSO [19].

TSPM (n=83), surface contamination (n=104) and urine (n=232) extracts were assayed for mutagenic activity with *S. typhimurium* YG1041 with S9-mix of aroclor 1254 induced rat liver [21; 143]. Mutagenic activity was calculated based on the dose-response curve acquired at different dose levels. The slope of the linear component was used as an estimate of the mutagenic potency [144]. Mutagenicity of TSPM and surface contamination was expressed as revertants/m^3 and as revertants/cm^2, respectively. Urinary mutagenic activity was expressed as revertants/g creatinine.

Cotinine analysis

Mainstream tobacco smoke (MS) and environmental tobacco smoke (ETS) intake was evaluated by assessing cotinine levels in both the Sunday and weekday urine samples [175].

Urinary cotinine was quantified by high performance liquid chromatography according to the method of Barlow *et al.* [141] with modifications by Parviainen *et al.* [142]. Urinary cotinine levels were expressed as µg/g creatinine.

NAT2 and CYP1A2 biotransformation phenotype

An aliquot of the Wednesday urine spot sample was taken and analyzed for caffeine and metabolites, according to the procedure described by Grant *et al.*[176]. Designation of the acetylator (NAT2) phenotype was based on the ratio between 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X). Results showed a clear bimodality of *N*-acetylation capacity with a ratio cut point at 1.7 resulting in 61.5% slow and 38.5% fast acetylators. *N*-oxidation phenotype (CYP1A2) was determined by the ratio of urinary paraxanthine (17X) and caffeine (137X) as described by Lang and Kadlubar [177]. As the ratio was symmetrically distributed, the median (5.5) was used as cut point to assign slow and rapid *N*-oxidation phenotypes.

Calculations & statistics

Mean personal inhalable particulate, inhalable CSM and dermal CSM exposure was estimated using the James-Stein estimator (B_g) [178; 179] to maximize accuracy and precision of the exposure estimates [180]. In this computation a weighted mean (Θ_{gi}), based on the between- and within-worker variability, of the individual (z_{gi}) and group mean (z_g) exposure is calculated (Eq. 1). Subjects were grouped by production function (n=8) and subsequently group means were calculated based on the individual mean exposures derived from the repeated personal exposure measurements (n=3). The weighing factor (B_g) was estimated for a fixed number of repeated measurements (J) on each individual using equation 2.

$$\Theta_{gi} = (B_g)\bar{z}_g + (1 - B_g)\bar{z}_{gi} \qquad (1)$$

$$\hat{B}_g = \frac{(S^2_{ww})J^{-1}}{S^2_{bw} + (S^2_{ww})J^{-1}} \qquad (2)$$

Surface mutagenic contamination was calculated by averaging the mutagenic activity levels of relevant contact surfaces for each subject. In these calculations, surface wipe samples were weighed equally regardless of the frequency and duration of contact with the particular surface. Mean ambient TSPM mutagenicity was calculated based on the pooled TSPM samples according to the work areas the subject was employed in. Cotinine corrected Sunday and weekday urinary mutagenicity levels were calculated based on the algorithms found for the relation between urinary cotinine and urinary mutagenicity levels as described previously [175].

Within- and between-worker components of exposure variance were estimated from the ln-transformed exposure concentrations employing a one-way nested random-effects ANOVA model. Comparisons of geometric means of cotinine corrected mutagenicity levels

between Sunday and weekdays and a-priori defined exposure groups were tested with the Student's t-test. Finally, a multivariate regression model was built to identify factors, which contributed significantly to ln-transformed urinary mutagenicity levels. The obtained results are presented separately for the Sunday and weekday urine samples. Possible differences in the regression coefficients (β) of the identified determinants between the Sunday and weekday urinary mutagenicity levels were studied in a mixed effect model with the additional inclusion of interaction terms between day (Sunday or weekday) and the identified determinants. The random effects part of the mixed effect model was used to allow for the fact that Sunday and weekday urinary mutagenicity levels were repeated urine samples from the same subject.

Estimation of the effect of the identified determinants on urinary mutagenicity was calculated based on the used multivariate regression models. In these calculations the 25%- and 75%-percentile of the ln-transformed parameters were used to estimate the percentage increase in urinary mutagenicity levels. For dichotomous variables the exponent of the estimated β-coefficient (e^{β}) was used to estimate the percentage increase in urinary mutagenicity levels. All statistical analyses were performed using SAS version 6.12 software [79].

Results

Of the 116 selected subjects, 105 subjects (90.5%) had complete data concerning the ambient exposure estimates, current skin condition, urinary cotinine and urinary mutagenic activity levels. Subsequently, results presented in this paper are based on these 105 subjects. For some of the subjects (20%) one of the three repeated personal inhalable or dermal exposure measurements were missing due to equipment failure or analytical errors. In these instances the individual mean exposure (z_{gi}) was calculated based on the two remaining measurements. Subjects were all male with a mean age of 37.9 (sd. 9.0) year.

An overview of the study population and mean mutagenic TSPM and surface contamination levels for the a-priori defined exposure groups is presented in Table 4.4-1. The exposure groups HL and HH revealed both statistically significant higher TSPM mutagenicity levels than exposure group LL (*t*-test, $p<0.0001$). Exposure group HH exhibited statistically significant higher surface mutagenic contamination levels than exposure groups LL and HL (*t*-test, $p<0.0001$). No statistical difference in TSPM mutagenicity was observed between exposure group HL and HH. These results confirmed the intended differences in ambient mutagenic exposure profile between the a-priori defined exposure groups.

Table 4.4-1 Mean mutagenic activity of total suspended particulate matter (TSPM) (rev/m^3) and mutagenicity of surface contamination (rev/cm^2) levels for the a-priori defined exposure groups.

Exposure group	NS / S [a]	TSPM GM (GSD) [b]	Surface GM (GSD) [b]
- Low TSPM and low surface mutagenic contamination levels (LL)	18 / 18	73 (2.13)	19 (1.02)
- High TSPM and low surface mutagenic contamination levels (HL)	19 / 0	449 (1.73)	20 (1.12)
- High TSPM and high surface mutagenic contamination levels (HH)	20 / 16	514 (1.73)	147 (2.60)
- Technical engineers (TD)	10 / 4	N.Q [c]	N.Q

a) Number of subjects. NS= non-smokers; S= smokers
b) Geometric mean (gm) and geometric standard deviation (gsd) of mutagenic exposure levels
c) Not quantified

Variability in personal exposure measures was almost equally distributed between and within subjects (Table 4.4-2). The estimated between- and within variance components resulted in a James-Stein estimator (B_g) of 0.23, 0.23 and 0.26 for the inhalable particulate, inhalable CSM and dermal CSM exposure measures, respectively. Mean individual exposure estimates (Θ_{gi}) were therefore largely based on the individual mean exposure. The observed within- and between-worker variance components were large for urinary mutagenicity levels. Correction of urinary mutagenicity for urinary cotinine levels reduced the between-variance component significantly (48%). This indicates that differences between subjects in urinary mutagenicity are to a large extent explained by tobacco smoke intake.

Geometric mean of cotinine corrected urinary mutagenicity levels for the a-priori defined exposure groups are presented in Figure 4.4-1. Non-smokers [A] and smokers [B] revealed similar patterns in Sunday and weekday urinary mutagenicity for the different a-priori defined exposure groups with the exception of a borderline significant difference in cotinine corrected Sunday urinary mutagenicity levels between smokers and non-smokers within the exposure group HH (*t*-test, p=0.10). In order to increase the number of subjects per exposure group smokers and non-smokers were subsequently pooled [C]. A borderline significant increase in cotinine corrected urinary mutagenicity between Sunday and weekday urine samples was observed for the total population (+1605 rev./g creat; *t*-test, p=0.08) and technical engineers (+4196 rev./g creat; t-test, p=0.08). The exposure group HH showed also an increase in urinary mutagenicity levels during the week, however, statistically non-significant (+3206 rev./g creat; *t*-test, p=0.12).

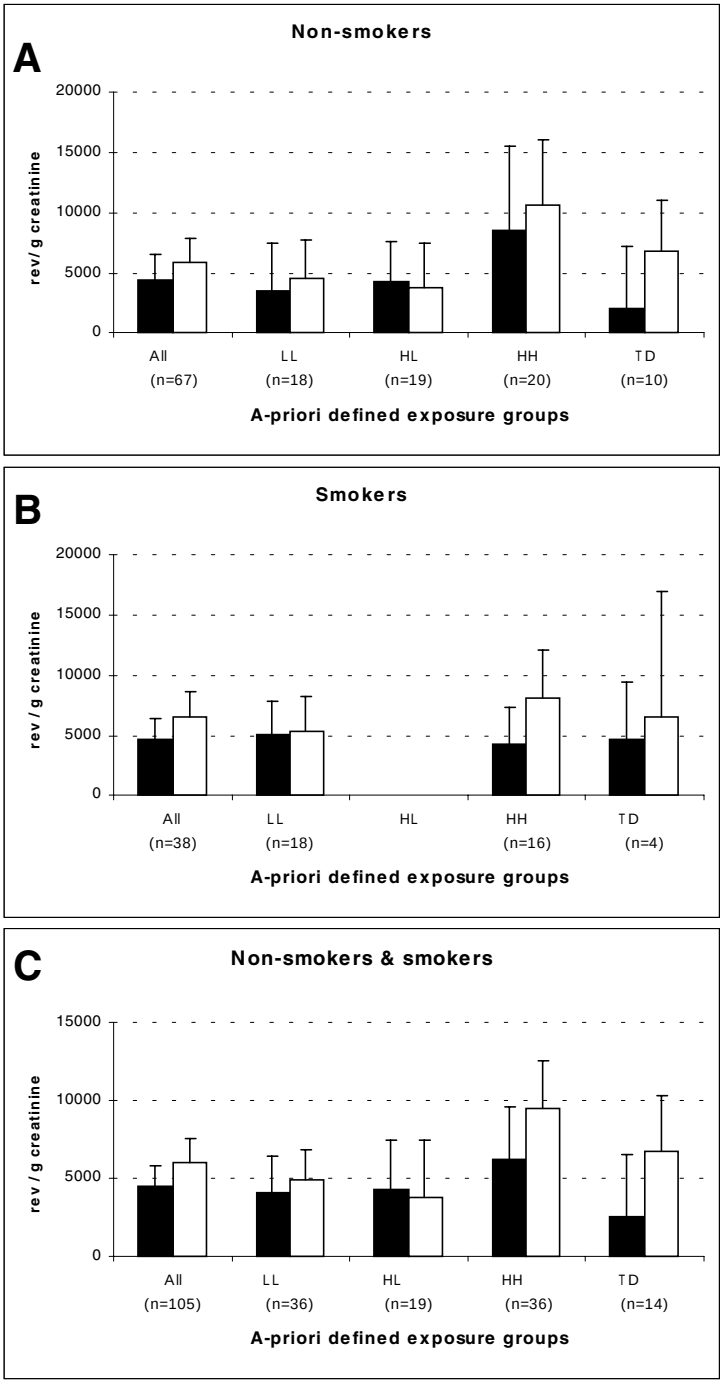

Figure 4.4-1 Geometric mean (GM) and 95% upper confidence limit of cotinine corrected urinary mutagenicity levels (rev/g creatinine) for the a-priori defined exposure groups. ■, Sunday urinary mutagenicity; □, Weekday urinary mutagenicity.

Table 4.4-2 Total, between- and within-worker variability of several personal exposure measures and urinary mutagenicity.

	N [a]	S^2_t [b]	S^2_{bw} [c]	S^2_{ww} [d]
Inhalable particulate (mg/m^3)	290	0.79	0.42	0.38
Inhalable CSM ($\mu g/m^3$)	284	0.37	0.19	0.18
Dermal CSM ($\mu g/cm^2$)	304	1.55	0.74	0.82
Urinary mutagenicity (rev/ g. creatinine)	210	2.23	1.38	0.86
Urinary mutagenicity (rev/ g. creatinine) (cotinine corrected)	210	1.62	0.72	0.90

a) Number of measurements, number of subjects is 105
b) Estimated variance component of total distribution
c) Estimated variance component of the between-worker distribution
d) Estimated variance component of the within-worker distribution

Possible relations between exposure measures, skin aberrations and biotransformation phenotypes were further investigated by means of multivariate regression analyses. These analyses were allowed for urinary cotinine concentration, skin aberrations and NAT2 phenotype as these factors were statistically significant associated with Sunday and/or weekday urinary mutagenicity levels (Table 4.4-3). Incidence of minor dermatitis and skin injuries were associated with increased urinary mutagenicity levels. Major dermatitis exhibited no relationship with urinary mutagenicity (data not shown). Consequently, only workers with minor dermatitis and traumata of the skin at time of the survey were classified as subjects with skin aberrations (n=36). This classification was subsequently used in all statistical analyses. Age and CYP1A2 biotransformation phenotype revealed no association with the observed urinary mutagenicity levels and were therefore not included in the multivariate regression models.

Exposure group HH revealed a significant increase (β=0.62; p=0.01) in weekday urinary mutagenicity levels relative to the LL exposure group (Table 4.4-3). No statistical differences between the a-priori defined exposure groups were found in Sunday urinary mutagenicity levels. The observed associations between the a-priori defined exposure groups, skin aberrations and urinary cotinine with Sunday and weekday urinary mutagenicity were not statistically significantly different. For slow acetylation phenotype a statistical significant different relationship with urinary mutagenicity for the Sunday and weekday urine sample was present.

Table 4.4-3 Relationship between urinary cotinine, NAT2-phenotype, skin aberrations and the a-priori defined exposure groups and Sunday and weekday urinary mutagenicity levels (rev/g. creatinine) of rubber manufacturing workers (n=105).

	Sunday urinary mutagenicity		**Weekday urinary mutagenicity**	
	β (SE) [a]	*p*-value	β (SE) [a]	*p*-value
Intercept	8.24 (0.34)	0.0001	8.59 (0.25)	0.0001
Urinary cotinine (µg /g. creatinine)	0.27 (0.05)	0.0001	0.26 (0.04)	0.0001
Slow acetylation (NAT2) [b]	0.08 (0.28)	0.78	0.45 (0.21)	0.03
Skin aberrations [c]	0.52 (0.29)	0.08	0.42 (0.22)	0.06
High TSPM mutagenicity and low surface mutagenicity (HL) [d]	0.05 (0.42)	0.90	-0.20 (0.31)	0.52
High TSPM mutagenicity and high surface mutagenicity (HH) [d]	0.39 (0.33)	0.23	0.62 (0.25)	0.01
Technical service (TD) [d]	-0.51(0.44)	0.25	0.29 (0.33)	0.39

a) β-coefficient and standard error of estimate
b) Relative to fast-acetylation phenotype
c) Minor dermatitis and/or traumata of the skin
d) Relative to low TSPM mutagenicity and low surface mutagenicity (LL) exposure group

Further statistical analyses with ambient and personal exposure measures resulted in the model presented in Table 4.4-4. No relationship was found between TSPM mutagenicity and urinary mutagenicity levels. Also, personal inhalable CSM and dermal CSM exposure estimates revealed no statistically significant associations with urinary mutagenicity. TSPM mass (mg/m^3) and personal inhalable particulate exposure, however, showed a significant association with both Sunday and weekday urinary mutagenicity levels. Both particulate exposure measures were significantly correlated ($r=0.55$, $p<0.0001$) and therefore only the personal inhalable particulate exposure estimate was used in the multivariate regression model as this exposure estimate showed statically stronger associations with urinary mutagenicity than TSPM mass. The observed relationships between urinary cotinine, skin aberrations and exposure measures were again not statistically significant different for the weekday and Sunday urinary mutagenicity levels except for slow acetylation phenotype, which exhibited only a significant relationship with the weekday urinary mutagenicity levels. The constructed multivariate models explained 34% and 46% of the variance in Sunday and weekday urinary mutagenicity levels, respectively. Interestingly the intercept of the models showed a slight increase in urinary mutagenicity during the week, which was not explained by the determinants included in the model.

Estimates of the percentage increase in Sunday and weekday urinary mutagenicity for the models presented in Table 4.4-4 are given in Table 4.4-5. High versus low tobacco smoke exposure resulted in the largest percentage increase of the studied determinants in both Sunday and weekday urinary mutagenicity levels, 267% and 289%, respectively.

Of the exposure measures surface mutagenicity revealed an almost three times stronger effect on Sunday and weekday urinary mutagenicity than inhalable particulate exposure (51% and 67% versus 21% and 23%). In addition, skin aberrations resulted in an increase of 51% and 42% for Sunday and weekday urinary mutagenicity levels, respectively. Percentage increase in urinary mutagenicity during the workweek as a result of slow-acetylation phenotype was estimated at 52%.

Table 4.4-4 Relationship between urinary cotinine, NAT2-phenotype, skin aberrations and exposure measures and Sunday and weekday urinary mutagenicity (rev/g. creatinine) of rubber manufacturing workers, excluding technical engineers (n=91) [a].

	Sunday urinary mutagenicity		**Weekday urinary mutagenicity**	
	β (SE) [b]	*p*-value	β (SE) [b]	*p*-value
Intercept	7.86 (0.50)	0.0001	7.95 (0.36)	0.0001
Urinary cotinine (µg /g. creatinine)	0.22 (0.05)	0.0001	0.23 (0.04)	0.0001
Slow acetylation (NAT2) [c]	0.09 (0.28)	0.75	0.42 (0.23)	0.03
Skin aberrations [d]	0.41 (0.29)	0.17	0.35 (0.24)	0.14
Inhalable particulate dust (mg/m^3)	0.42 (0.23)	0.07	0.46 (0.19)	0.02
Surface mutagenicity (rev/cm^2)	0.24 (0.12)	0.05	0.30 (0.10)	0.0028

a) Technical engineers were not included in the analyses due to missing ambient mutagenic exposure estimates
b) β-coefficient and standard error of estimate
c) Relative to fast-acetylation phenotype
d) Minor dermatitis and/or traumata of the skin

Table 4.4-5 Estimation of the effect (percentage increase) of the identified determinants on Sunday and weekday urinary mutagenicity levels[a].

	Exposure distribution		**Sunday urinary mutagenicity**	**Weekday urinary mutagenicity**
	25%-percentile	75%-percentile	%-increase	%-increase
Urinary cotinine (µg /g. creatinine)	2	693	266.8	289.1
Slow acetylation [b]	NA [c]		9.4	52.2
Skin aberrations [d]	NA [c]		50.7	41.9
Inhalable particulate dust (mg/m^3)	0.52	0.82	21.2	23.4
Surface mutagenicity (rev/cm^2)	19	106	50.9	67.2

a) Based on the multivariate regression model presented in table 4.4-4
b) Relative to fast-acetylation phenotype
c) Not applicable, dichotomous variable
d) Minor dermatitis and/or traumata of the skin

Discussion

We studied mutagens in urine of rubber workers in relation to inhalable and dermal exposure. In addition, the potential influence of skin aberrations and biotransformation polymorphisms on urinary mutagenicity levels was addressed. Smoking is almost invariably the most important contributor to urinary mutagenicity levels. Therefore, adequate adjustment of daily tobacco smoke exposure is a necessity when using the urinary mutagenicity assay to evaluate possible genotoxic exposures in the workplace [175]. In this study urinary mutagenicity levels were corrected for urinary cotinine concentrations based on the algorithms described previously [175] or by inclusion of the urinary cotinine concentrations in the multivariate regression analyses. The observed relationships between urinary cotinine and urinary mutagenicity in these regression analyses were similar to the previously published algorithms. After urinary cotinine correction the patterns in Sunday and weekday urinary mutagenicity levels were remarkably similar with no indication of a synergistic effect between smoking and occupational exposure. Therefore, cotinine corrected urinary mutagenicity data of non-smokers and smokers could be pooled to increase sample size in further statistical analyses.

Inhalable and dermal exposure assessment was based on ambient and personal exposure measurements. Ambient measurements were used to assess potential mutagenic exposure conditions encountered in the rubber manufacturing industry as personal exposure samples did not result in enough collected material to be tested in the Ames mutagenicity assay. The collected TSPM and surface contamination samples were tested for mutagenicity with *S. typhimurium* YG1041 in the presence of a metabolic activation system. The strain YG1041, with elevated levels of nitroreductase and *O*-acetyltransferase was shown in a previous study to be highly sensitive for detecting mutagenicity in rubber dust and fume samples [168]. Therefore, this strain was used in the current survey to test mutagenic activity in TSPM, surface wipe samples and in urine of rubber workers under identical test conditions. Assignment of ambient mutagenic exposure levels to the individuals was based on the work area the subject was mainly employed in and actual tasks performed. Mean individual TSPM and surface mutagenicity levels were calculated independent of duration of contact with a particular surface or residence time in a particular work area. Therefore, these estimates could have been imprecise. However, TSPM and surface mutagenic exposure levels are to a large extent determined by company specific factors and production functions within a company [171]. Therefore, individual estimates of ambient mutagenic TSPM and surface contamination will have resulted in a justifiable relative ranking of subjects from different companies and production functions regarding external mutagenic exposure conditions. Personal exposure estimates of inhalable particulate, inhalable CSM and dermal CSM exposure were calculated based on a weighted mean of the individual and group mean exposure to maximize accuracy and precision of the exposure estimates [180]. Estimates of exposure based on individual means may result in attenuation of the exposure-response

relationship, while grouped estimates may control attenuation bias but may result in a decrease of the precision [181]. Combining individual and group estimates can simultaneously control both types of error.

The results of this study showed an increase in urinary mutagens of rubber workers during the workweek relative to Sunday. The increase in urinary mutagenicity was most pronounced among technical engineers and subjects with potential high TSPM and surface mutagenic exposure levels. No increase in urinary mutagenicity was found for subjects with a high TSPM but low surface mutagenic activity. Based on these results it can be concluded that dermal contact with mutagenic surface contamination is probably the most likely exposure route. Further statistical analysis using mutagenic activity of contact surfaces (rev/cm^2) as a continuous variable confirmed the relationship between mutagenicity of possible contact surfaces and Sunday and weekday urinary mutagenicity levels, while again no association was found between TSPM mutagenic activity levels and mutagens in urine of rubber workers. However, exposure estimates of both ambient and personal particulate mass revealed a significant association with urinary mutagenicity levels. That a relationship with particulate exposure was observed and not with inhalable CSM or TSPM mutagenic activity suggests significant non-extractable particle bound mutagenicity. The existence of non-extractable bound bioactive material to the surface of airborne rubber dust particles was previously demonstrated in a study by Alink *et al.* [122]. In this study, 83% of the inhibition of the gap-junctional intercellular communication could be attributed to bio-active material tightly bound to the surface of the particulate matter that originated from a mixing department of a rubber tire company. As the highest personal inhalable particulate exposure levels were measured in the mixing departments of the involved companies the a-priori defined exposure groups might have had limited value with regard to airborne mutagenic exposure conditions. Interestingly, personal dermal CSM exposure showed no relationship with urinary mutagenicity levels, while surface mutagenicity levels did. Presumably, quantification of the personal dermal CSM exposure levels at the wrist did not result in a more accurate exposure measure for dermal mutagenic exposure conditions.

Identified associations between occupational exposure estimates and urinary mutagenicity were not statistically different for the pooled weekday urine samples and Sunday urine samples, though in most instances the observed associations with Sunday urinary mutagenicity were weaker and statistically less significant. If the biological half-life of the detected mutagenic compounds is longer than successive exposure intervals, accumulation of these compounds will occur resulting in a steady-state concentration. As significant occupational related (residual) urinary mutagenic activity was detected in the Sunday urine samples, these samples cannot be regarded as a ‘clean’ baseline sample. Therefore, the ratio or absolute difference between Sunday and weekday urinary mutagenicity levels would have certainly resulted in an underestimation of the mutagenic effect.

Subjects with skin aberrations showed statistically significant higher urinary mutagenicity levels than subjects with a normal skin barrier function. This association was stronger in the regression analyses that included also the technical engineers. Technical engineers have a high prevalence of minor dermatitis and traumata of the skin, 33% and 22%, respectively. Exclusion of this production function due to missing ambient mutagenic exposure estimates, from subsequent regression analyses resulted in the loss of statistical power and consequently the loss of statistical significance. The observed trend between skin aberrations and urinary mutagenicity remained, however, the same. Volunteer studies have shown that even mild skin barrier damage could dramatically increase percutaneous penetration [172; 182-184]. However, the magnitude of the effect depends greatly on the chemical properties and clinical manifestation of the disease [185]. In this study, increased penetration was observed for subjects with mild dermatitis and traumata of the skin at time of the survey but not for subjects with major dermatitis. In a previous study it was shown that rubber workers often do not regard their impaired skin (e.g. minor dermatitis) as a disease and will therefore not alter their working practice [75]. However, in the case of major dermatitis changes in behavior were found with regard to skin aggravating risk factors [167]. The results of this study suggest that subjects with major dermatitis consciously minimized direct skin contact with contaminated surfaces and therefore did not reveal the same positive trend in urinary mutagenicity as subjects with less severe skin aberrations. More importantly, the observed association between skin aberrations and urinary mutagenicity is another indication of the importance of the dermal exposure route in this particular industry.

Slow-acetylators revealed significantly higher weekday urinary mutagenicity levels than fast-acetylators. NAT2 modulation of urinary mutagenicity has been found in several studies of subjects exposed to aromatic amines [32; 33; 186]. In addition, slow-acetylation status has been associated with an increase in urinary bladder cancer risk among subjects with past occupational exposure to aromatic amines [38]. NAT2 selectively *N*-acetylates arylamines and can also catalyze the *O*-acetylation of the *N*-hydroxy-arylamine metabolites. For urinary mutagenicity *N*-acetylation of arylamines represents in most instances a competing pathway for *N*-oxidation, a necessary metabolic activation step occurring in the liver [163]. However, no indication was found in this study for this competing pathway as *N*-oxidation capacity revealed no association with urinary mutagenicity. However, there is considerable debate whether the urinary metabolite ratio between paraxanthine and caffeine accurately reflects CYP1A2 activity as paraxanthine is both a product and a substrate of CYP1A2 and is significantly influenced by urine-flow [126]. Remarkably, NAT2 phenotype only influenced the urinary mutagenicity levels during the workweek, while the other determinants significantly affected both weekday and Sunday urinary mutagenicity levels. The observed relationship between urinary mutagenicity and the exposure estimates were therefore probably not modulated by NAT2-dependent enzyme activities. This point towards other NAT2 modulated (occupational) exposures that were not quantified as such with the used sampling and/or analytical techniques. Also, the observed slight increase in intercepts of the applied

multivariate regression models for weekday urinary mutagenicity compared to Sunday urinary mutagenicity levels suggest unmeasured exposure routes for mutagenic substances, like the gaseous phase or oral exposure route.

In conclusion, the dermal exposure route seemed to contribute more to urinary mutagens of rubber workers than the inhalable route, especially when taken into account that subjects with skin aberrations revealed an additional increase in urinary mutagenicity of about 40%. In addition, levels of mutagens in urine were partly modulated by NAT2-dependent enzyme activity with slow acetylators having higher levels of mutagens in their urine. Prevalence rates of these unfavorable skin and biotransformation conditions are, however, high, 40% and 60%, respectively. Therefore the increase in urinary mutagenicity due to these factors is of real importance.

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Section 4.5
Urothelial cell DNA adducts among rubber manufacturing workers

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Workers employed in the rubber manufacturing industry appear to have a significant excess cancer risk in a variety of sites, including cancer of the urinary bladder. In this cross-sectional study, we investigated the occurrence of DNA adducts in exfoliated bladder cells of currently exposed, non-smoking rubber workers (n=52) and their relationship with occupational exposure estimates and biotransformation phenotypes (NAT2 and CYP1A2). Four DNA adducts were identified with the proportion of positive samples ranging from 3.8% to 79%. The highest proportion of positive samples and the highest relative adduct labeling (RAL) levels were in workers involved in the production functions 'mixing' and 'curing', areas with potential for substantial exposure to a wide range of compounds used in rubber manufacturing ($p < 0.05$ for adducts #2 and/or #3, compared to all other departments). However, no statistically significant relationships were found between identified DNA adducts and non-specific personal inhalable and dermal CSM exposure estimates or urinary mutagenicity. Interestingly, subjects with a fast NAT2 acetylation phenotype tended to have higher levels of DNA adducts. Aryldiamines, common in mixing and curing, might represent a potential exposure consistent with these data. This study suggests that workers in mixing and curing may be exposed to compounds that can adduct DNA in urothelial cells. Larger studies among rubber workers should be conducted to study in more detail the potential carcinogenicity of exposures encountered in these work areas.

Submitted for publication

Introduction

Epidemiological studies of workers employed in the rubber manufacturing industry have indicated a significant excess cancer risk in a variety of sites (e.g. lung and urinary bladder cancer and leukemia) [7; 8]. Urinary bladder cancer, in this industry has been attributed traditionally to exposure to antioxidants such as Nonox 'S' that contained free β–naphthylamine as a contaminant [2]. The use of this antioxidant and similar compounds contaminated with β-naphthylamine was subsequently discontinued in the 1950's. However, several recent epidemiological studies have found a moderate excess risk of urinary bladder cancer among workers with supposedly no concomitant exposure to β–naphthylamine [9; 187]. These observations placed in doubt the exclusive link between β–naphthylamine exposure and urinary bladder cancer in the rubber manufacturing industry. Interestingly, an excess of urinary bladder cancer was also reported in two recent cohort studies of workers manufacturing chemicals for the rubber manufacturing industry; these workers were exposed to neither benzidine nor β-naphthylamine [188; 189].

Workers in the rubber manufacturing industry are exposed to a wide range of potentially carcinogenic compounds [7; 188]. The chemical composition and toxic properties of the complex exposure mixture is, to a large extent, unknown. Non-chemical specific exposure- and biomarkers are often applied in these exposure conditions [20; 188]. Examples of such markers are the *S. typhimurium* mutagenicity assay and ^{32}P-postlabelling method for bulky carcinogen-DNA adducts. In several studies in the rubber manufacturing industry, exposure to mutagenic compounds was established with the *S. typhimurium* mutagenicity assay either by measuring mutagenicity of airborne particulates and fumes or by measuring the mutagenicity in urine of workers [14; 17; 19; 25; 26; 168; 188]. Although, the ^{32}P-postlabeling method has been used in a large number of studies on occupational exposures to detect carcinogen-DNA adducts [190], it has to our knowledge so far not been applied in the rubber manufacturing industry.

Biotransformation polymorphisms may play an important role in the individual variability in carcinogen metabolism. *N*-acetyltransferase capacity plays a significant role in the biotransformation of arylamines, of which some are known bladder carcinogens [163; 164]. Slow NAT2 acetylation status has been associated with increased urinary mutagenicity [31; 31; 32; 149; 188; 188], DNA adducts [34-37; 149; 188] and a modest increase in urinary bladder [38] and several other cancers [39]. Fast acetylators have been suggested to be at higher risk of developing cancer of the colon [40]. Although the increase in biomarkers or cancer risk due to specific biotransformation polymorphisms in most cases is small, the polymorphisms are widespread in the general population and may be of importance for individuals with a particular exposure [191].

We studied the relationship between inhalable and dermal exposure and urinary mutagenicity and urothelial cell DNA adduct levels in a population of rubber manufacturing workers, which were selected from a large cross-industry survey in The Netherlands [166; 167]. Subjects were selected based on smoking status (non-smokers) and ambient mutagenic exposure profile. Ambient mutagenic exposure levels were measured with strain *S. typhimurium* YG1041 in the presence of a metabolic active rat liver S9-fraction. In addition the influence of NAT2 and CYP1A2 biotransformation phenotype on DNA adduct levels was investigated.

Materials & methods

Cross-sectional study

The field phase of this study was conducted from January 1997 through July 1997, among subjects working in the rubber manufacturing industry in the Netherlands. Subjects (n=225) were employed in 9 companies (3 rubber tire, 5 general rubber goods and 1 retreading company) and were randomly selected based on their production function (e.g. compounding and mixing, pre-treating, moulding, curing, finishing, shipping, engineering service and laboratory) [166; 167]. Fieldwork was carried out within

one week per company and comprised a medical survey, ambient and personal exposure measurements and collection of biological samples. Informed consent was obtained after the study was explained to the subjects.

After completion of the field phase of the survey and preliminary analysis, subjects were selected from the original population based on their ambient mutagenic exposure profile. The study population was divided into 4 a-priori exposure groups using median levels of total suspended particulate matter (TSPM) mutagenicity and surface mutagenic contamination levels. The exposure groups are characterized as follows:

[a] low TSPM mutagenicity and low surface mutagenic exposure levels (LL);

[b] high TSPM mutagenicity and low surface mutagenic exposure levels (HL);

[c] low TSPM mutagenicity and high surface mutagenic exposure levels (LH);

[d] high TSPM mutagenicity and high surface mutagenic exposure levels (HH).

No subjects matched the a-priori categorization of low TSPM mutagenicity and high surface mutagenic exposure levels (LH) leaving three exposure groups (LL, HL, HH). Twenty non-smokers were randomly selected from the three remaining exposure groups resulting in a study population of 60 subjects of which 56 subjects (93.3%) had retrievable 24h-urine samples.

All subjects were male and were administered a questionnaire, as part of the initial survey to collect information regarding age, medical history, alcohol and roasted meat consumption and use of open fire place. Environmental tobacco smoke (ETS) intake was evaluated by assessing urinary cotinine levels by high performance liquid chromatography according to the method of Barlow *et al.* [141] with modifications by Parviainen *et al.* [142]. Urinary cotinine levels were expressed as µg/g creatinine.

Occupational exposure measures

Ambient exposure measurements were collected as described previously [165; 168]. Briefly, TSPM exposure was measured on random selected days (Tue. – Thurs.) using a high-volume sampler [94]. On average 1.8 samples were collected per department within a company; samples within a department were subsequently pooled for mutagenicity analysis. Surface contamination was estimated by obtaining wipe samples of potential contact surfaces. Samples were taken by a modification of the OSHA wipe sampling procedure [116]. Personal inhalable particulate exposure was measured on three consecutive days (Tue. – Thurs.) by means of a PAS6 sampling head mounted near the breathing zone of the worker [94; 95]. Personal dermal exposure to cyclohexane soluble matter (CSM) was measured by means of a dermal pad sampler worn at the lower part of the wrist of the hand of preference on the same days as the personal inhalable exposure measurements were taken [10; 76]. CSM contents of inhalable and dermal exposure samples were determined by means of the NIOSH PCAM 217 method [77; 95]. In short, samples were placed in cyclohexane (Merck, Darmstadt, Germany) and sonificated for 20 minutes. The suspension was filtered through a glassintertube G4 (Schott, Germany) and collected in a pre-

weighed 10 ml-vial. After evaporation of cyclohexane under nitrogen and subsequent drying for 2h at 40°C the residue was weighed by means of a microbalance [166]. No chemical analysis of the extract was performed.

Mutagenicity assays

TSPM and surface contamination samples were extracted consecutively with cyclohexane (Merck), dichloromethane (Merck) and methanol (Merck) as described previously [168]. After evaporation of organic solvents under nitrogen at 40°C, the residue was dissolved in 2.5 ml of dimethylsulfoxide (DMSO) (Merck) [19]. Spot urine samples were collected on Wednesday and Thursday after work had ended (± 4 PM). Volumes, corresponding with 0.5 mmol of creatinine of the Wednesday and Thursday urine samples were pooled for each subject prior to mutagenicity analysis. Pooled urine samples were neutralized to pH 7.0 and extracted with XAD-2 resin (6-cm^3 bed volume) and eluted with 10 ml of acetone. After evaporation of acetone under nitrogen at 40°C, the residue was dissolved in 2.5 ml of DMSO [19].

TSPM, surface contamination and urine extracts were assayed for mutagenic activity with *S. typhimurium* strain YG1041 with S9-mix of aroclor 1254 induced rat liver [21; 143]. Mutagenic activity was calculated based on the dose-response curve acquired at different dose levels. The slope of the linear component was used as an estimate of the mutagenic potency [144]. Mutagenicity of TSPM and surface contamination was expressed as revertants/m^3 and as revertants/cm^2, respectively. Urinary mutagenic activity was expressed as revertants/g creatinine.

Urothelial cell DNA adduct analysis

Twenty-four hour urine samples were collected from Monday (after first morning void) to Tuesday (including first morning void). Samples were kept cold (± 4°C) during collection and made 20% glycerol (v/v) to minimize cell loss due to lysis after freezing. Consequently, samples were stored at –20°C until analysis.

Frozen samples were thawed, mixed and urinary pH was assessed. A 500 ml aliquot of the urine samples was filtered through sequential 500 and 250 µm sieves, then exfoliated urothelial cells were isolated by vacuum filtration using 5 µm nylon filters (Osmonics, USA). Cells were washed of the filters with a phosphate buffered saline solution (PBS, pH 7.4) and washed several times by re-suspension in the same buffer followed by centrifugation at 800 RPM for 15 minutes in a refrigerated centrifuge. DNA was isolated from the cell pellets by a solvent extraction technique and quantitated by normal nucleotide analysis with the ^{32}P-postlabeling method as described by Talaska *et al.* [192]. ^{32}P-postlabeling of the exfoliated urothelial DNA samples was performed under conditions of radiolabelled adenosine 5'-triphosphate (ATP) excess [193]. Necessary excess of ATP was provided by the addition of 150 µCi to each sample. Carcinogen-DNA adduct levels were calculated by determining the relative adduct labeling (RAL), which was defined as: counts per minute (c.p.m.) in adduct spots / c.p.m. in normal nucleotides.

^{32}P-postlabeling analyses were performed with blind-coded samples and each sample was analyzed in duplicate, at least. Samples that had insufficient amounts of DNA (arbitrary cut-off point 20x10^6 nucleotides) were reanalyzed by increasing the amount of DNA-sample to be tested or by re-isolation of larger volumes (>500 ml) of the same 24h urine sample (n=19).

NAT2 and CYP1A2 biotransformation phenotype

An aliquot of the Wednesday urine spot sample was taken and analyzed for caffeine and metabolites according to the procedure described by Grant *et al.*[176]. Designation of the acetylator (NAT2) phenotype was based on the ratio between 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X). Results showed a clear bimodality of *N*-acetylation capacity with a ratio cut point of 1.7. *N*-oxidation phenotype (CYP1A2) was determined by the ratio of urinary paraxanthine (17X) and caffeine (137X) as described by Lang and Kadlubar [177]. As the ratio was symmetrically distributed, the median (5.5) was used as cut point to assign slow and fast *N*-oxidation phenotypes.

Calculations and statistical analysis

Surface mutagenic contamination was calculated by averaging the mutagenic activity levels of relevant contact surfaces for each subject. In these calculations, surface wipe samples were weighed equally regardless of the frequency and duration of contact with the particular surface. Mean ambient TSPM mutagenicity was calculated based on the pooled TSPM samples according to the work areas the subject was employed in. Cotinine corrected pooled weekday urinary mutagenicity levels were calculated based on the algorithms found for the relation between urinary cotinine and urinary mutagenicity levels as described previously [175].

Mean personal inhalable particulate, inhalable CSM and dermal CSM exposure was estimated using the James-Stein estimator (B_g) [178; 179] to maximize accuracy and precision of the exposure estimates [180]. In this computation a weighted mean of the individual and group mean exposure is calculated, based on the between- and within-worker exposure variability. Subjects were grouped by production function (n=8) and subsequently group means were calculated based on the individual mean exposures derived from the repeated personal exposure measurements (n=3).

RAL levels were not normally distributed. Therefore, two-tailed non-parametric tests were used throughout the study. Spearman correlation coefficients were used to investigate the relationship between identified DNA adducts and several exposure indices. Differences in DNA adduct levels between exposure groups were tested with the Wilcoxon rank sum test. Trends in proportion of positive samples by phenotype or production function were investigated using the Cochran-Armitage test for trend. Because of the

small numbers in most analyses exact p-values were calculated for the analyses with the proportion of positive DNA adduct samples [82]. All statistical analyses were performed using SAS version 6.12 software [79].

Results

An overview of the study population and ambient mutagenic exposure levels of the a-priori defined exposure groups is presented in Table 4.5-1. Sufficient DNA (> $20x10^6$ nucleotides) for ^{32}P-postlabeling analysis was obtained from 52 of 56 available 24h-urine samples (93%). There was a wide range in the quantity of radiolabeled nucleotides ($20x10^6$ – $256x10^6$), as would be expected since the number of DNA in each sample was not known prior to the analysis. An excess of radiolabel was seen in the normal nucleotide maps for each sample, indicating that sufficient [^{32}P]ATP was available to label all nucleotides in the samples.

Table 4.5-1 Mean mutagenic activity of total suspended particulate matter (TSPM) (rev/m^3) and mutagenicity of surface contamination (rev/cm^2) levels for the a-priori defined exposure groups.

Exposure group	N (n) [a]	TSPM GM (GSD) [b]	Surface GM (GSD) [b]
- Low TSPM and low surface mutagenic contamination levels (LL)	19 (18)	73 (2.13)	19 (1.02)
- High TSPM and low surface mutagenic contamination levels (HL)	20 (18)	449 (1.73)	20 (1.12)
- High TSPM and high surface mutagenic contamination levels (HH)	17 (16)	514 (1.73)	147 (2.60)

a) Number of subjects selected. Between parenthesis number of subjects of whom sufficient amounts of DNA were retrieved
b) Geometric mean (GM) and geometric standard deviation (GSD) of mutagenic exposure levels

During the analyses, eleven different possible DNA adducts were observed (numbering in temporal order of detection). Adducts 5, 7 and 8 were later identified as un-adducted deoxyguanosine nucleotides. Adducts 4 (number of positive samples=1), 6 (n=2), 9 (n=5) and 10 (n=2) were detected in samples that had DNA amounts lower than the a-priori minimum level of $20x10^6$ normal nucleotides. Repeated analysis of the same samples, however, with increased DNA concentrations did not show the previously mentioned adducts and these DNA adducts were therefore not included. In Table 4.5-2, Spearman correlation coefficients for the four remaining adducts (1,2,3, and 11) and mean relative

adduct labeling levels (RAL x 10^7) are shown. Mean RAL levels were inversely related to the proportion of positive samples. Significant correlation coefficients were observed between adducts 1 and 3 (r=0.52, p=0.0001) and adducts 2 and 11 (r=0.32, p=0.02).

Table 4.5-2 Spearman correlation coefficients (and p-values) between identified DNA adducts and mean relative adduct labeling (RAL x 10^7) among rubber manufacturing workers (n=52).

Adduct No.	1	2	3	11
2	0.23 (0.11)			
3	0.52 (0.0001) [a]	0.25 (0.08)		
11	0.08 (0.55)	0.32 (0.02) [a]	0.14 (0.33)	
N [b]	41	13	29	2
Mean (sd) [c]	2.85 (2.99)	5.77 (5.17)	3.68 (3.42)	12.55 (5.30)

a) Correlation coefficient significant at the 0.05 level
b) Number of subjects tested positive for a particular DNA adduct
c) Mean RAL and standard deviation of the positive DNA samples for a particular DNA adduct

In Table 4.5-3, median and mean RAL levels and proportion of positive samples for DNA adducts by the a-priori defined exposure groups are presented. No significant differences in RAL or the proportion of positive samples were observed between the three a-priori defined exposure groups.

Table 4.5-3 Median and mean relative adduct labeling levels (RAL x 10^7) and proportion of positive samples stratified by the a-priori defined exposure groups.

Exposure group [a]	Adduct 1 (n=41)				Adduct 2 (n=13)			Adduct 3 (n=29)			Adduct 11 (n=2)		
	N	Median	Mean	% [b]	Median	Mean	% [b]	Median	Mean	% [b]	Median	Mean	% [b]
LL	18	1.15	2.58	83.3	0.00	0.26	22.2	0.40	2.15	55.6	0.00	0.49	5.6
HL	18	1.38	1.32	72.2	0.00	0.74	27.8	0.08	1.42	50.0	-	-	0.0
HH	16	1.98	2.19	81.3	0.00	2.49	25.0	1.15	1.57	62.5	0.00	1.02	6.3

a) A-priori defined exposure groups: [LL] potential low TSPM mutagenicity and low surface mutagenic exposure levels; [HL] potential high TSPM mutagenicity and low surface mutagenic exposure levels; [HH] potential high TSPM mutagenicity and high surface mutagenic exposure levels.
b) Percentage of positive samples of DNA from exfoliated bladder cells

Possible influence of potential confounders or effect-modifiers on RAL levels or the proportion of positive samples was studied by linear and logistic regression, respectively. Age, alcohol- and roasted meat consumption, urinary-pH and -cotinine concentration and levels of normal nucleotides were not associated with the observed RAL levels or frequency of the four major adducts. A consistent, moderate increase in the

proportion of positive samples for all four identified DNA adducts was observed for subjects with a fast *N*-acetylation status (Figure 4.5-1). The observed difference was statistically significant for adduct 1 (p=0.04) but non-significant for the other adducts, presumably due to the limited number of positive samples. CYP1A2 activity was not related to the proportion of positive samples or RAL levels of DNA adducts. Several quantitative external and internal exposure indices, which were independent of the initial grouping scheme were consequently studied in relation with the observed RAL levels (Table 4.5-4). Personal inhalable particulate, inhalable CSM and dermal CSM exposure indices were not related with the observed DNA adducts, except for inhalable CSM exposure and adduct 3 (r=0.31, p=0.03). No consistent patterns could be discerned.

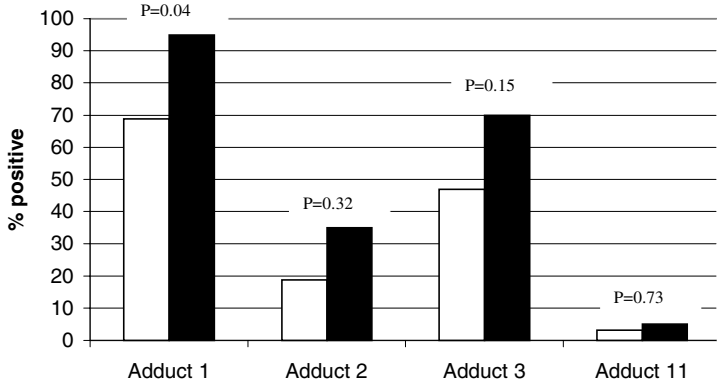

Figure 4.5-1 Proportion of positive samples of DNA from exfoliated urothelial cells by *N*-acetyltransferase activity. □, slow *N*-acetylation phenotype (n=32); ■, fast *N*-acetylation phenotype (n=20). Fisher exact p-values given for the difference in proportion of positive samples between slow and fast acetylators.

Table 4.5-4 Spearman correlation coefficients (and p-values) for several exposure indices and urothelial DNA adducts among rubber manufacturing workers (n=52).

Adduct No.	Inhalable dust	Inhalable CSM	Dermal CSM	Urinary mutagenicity
1	0.02 (0.91)	0.06 (0.69)	0.17 (0.23)	0.06 (0.65)
2	0.16 (0.25)	0.21 (0.13)	0.08 (0.58)	0.17 (0.25)
3	0.14 (0.32)	0.31 [a] (0.03)	0.06 (0.67)	0.04 (0.80)
11	-0.02 (0.91)	0.23 (0.11)	0.09 (0.55)	0.04 (0.78)

a) Correlation coefficient significant at the 0.05 level

Figure 4.5-2 shows the distribution of the proportion of positive DNA samples by production function. Samples from workers involved in 'mixing' had the highest proportion of positive samples for DNA adducts followed by the production function 'curing' and 'miscellaneous'. The production functions 'mixing' and 'curing' were investigated further as these production functions differ substantially from each other and the remaining production functions regarding chemical nature of and level of exposure.

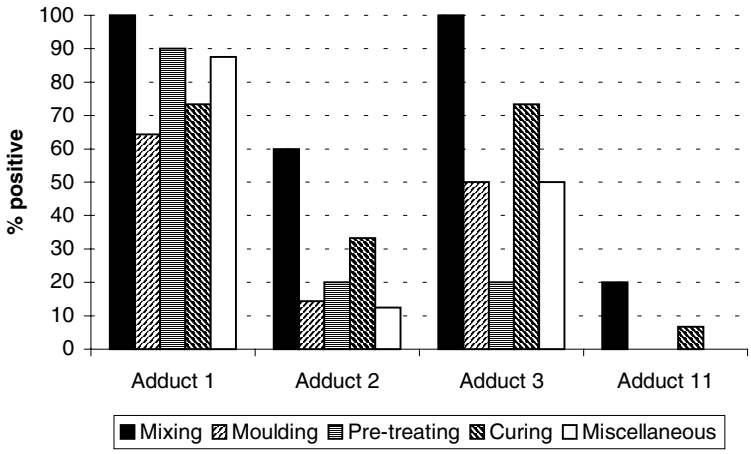

Figure 4.5-2 Proportion of positive samples of DNA from exfoliated urothelial cells by production function. The number of samples obtained from each function are: mixing, n=5; Moulding, n=10; Pre-treating, n=14; Curing, n=15; Miscellaneous, n=8.

Table 4.5-5 Median and mean relative adduct labeling levels (RAL x 10^7) and proportion of positive samples stratified by production functions 'mixing' and 'curing'. # p<0.10; * p<0.05.

Production function		**Adduct 1** (n=41)			**Adduct 2** (n=13)			**Adduct 3** (n=29)		
	N	Median	Mean	% [a]	Median	Mean	%	Median	Mean	%
Mixing	5	1.4	2.3	100	1.4 * [b]	5.0	60.0 # [c]	4.1 * [b]	3.8	100 * [c]
Curing	15	2.6	2.0	73.3	0	1.4	33.3	1.9 * [b]	2.4	73.3 # [c]
Other	32	1.1	2.0	81.3	0	0.4	15.6	0	1.1	40.6

a) Percentage of positive samples of DNA from exfoliated bladder cells
b) Relative adduct labeling levels in the production functions 'mixing' and 'curing' versus the 'other' production functions were evaluated by the Wilcoxon rank sum test
c) Fisher exact p-values for difference in proportion of positive samples between the production functions 'mixing' and 'curing' versus the 'other' production functions

At least, estimated mean personal inhalable dust levels were significantly higher in 'mixing' than in the other production functions (Wilcoxon, p=0.035) and rubber fume exposure quantified as CSM exposure was significantly higher in 'curing' than in the other

production functions (Wilcoxon, p=0.0006). DNA adduct 11 was not included in these analyses as the number of positive samples (n=2) was too small to enable any further statistical analyses. Stratified analysis by production function showed a significant difference in RAL levels and proportion of positive samples between the 'mixing' and 'other' production functions for both adduct 2 (p=0.03 and p=0.06, respectively) and adduct 3 (p=0.01 and p=0.02, respectively) (Table 4.5-5). For the production function 'curing' a statistical significant difference in RAL level and proportion of positive samples was only found for adduct 3 (p=0.03 and p=0.06, respectively). Further stratification by both production function and NAT2 phenotype produced statistically significant trends both in the proportion of positive samples and median RAL for adducts 2 and 3 within 'mixing' and 'curing' (Table 4.5-6).

Table 4.5-6 Trends in median and mean relative adduct labeling levels (RAL x 10^7) and proportion of positive samples stratified by production functions 'mixing' and 'curing' and N-acetylation status (NAT2).

NAT2	Production function		**Adduct 1** (n=41)			**Adduct 2** (n=13)			**Adduct 3** (n=29)		
		N	Median	Mean	% [a]	Median	Mean	%	Median	Mean	%
Fast	Mixing	1	1.0	1.0	100	5.6	5.6	100	4.1	4.1	100
Slow	Mixing	4	2.8	2.7	100	0.7	4.8	50.0	3.6	3.7	100
Fast	Other [b]	13	1.7	1.9	92.3	0	0.6	23.1	0.2	2.4	53.8
Slow	Other [b]	19	0.8	2.1	68.4	0	0.2	10.5	0	0.2	31.6
					P=0.062 [d]			P=0.020			P=0.009
Fast	Curing	6	2.9	3.2	100	1.4	1.8	50.0	3.3	3.9	100
Slow	Curing	9	0.6	1.2	55.6	0	1.3	22.2	1.1	1.3	55.6
Fast	Other [c]	13	1.7	1.9	92.3	0	0.6	23.1	0.2	2.4	53.9
Slow	Other [c]	19	0.8	2.1	68.4	0	0.2	10.5	0	0.2	31.6
					P=0.520			P=0.066			P=0.008

a) Proportion of positive samples
b) Excluding subjects employed in the production function 'curing'
c) Excluding subjects employed in the production function 'mixing'
d) Exact p-value for trend (Cochran-Armitage test for trend) for percentage of positive samples

Overall, production functions 'mixing' and 'curing' (i.e. occupational exposure) seemed to be more important than NAT2 phenotype. Stratified analysis within the production function 'curing' revealed a moderate, but non-significant correlation between dermal CSM exposure and adducts 1 and 3 (r=0.45;p=0.09 and r=0.42;p=0.11). In contrast, for adduct 2 a moderate, but again non-significant correlation with inhalable CSM exposure was noted (r=0.43;p=0.11). Unfortunately, the number of subjects employed within the production function, 'mixing', were too small to explore possible relationships between quantitative external occupational exposure estimates and the detected DNA adducts.

Discussion

Excess risk of cancer of the urinary bladder in the rubber industry has been reported since the 1950's with SMR's ranging from 250 to 2000 in studies published until 1965 [2; 194]. The majority of the excess of urinary bladder cancer appeared attributable to antioxidants that contained a contamination of free naphthylamines, including β-naphthylamine, a known bladder carcinogen. These antioxidants were removed from the manufacturing process around 1950 [2]. However, several more recent epidemiological studies among workers with no recorded exposure to β-naphthylamine found a moderate excess risk of urinary bladder cancer with SMR's ranging from 159 to 214 [8; 9]. These results placed in doubt the exclusive link between β-naphthylamine exposure and urinary bladder cancer in the rubber manufacturing industry.

We studied urothelial carcinogen-DNA adducts levels in a population of non-smoking occupationally exposed rubber workers. Subjects were selected from a large cross-industry survey based on ambient mutagenic total suspended particulate matter and mutagenic surface contamination and history of non-smoking. The rationale for this selection was to increase contrast in both inhalable and dermal mutagenic exposure conditions, to evaluate specific exposure routes. In addition, the exposure group with low TSPM mutagenicity and low surface mutagenic exposure levels was intended to be used as an internal comparison group. Usually, an internal control group is desirable as long as there is a wide range of clearly identifiable exposures of interest within a work place, since selection bias and confounding are likely to be less of a problem than could be encountered when using an external comparison group [83]. However, external mutagenicity turned out not to be related to the observed DNA adducts. Therefore, the a-priori grouping scheme based on total dose measures and the internal comparison group were probably of limited value.

Four DNA adducts (adduct 1,2,3 and 11) were detected with prevalence rates of 79%, 25%, 56% and 3.8%, respectively. Relative adduct labeling levels were, however, low. As adduct 1 and 3 and adduct 2 and 11 were moderately correlated, the correlated adducts might reflect metabolites of the same compound or of compounds of similar exposure paths. Identified DNA adducts were not associated with the a-priori defined exposure groups or quantitative personal exposure estimates except for a significant correlation between inhalable CSM exposure and adduct 3, which probably can be attributed to chance. Only stratification by production function showed statistically significant different RAL levels and proportion of positive samples, with the production functions 'mixing' and 'curing' showing the highest DNA adduct levels. This trend was most pronounced for adducts 2, 3 and 11. The relationship between mixing and curing and adduct 1 was less clear and it can be argued that this particular DNA adduct might not be occupationally related although it was significantly correlated with adduct 3. The high prevalence of adduct 2 and 3 (25 and 56%, respectively) and the observation that

the adducts were found for all production functions suggested that the responsible exposures may be common and widespread in the rubber manufacturing industry, but occur more frequently and at higher levels in the mixing and curing departments.

Occupational exposure to chemical compounds in the rubber manufacturing industry essentially arises by handling of bulk raw materials and chemical additives and due to the generation of rubber fumes and gases because of high process temperatures during activities like curing. Highest exposure levels to specific chemicals are likely to occur in the mixing department where most handling, weighing and mixing of raw materials takes place and the curing department where the highest process temperatures are reached (e.g. generation of rubber fumes and gases). Indeed, significant higher inhalable dust levels were detected in the mixing department and for curing significant higher inhalable CSM exposure concentrations were observed. However, these exposure measures are non-specific and do not necessarily reflect variability in chemical-specific exposure levels. When these non-specific exposure measures are used across production functions and companies, misclassification due to differences in chemical composition of the complex mixture will obscure relations with DNA adducts. The numbers of workers in a production function in a company were too small to study the relation between external exposure measures and the observed DNA adducts for each department separately. Stratified analysis for the production function curing (across 7 companies) revealed nevertheless a moderate, but non-significant correlation between dermal CSM exposure and adducts 1 and 3 and likewise for adduct 2 with inhalable CSM exposure.

As no real unexposed control group was present in this study and no strong unambiguous associations were found with occupational exposure estimates, the observed increase in DNA adduct levels for the production functions 'mixing' and 'curing' have to be interpreted with caution. Although exposure to non-occupational (concomitant) genotoxic compounds cannot be ruled out it seems unlikely that these would have resulted in the consistent DNA adduct patterns observed for the several production functions, particularly in a group of non-smokers. Interestingly, the observed differences in DNA adduct levels by production functions were comparable to the distribution of the urinary bladder cancer risk in the past. Urinary bladder cancer risk was then observed in various work areas but mostly in the compounding and mixing department [3; 195], which might suggest that the observed DNA adducts in this study are related to exposure to one or more raw rubber chemicals or unknown contaminants.

Observed DNA adducts levels tended to be moderately increased for subjects with a fast *N*-acetylation phenotype. In a previous study we showed that both Sunday and weekday urinary mutagenicity levels were related to both dermal and inhalable exposure conditions. Furthermore, subjects with skin aberrations such as minor dermatitis and skin injuries like cuts and burns revealed higher levels of urinary mutagenicity, substantiating the importance of the dermal exposure route in this industry. Adduct levels were not correlated with urinary mutagenicity, but these two biomarkers integrate exposure over different periods; urinary mutagenicity being more responsive to daily

exposure variations and adduct levels integrating exposure over urothelial cell lifespan. In addition, levels of mutagens in urine were modulated by NAT2-dependent enzyme activity differently than were adduct levels, with slow acetylators having higher levels of mutagens in their urine [196]. The different modulation by *N*-acetylation activity and absence of a relationship between urinary mutagenicity and urothelial cell DNA adducts suggest that urinary mutagenicity may be due to, at least for a significant part, by different compounds or metabolites than the bulky DNA adducts detected with the ^{32}P-postlabeling method.

Fast-acetylation was marginally associated with an increased risk of urinary bladder cancer in workers exposed only to benzidine [197]. *N*-acetylation is normally considered as a detoxification step in arylamine biotransformation. However, benzidine is an aryldiamine and *N*-acetylation is required to produce the active *N*-hydroxy, N'-acetylbenzidine metabolite [198; 199]. Although, the participating rubber companies did not use benzidine, several other aryldiamines were widely used as antidegradants like for example, several derivates of *p*-phenylenediamine. These aryldiamines are generally considered to be non-mutagenic which could explain the discrepancy between urinary mutagenicity and observed DNA adduct levels [159].

In conclusion, this study showed low, but significantly increased levels of urothelial DNA adducts in certain groups of rubber workers. The study was too small to explore in depth possible relationships between external occupational exposure estimates and detected DNA-adducts within the identified high risk production functions 'mixing' and 'curing'. The potential ambiguous role of *N*-acetylation activity in relation to urinary mutagenicity and urothelial DNA adduct levels needs more clarification, however. Larger studies among rubber workers employed in these production functions with inclusion of an unexposed control group should be conducted to study in more detail the potential carcinogenicity of the exposures encountered in these work areas.

Acknowledgements

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Chapter 5
General discussion

In this chapter the main findings of this thesis are evaluated and methodological problems interfering with the interpretation of the results are considered. Finally, implications of the presented results for the rubber manufacturing industry and future (epidemiological) research are addressed.

General discussion

Introduction

The main objective of this study was to investigate the relevance of the dermal exposure route for genotoxic exposure and biological effects among rubber manufacturing workers. To this end, an industry-wide exposure survey comprising 225 subjects from 9 different rubber manufacturing companies was initiated.

Workers in the rubber manufacturing industry are exposed to a complex mixture of chemical compounds. The chemical complexity and unknown toxic properties of these exposures precludes selection and detailed chemical analysis of individual genotoxic components. A stepwise approach for the assessment of the potential carcinogenic risk of complex mixtures was used as an outline for this study [20]. In this approach non-chemical specific exposure estimates are used to characterize the exposure conditions. Four tiers were discerned in this study. In the first tier, personal exposure to inhalable particulates, inhalable cyclohexane soluble matter (CSM) and dermal CSM contamination was investigated. In the second tier, mutagenicity of ambient exposure measurements was used to estimate the genotoxic exposures encountered in the rubber manufacturing industry. The first two tiers were used to characterize the pattern and level of external exposure to the complex mixture. In the third tier, urinary mutagenicity was assessed to estimate the internal genotoxic dose. In the fourth and last tier DNA adduct levels in exfoliated urothelial bladder cells were studied as an early biological effect marker. Based on the results obtained in the third and fourth tier the relevance of the dermal exposure route was assessed.

In this chapter key findings of the study are critically discussed and will encompass choice of study design, selection of the study population and exposure assessment methods, which may have influenced the results of this study. Implications and recommendations for the rubber manufacturing industry and future epidemiological research are presented in the last part of this chapter.

Choice of study design

Selection of companies and production functions

For the purpose of this study, an industry wide exposure survey in 9 different rubber manufacturing companies was initiated. The selected companies formed a representative cross-section of the rubber manufacturing industry in The Netherlands (3 rubber tire, 5 general rubber goods and 1 retreading company) (For an overview see Table 2.2-1). All production functions (basic manufacturing operations), which can be distinguished in any rubber company, were included in the survey. These production functions were

Compounding and mixing, Moulding, Pre-treating, Curing, Finishing, Engineering service, Laboratory and Shipping. The inclusion of different rubber companies and all eight production functions enabled a complete overview of all production processes and related exposures that could be encountered in the present-day rubber manufacturing industry in the industrialized world.

Selection of the study population

In total 1,355 workers were employed in the selected companies at time of the survey, which comprised about 35% of the workers directly involved in the production process in the rubber manufacturing industry in The Netherlands in 1997. A random sample of the total workforce was selected (n=224), stratified by production function. For these subjects personal exposure to inhalable particulates, inhalable CSM and dermal CSM exposure was assessed. As only 15% of the total worker population in these companies participated in the survey there is a potential for selection bias. Although it cannot be ruled out, there were no indications during the fieldwork that this has occurred. Furthermore, for most small and medium-sized companies (n=5), a significant part of the workforce (>35%) was included in the survey, which will have limited the possibility of selection bias.

Based on the measured ambient mutagenic exposure conditions a-priori defined exposure groups were constructed and a selection of urine samples of smoking and non-smoking rubber workers (n=116) was analyzed for urinary mutagens. The rationale for this selection was to increase contrast in both inhalation and dermal mutagenic exposures in order to facilitate the disentangling of the inhalation and dermal exposure route. In addition, the exposure group with low total suspended particulate matter (TSPM) mutagenicity and low surface mutagenic exposure levels was intended to be used as an internal comparison group. The validity of the a-priori defined exposure groups and subsequently the selection of subjects will be discussed later. Of the 116 subjects who were selected in the third tier, 24h-urine samples of 60 non-smokers were selected in the fourth tier to study the possible presence of DNA adducts in exfoliated urothelial bladder cells. Non-smoking rubber workers were selected as main stream tobacco smoke exposure has been shown to induce DNA adducts in exfoliated bladder cells which migrate along the diagonal radioactive zone on the thin-layer chromatography (TLC) plates [200]. This strong diagonal zone of DNA adducts (background) would have made the identification of the relatively low levels of DNA adducts expected in this study extremely difficult. By a-priori exclusion of smokers a synergistic or additional effect of cigarette smoking on detected DNA adduct levels could not be investigated. However, in the literature no synergistic effects of smoking and occupational exposures on adduct formation in lymphocytes or exfoliated bladder cells have been described. Synergistic effects of smoking and occupational exposure on urinary mutagenicity, however, has been observed for several exposures [155; 201] and in several industries [202; 203] among which the rubber industry [17; 159]. A synergistic effect of smoking and occupational exposure on

urinary mutagenicity might be due to induction of enzymes involved in the mutagenic bioactivation or due to oral absorption of mutagens by a finger-shunt effect [155]. The results of our study, however, did not show any synergistic effect of smoking on the observed urinary mutagenicity levels. Adjustment of urinary mutagenicity for tobacco smoke intake based on urinary cotinine levels may, however, have controlled for the effect of induced enzyme systems and oral absorption of mutagenic compounds, masking a possible synergistic effect.

Exposure assessment methods

Selection of appropriate biomarker(s), as an index of exposure to carcinogens is difficult as complex mixtures frequently contain more than one carcinogen and in addition the concentration of the marker in the mixture often varies within workplaces and time. In most instances, the exact individual components of the complex mixture are unknown. In such exposure situations non-specific exposure parameters have to be used, which give an estimate of the integrated carcinogenic potency of the exposure. In this study the *Salmonella* microsome assay for detection of mutagens [204] and the ^{32}P-postlabeling method for detection of DNA adducts [205] were used. These markers integrate exposure to a variety of genotoxic agents into a single measurement.

Genotoxic exposures

Many carcinogens are genotoxic and mutations are likely to be involved in the initiation of carcinogenesis. Epigenetic carcinogens, which raise the incidence of cancer by action through non-genotoxic mechanisms on cell populations, which have been previously exposed to initiating doses of other carcinogens were outside the scope of this investigation. Mutagenic activity was assessed with the Ames *Salmonella* microsome assay [204]. Although various limitations might be encountered in detecting mutagens with the Ames assay it is generally considered a valuable tool in testing the genotoxic potency of complex mixtures [132]. Such limitations include interaction of extraction solvents with test compounds [206], toxicity of substances to bacteria and liver homogenates, competition of indirect mutagens for enzyme sites and inadequacy of the activation system to reflect the in-vivo situation [207; 208]. Despite these shortcomings, a high correlation between mutagenicity and carcinogenicity within chemical groups such as aromatic amines and polycyclic aromatic hydrocarbons (PAH) has been observed [209]. These chemical groups are also of main interest in the rubber manufacturing industry [8; 14; 15] and therefore the *S. typhimurium* mutagenicity test was deemed appropriate for the evaluation of genotoxic exposures in this industry.

Predominantly, low levels of mutagenic activity in rubber dust and fume samples were detected with *S. typhimurium* TA98 in the pilot study (Section 4.2). Use of more sensitive derivatives of TA98 revealed a high mutagenic activity in *S. typhimurium* strain YG1024, which has elevated levels of *O*-acetyltransferase activity while no mutagenic

activity could be detected in *S. typhimurium* strain YG1021, which has elevated levels of nitroreductase activity. These results were indicative for the presence of mutagenic aromatic amines in rubber dust and fume samples. However, as this observation was based on samples originating from the mixing and curing department of only one rubber tire company use of YG1024 throughout the study could have resulted in biased results towards aromatic amines. Therefore, *S. typhimurium* strain YG1041, which has elevated levels of both enzymes was used as screening strain in this study.

Biological effects

Chemical carcinogens or their metabolites can interact with macromolecules such as DNA, forming adducts [210]. Unless repaired, such DNA lesions may result in mutations at critical genomic sites during DNA replication, ultimately leading to cancer through the multistage process of carcinogenesis. Measurement of DNA adducts formed from a genotoxic agent is a measure of its initiating potential. The used ^{32}P-postlabeling assay has been shown to be a very sensitive method to detect DNA adducts induced by structurally diverse carcinogens, particularly bulky aromatics [211].

As described above the two non-selective methods that were used in this study, are able to detect a wide range of genotoxic compounds. However, both methods are less suitable for measuring the genotoxicity of *N*-nitrosamines [212; 213], which have been shown to be generated during and after curing of rubber products [214]. Estimates of genotoxic exposure and biological effects in this study have therefore limited significance for the possible genotoxic risk exerted by volatile *N*-nitrosamines in the rubber industry.

Exposure grouping

In the third tier of the study 116 subjects were selected based on ambient mutagenic TSPM and mutagenic surface contamination and smoking habits. The rationale for this selection was to increase contrast in both inhalation and dermal mutagenic exposure in order to facilitate the disentangling of the inhalation and dermal exposure route. In addition, the exposure group with low TSPM mutagenicity and low surface mutagenic exposure levels was intended to be used as an internal comparison group. Usually, it is desirable to select internal comparison groups because comparisons of exposed to non-exposed workers tend to be less influenced by selection bias and confounding than when an external comparison group is used [83].

The three a-priori defined exposure groups exhibited a significant difference in TSPM mutagenicity and mutagenic surface contamination (Table 4.4-1). The highest exposed subjects also revealed the largest increase in urinary mutagenicity during the working week, which was attributed to the mutagenic surface contamination levels (Figure 4.4-1). No relation was found between TSPM mutagenicity and urinary mutagenicity. However, further statistical analyses revealed a significant relation between urinary mutagenicity and both ambient and personal inhalable particulate mass. This suggested substantial

particle bound mutagenic activity that was not detected in extracts of TSPM samples on which the a-priori exposure categorization was based. Therefore, the a-priori defined exposure groups might have had limited value with regard to airborne mutagenic exposure conditions.

Frequency and timing of sampling

Knowledge of toxicokinetics is important for determining the frequency and timing of biological sampling, and for selection of fluids most appropriate for study [215]. Due to the unknown chemical composition of the exposure, the toxicokinetics involved were unknown. Therefore spot urine samples collected at the end of the workshift on Wednesday and Thursday were pooled in order to collect a sample that would reflect occupational exposures with a range of half-lives. As a consequence ambient external exposure samples were pooled before analyses and personal exposure estimates were averaged to derive mean exposure estimates, which were intended to be used to explain the change in urinary mutagenicity during the working week relative to Sunday. In this study design, subjects function as their own reference without the need to control for factors like smoking and dietary habits. Underlying assumption in this approach is that behavioral patterns with regard to these confounding factors are similar during the weekend and weekdays. This was shown not to be the case for main stream and environmental tobacco smoke intake, which was clearly higher during the weekend than during the working week (Figure 4.1-1). Therefore, urinary mutagenicity levels were corrected for tobacco smoke intake based on urinary cotinine concentrations. After this correction, urinary mutagenicity levels were comparable between smokers and non-smokers and an almost similar increase in urinary mutagenicity of respectively 39% and 34% was observed for smokers and non-smokers respectively in relation to occupational genotoxic exposures (Table 4.1-3).

Dermal exposure

Dermal exposure was assessed with a single pad sampler on the hand of preference. In a small study we showed that the use of more pad samplers on different areas of the body would not have resulted in another exposure classification than based on the one pad sampler on the hand of preference (Table 3.2-4). However, if significant differences in permeability of the skin exist between anatomical regions then CSM contamination at the lower wrist does not necessarily reflect total dermal uptake [125]. Surrogate skin techniques (e.g. placing a chemical collection medium against the skin supposedly mimicking the properties of the skin) like a pad sampler measures potential dermal exposure, which is by definition an overestimation of the actual dermal uptake [102; 113]. Use of other dermal exposure techniques (e.g. hand washing) or surrogate skin techniques with a larger surface area (e.g. gloves) would, however, have interfered with the dermal absorption of skin contaminants making simultaneous unbiased assessment of the internal dose impossible.

Evaluation of main findings

Genotoxic exposures

The presence of genotoxic compounds in the air and on possible contact surfaces, like machines and rubber products, which a worker would most likely touch during normal work practice, was demonstrated by the detection of substantial mutagenic activity in TSPM and in surface wipe samples (Section 4.3). Also, elevated levels of mutagens were detected in urine samples of rubber workers (Section 4.4). These elevated levels of urinary mutagenicity were related to the mutagenicity found on contaminated surfaces and to the airborne particulate mass. No relation was found between TSPM mutagenicity and urinary mutagenicity.

The mutagenic potency of the TSPM, surface wipe and urine samples are difficult to compare with those found in other studies because no other published study in the rubber industry used the *Salmonella* strain YG1041. In section 4.2 it was estimated that the strain YG1041 was about 5 to 30 times more sensitive in the detection of mutagenic activity in the collected TSPM samples than the conventional strain TA98. However, this observation was based on only 2 samples in which mutagenic activity could be detected with TA98. As these samples originated from only one production function of one company, the derived estimates of increased sensitivity have limited validity for other companies and other production functions. Hagiwara *et al.* [143] have shown that the increase in sensitivity of the YG1041 strain could vary from 1 to 1700 times for various mutagenic chemicals relative to TA98.

Biological effects

Four specific DNA adducts were identified in exfoliated urothelial bladder cells among non-smoking rubber manufacturing workers. The identified DNA adducts were prevalent and were observed in workers from all production functions but predominantly in workers in the production functions 'mixing' and 'curing'. No relations were observed between inhalable particulate, dermal CSM exposure, ambient- and urinary mutagenicity and the identified DNA adducts. Because no relations were found, it can be argued that genotoxic exposures through other exposure routes (e.g. gaseous phase or oral route) may have contributed to the identified DNA adducts. Yet, inhalable particulate, dermal CSM exposure and ambient and urinary mutagenicity are all non-chemical specific exposure estimates. Although the ^{32}P-postlabeling method as such is non-selective, the detected DNA adducts are in principle an addition product of a single agent and thus chemical specific. The absence of a relation between the non-chemical specific exposure estimates and DNA adducts might therefore not be surprising. However, as no really unexposed control group was included in the survey and no dose-response relationship was found between one or more exposure estimates and DNA adducts, it cannot be excluded that the observed DNA adducts are non-occupationally related. However, as this

is the first study to show DNA adducts in bladder cells of rubber workers, the results are not without significance. The DNA adducts were present at the highest levels in the mixing and curing departments suggesting that workers in these areas may be currently exposed to bladder carcinogens.

Studies in other occupational settings have shown that adducts are quantitatively related to genotoxic exposures. These studies were performed in occupational settings with known unique chemical exposures and previously identified DNA adducts, mostly PAH related [216]. However, even in these situations correlations between exposure estimates and DNA adducts are often weak [217]. Only, among benzidine exposed workers in India a significant correlation (r=0.59) between urinary mutagenicity and urothelial DNA adducts has been found [24]. However, these workers had extensive dermal and respiratory contact with excessive amounts of benzidine or benzidine-dyes. Therefore, the majority of urinary mutagens were benzidine related, which was demonstrated by the high correlation between benzidine metabolites and urinary mutagenicity (r=0.88). Genotoxic exposure levels in the rubber manufacturing industry are much lower and most likely due to their complex nature less dominated by a single compound. The absence of a relation between urinary mutagenicity and DNA adducts in exfoliated bladder cells might therefore not be surprising.

Relevance of the dermal route

The relevance of the dermal route for genotoxic exposure and biological effects among rubber manufacturing workers was estimated based on the relations found between inhalation and dermal exposure route estimates and the two end-points urinary mutagenicity and DNA adducts.

As no relations were observed between inhalable particulate, dermal CSM exposure and ambient mutagenicity levels on the one hand and the four identified DNA adducts on the other the relevance of the dermal exposure route for the observed biological effect could not be deduced. Although, animal experiments have shown that topical application of used gasoline engine oils containing PAH related carcinogens could lead to the formation of DNA adducts in critical internal organs [218; 219].

Mutagen levels in urine of rubber workers were clearly associated with mutagenicity of contaminated surfaces and the mass of airborne particulate exposure levels. The two exposure estimates were not interrelated (r= -0.13; p=0.21) and therefore evidently reflect different exposure routes. Estimation of the contribution of the two exposure routes showed that mutagenicity of contaminated surfaces contributed more to the observed urinary mutagenicity levels than the mass of the airborne particulate exposure levels, approximately by a factor of three. The importance of the dermal exposure route was underlined by the observation that subjects with an impaired skin barrier function revealed almost two times higher levels of urinary mutagens than subjects with an intact skin. Prevalence rates of these unfavorable skin conditions were high in this population

with 44% of the subjects diagnosed with major or minor hand dermatitis and/or traumata of the skin at time of the survey. Increase in urinary mutagenicity due to an impaired skin is therefore relevant for a large group of rubber workers.

The relation between urinary mutagenicity and contaminated surfaces is remarkable, as this exposure measure is a very indirect measure for dermal exposure. Actual personally measured dermal CSM exposure levels on the other hand did not reveal a significant association with urinary mutagenicity. The existence of mutagenic compounds on surfaces is probably more discriminatory than personally measured dermal CSM exposure in relation to urinary mutagenicity. Furthermore, due to the high day-to-day variability in personally measured dermal CSM exposure the dermal CSM exposure estimates might have led to attenuation of the exposure-response relation.

Already in the early eighties dermal absorption of genotoxic compounds in this particular industry was suggested by Falck *et al.* [17], Kilpikari *et al.* [18] and a few years later by Bos *et al.* [19]. In section 3.1 it was shown that personal dermal exposure levels have dropped in the same rate as the personal inhalable particulate exposure levels over the last decade (approximately 6-7% per year). Therefore, the suggestion that because of declining airborne exposure levels, the dermal exposure route is becoming increasingly more important [102; 103] does not hold true for the 7 longitudinally studied rubber manufacturing companies in this survey. As this is the only longitudinal dermal exposure survey to date, it is unknown whether this observation is relevant for other companies or industries as well. However, reducing the overall environmental contamination by control or elimination of the source of the contaminant is likely to result in a decrease in exposure through inhalation and dermal uptake [109; 220]. Although the downward trend in inhalable particulate and dermal contamination was similar, inhalable particulate and dermal CSM exposure levels were only moderately correlated ($r=0.22$; $p<0.001$) (Section 3.2). The low correlation between the inhalation and dermal exposure estimates has also large implications for previously conducted epidemiological research, which has focussed primarily on the inhalation route. Estimates of exposure through inhalation do not necessarily reflect the dermal exposure route, which could result in potentially severe misclassification of total exposure.

Influence of biotransformation polymorphisms

The observed levels of urinary mutagenicity and DNA adducts were both moderately modulated by NAT2-dependent enzyme activity. Slow acetylators revealed significantly higher levels of urinary mutagenicity, but in contrast borderline significantly lower levels of DNA adducts. The possible different modulation by *N*-acetylation activity and absence of a relationship between urinary mutagenicity and urothelial DNA adducts suggest that urinary mutagenicity was caused, at least for a significant part, by different chemical compounds or metabolites than the identified DNA adducts. Increased levels of urinary mutagenicity among slow-acetylators has been observed in several studies among workers exposed to aromatic amines [32; 33; 186]. The existence of mutagenic aromatic amine

exposure in the rubber manufacturing industry was also suggested in section 4.2 as mutagenic activity was exclusively observed in *S. typhimurium* strains with elevated levels of *O*-acetyltransferase activity (strain YG1041 and YG1024), which are highly sensitive to mutagenic aromatic amines [143]. Fast-acetylators revealed higher levels of DNA adducts in exfoliated urothelial bladder cells. So far increased levels of DNA adducts have been predominantly associated with slow-acetylation status [34-37], which in turn has been shown to be associated with an increased risk of urinary bladder cancer [38]. However, for exposure to some specific chemicals exceptions to this observation exist. For example, for workers exposed to benzidine fast-acetylation status has been shown to be associated with an increased risk of urinary bladder cancer [197]. Benzidine is a biphenyldiamine and although benzidine exposure in this industry can be excluded, several other aryldiamines are widely used as antidegradants in the rubber manufacturing industry like for example, several derivatives of *p*-phenylenediamines.

Conclusions and recommendations

Based on the results described in this thesis it was concluded that workers in the rubber manufacturing industry in The Netherlands are currently exposed to genotoxic compounds and that the dermal exposure route is of major importance for the uptake of these compounds. Whether this holds true for DNA adduct formation in the urinary bladder remains unclear. Exposure to arylamines is being suggested as of particular importance in this industry. Implications and recommendations based on the obtained results for the rubber manufacturing industry and future epidemiological research are described below.

The rubber manufacturing industry

Since 1982 the rubber industry has been listed by IARC as 'an exposure circumstance that entails exposures that are carcinogenic to humans' [7]. After this date, workplace improvements have been implemented to improve the working circumstances in the rubber industry. These improvements have resulted in a significant decrease in both the inhalable particulate [74; 156] and dermal CSM exposure levels over the last decades [74]. Whether the observed decline in exposure levels has also reduced the observed cancer risk in this particular industry is unknown. Nevertheless, because genotoxic compounds and biological effects are still being detected in the present-day rubber manufacturing industry [14; 168; 171; 196] additional reductions in exposure levels are a necessity to limit the potential cancer risks in the rubber industry. When reducing external exposure levels special attention must be given to the dermal exposure route as this route has been shown of particular importance, especially for workers with an impaired skin. The prevalence rate of an impaired skin barrier function as a result of major or minor dermatitis and/or skin injuries are high in this population, 7%, 28% and 17%, respectively. The most frequent occurring skin problem 'minor dermatitis' was found to

be strongly associated with moderate and frequent hand washing especially with industrial surfactants containing scrubbing particles. The awareness of skin problems and the importance of the dermal exposure route has to be disseminated also to the rubber workers, as it was shown that skin problems were often not recognized by the workers as an adverse health effect (Section 2.1). Furthermore, sensible hand washing practices have to be introduced, which minimize irritant and mechanical insults to the skin but still can be frequently used to minimize the residence time of the genotoxic contamination on the skin.

Future research

Epidemiological research in the rubber manufacturing industry has always been hampered by the absence of detailed exposure assessment [8]. In only two case-control studies and in two cohort studies exposure measures other than job categories or work areas were used [195; 221-223]. In a case-control study of lymphatic and haematopoietic cancers seventy job groupings were categorized independently by industrial hygienists as to estimated solvent exposure [222]. Exposure to polycyclic hydrocarbons, *N*-nitrosamines, carbon black and talc were independently categorized for the individual jobs in a case control study of stomach cancer [221]. In a cancer mortality study in the British rubber industry occupational exposure was estimated by the cumulative duration of employment in jobs associated principally with exposure to either dust, fumes and/or solvents [195]. In a recent cohort study among German rubber workers crude semi-quantitative exposure estimates for *N*-nitrosamines were used to study the relationship between this exposure and cancer mortality [223]. Still, none of these exposure estimates used in these studies were based on actual quantitative measurement data.

The study presented in this thesis identified elevated exposure to mutagenic compounds and elevated levels of DNA adducts in the present-day rubber industry. Indicators of the variability in these exposure estimates between companies and production functions were derived. However, exposure assessment in the rubber industry would benefit most if specific chemicals or groups of chemicals, which are related to the observed cancer risks would be identified. The selective response in the used *S. typhimurium* strains and the NAT2 modulation of urinary mutagenicity and DNA-adducts pointed at the possible importance of arylamine exposure in the present-day rubber industry. Future toxicological and occupational hygiene research should therefore focus on the further chemical identification of the mutagenic compounds and observed DNA adducts. Subsequently, these specific chemical exposures will have to be measured and their exposure affecting factors assessed. Based on the occurrence of these identified factors historical exposure estimates could be derived and incorporated in new epidemiological research. These estimates should comprise both the inhalation and dermal exposure route. In addition, inclusion of skin quality and biotransformation polymorphisms like NAT2 status is of particular importance.

The recently observed cancer risks in the rubber industry are moderate to low [8]. As both the inhalation and dermal exposure levels have decreased significantly in the last decade, the observed cancer risks probably have, at least to a certain extent, as well. Therefore new epidemiological studies should preferably rely on cancer incidence rather than on mortality statistics to increase the number of cases and therefore the statistical power of the study. Two prospective cohort studies among rubber workers have recently been initiated in the United Kingdom [187] and Germany [224]. These studies are an excellent opportunity to incorporate more biologically relevant exposure measures and genetic susceptibility markers to determine the role of specific exposures and polymorphisms in the aetiology of cancer in this industry.

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Summary

Epidemiological studies among workers employed in the rubber manufacturing industry have indicated a significant excess cancer risk in a variety of sites. Although, several associations between exposures and observed cancer risks have been hypothesized, it has in general not been possible to identify specific agents for the majority of observed cancer risks. In several studies, however, the importance of the dermal route has been advocated. The main objective of the study described in this thesis was to investigate the relevance of the dermal exposure route for genotoxic exposure and biological effects among rubber manufacturing workers. To this end, an industry-wide exposure survey comprising 225 subjects from 9 different rubber manufacturing companies was initiated.

Workers in the rubber manufacturing industry are exposed to a complex mixture of chemical compounds. In chapter 1, basic concepts in the evaluation of exposure to complex mixtures of potentially carcinogenic chemicals are introduced. As the chemical composition and toxic properties of the complex mixture in the rubber industry is largely unknown non-specific exposure estimates were used in this survey. In the evaluation of potentially carcinogenic chemicals these estimates are usually derived from techniques that make use of the notion that one step in carcinogenesis is damage to or mutation in DNA.

The study described in this thesis can be divided according to the three major research questions formulated in chapter 1. Namely:

- to evaluate current inhalable particulate and dermal exposure levels in the rubber manufacturing industry in The Netherlands (Sections 3.1 & 3.2);
- to characterize external mutagenic exposure conditions in the rubber manufacturing industry in The Netherlands (Sections 4.2 & 4.3);
- to study the relation between inhalable particulate matter, dermal exposure levels, urinary mutagenicity and DNA adducts in exfoliated urothelial bladder cells (Sections 4.4 & 4.5).

Based on these research questions the relevance of the dermal exposure route for genotoxic exposure and biological effects was eventually estimated. As a decrease in the barrier function of the skin could lead to an increase in dermal uptake, adverse skin conditions and possible workplace related and personal factors were studied as well. In Section 2.1, we evaluated the applicability of a previously validated symptom-based questionnaire on hand dermatitis. The assessment of skin complaints based on the questionnaire was compared to a medical evaluation performed by a dermatologist. The 2 different diagnostic tools used for assessing hand dermatitis resulted in dissimilar estimates of the prevalence of active dermatitis, ranging from 6.9% to 38.1% of all workers (n=202). Using the medical evaluation as ‘gold standard’ we observed a moderate

sensitivity (71.4%) and specificity (76.1%), for the classification based on the self-administered questionnaire. Based on these results it was concluded that the use of a symptom-based questionnaire to assess skin complaints in a population of rubber workers was questionable. As a consequence, only the diagnosis of skin complaints made by the dermatologist was used in subsequent analyses. Prevalence of 'major' hand dermatitis (7%) among rubber workers was comparable with that in the general population, 'minor' signs of dermatitis, however, were more common among the surveyed population (28%) as were traumata's of the skin (17%) (Section 2.2). Dermal exposure to cyclohexane soluble matter (CSM) during work was related to the occurrence of 'major' hand dermatitis, but not to the occurrence of 'minor' hand dermatitis. Moderate and frequent hand washing especially with industrial surfactants containing scrubbing particles were found to be strongly associated with the occurrence of 'minor' dermatitis (OR=4.27; 95% C.I. 0.90 – 20.27 and OR=6.38; 95% C.I. 1.33 – 30.17, respectively).

Seven out of the nine companies, involved in the present study were previously surveyed in 1988. In both surveys sampling and analytical methodologies were kept identical which enabled the identification of exposure trends in both inhalable and dermal exposures (Section 3.1). In addition, changes in working organization and control measures taken after 1988 were identified and their effectiveness in reducing personal exposure levels were evaluated. Comparison of the exposure levels between 1988 and 1997 revealed a reduction rate of 5.7% and 6.7% per year for inhalable particulate and dermal exposure, respectively. Companies and production functions with the highest exposure levels in 1988 and workers with seniority (more experience) showed a steeper decline in exposure levels. Fifty-seven control measures, mostly designed to control the levels of inhalable exposure were identified. Using this information 80% of the historical decline in mean inhalable dust concentrations was explained. For dermal exposure, only 30% of the decline was explained by these factors. Elimination of the source was proven to be the most effective control measure for both inhalation and dermal exposures, reducing them by 67% and 66%, respectively. Control measures designed to control the levels of contaminants were somewhat less effective, but still were associated with statistically significant exposure reductions of 34% and 49%, respectively. Surprisingly, measures aimed at reduction of emission did not appear to contribute in general to a decrease in exposure levels. The results indicated that efforts taken to improve work conditions in the rubber manufacturing industry in The Netherlands between 1988 and 1997 had successfully reduced both inhalable particulate and dermal contamination.

In section 3.2, we applied a conceptual model for dermal exposure (see appendix) to identify compartments and mass transport processes relevant for dermal exposure in the rubber manufacturing industry. Identification of the spatial distribution of the dermal contamination among ten rubber workers showed high CSM surface concentrations for the upper body. Moreover, because of the high correlation between dermal exposure at the wrist and calculated total body exposure ($r=0.89$, $p<0.01$) an exposure assessment strategy based on only one pad sampler was subsequently employed to estimate CSM

surface concentrations in the skin contaminant layer of the total population of rubber workers. Qualitative and quantitative evaluation of the relevant compartments and related mass transport processes demonstrated the importance of deposition of airborne contaminants and direct transfer of contaminants from sources and surfaces to the skin contaminant layer. Interestingly, the importance of the different exposure pathways varied considerably between production functions.

Lifestyle factors such as smoking have been shown to influence urinary mutagenicity. Therefore, these factors have to be carefully considered when evaluating occupational genotoxic exposures. In section 4.1, we investigated day-to-day variability in active and passive tobacco smoke intake by studying urinary cotinine levels and determined their influence on observed urinary mutagenicity. Urinary cotinine was assessed for a sub-population of smoking and non-smoking rubber workers (n=105) on Sunday, Wednesday and Thursday. A sharp decrease in urinary cotinine concentration was observed during the week compared to Sunday for smokers (39%, $p<0.01$) and non-smokers (23%). Regression-coefficients for urinary cotinine and urinary mutagenicity, measured by the *S. typhimurium* strain YG1041 with metabolic activation, were similar for the Sunday and pooled weekday urine samples (Wed. and Thurs.). The derived estimates were subsequently used to adjust urinary mutagenicity for tobacco smoke intake. Cotinine adjusted urinary mutagenicity levels were comparable between smokers and non-smokers and a similar increase in urinary mutagenicity of respectively 39% and 34% was observed for both smokers and non-smokers due to occupational mutagenic exposures or other changes in lifestyle factors. The results indicated that adequate adjustment for daily tobacco smoke exposure is a necessity when using the urinary mutagenicity assay to evaluate possible mutagenic exposures in the workplace.

In section 4.2 mutagenic activity of ambient rubber dust and fume exposure in the mixing and curing department of two rubber tire companies situated in The Netherlands and Sweden were investigated. *S. typhimurium* strains YG1021, YG1024 and YG1041 were used to study the possible presence of mutagenic nitroarenes and aromatic amines. A large difference in mutagenic activity was found between the two companies. While the rubber tire company situated in The Netherlands revealed overall high mutagenic activity of rubber dust and fumes in the mixing and curing departments, respectively 430 and 279 rev/m^3 (YG1041), the Swedish company showed almost no mutagenic activity, respectively 18 and 54 rev/m^3 (YG1041). Further identification of the mutagenic profile showed that mutagenic activity was exclusively observed in *S. typhimurium* strains with elevated levels of *O*-acetyltransferase activity (YG1041 and YG1024) in the presence of a metabolic active liver S9 fraction, possibly indicating the presence of indirect mutagenic aromatic amines.

In section 4.3 mutagenic exposure conditions in eight production functions of several general rubber goods (n=5), rubber tire (n=3) and retreading companies (n=1) in The Netherlands were studied. Large differences in median mutagenicity of total suspended

particulate matter (TSPM) samples were observed between companies (range 49-1056 rev/m^3) and to a lesser extent between production functions (range 129 - 402 rev/m^3). 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 – 665 rev/cm^2. Mixing had the largest proportion of positive samples resulting in a median surface mutagenic contamination of 39 rev/cm^2. 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 exposure 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 indicated 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 skin contact surfaces supported the potential importance of the dermal route in the uptake of genotoxic compounds of workers in the rubber manufacturing industry.

In section 4.4, the relation between airborne particulate and dermal mutagenic exposure levels and mutagens in urine of rubber workers (n=105) were studied to determine the relevance of the inhalable and dermal exposure route. In addition, the potential influence of skin aberrations and biotransformation polymorphisms (NAT2 and CYP1A2) on urinary mutagenicity levels was addressed. A significant increase of 1605 rev./g. creat. in urinary mutagenicity during the working week relative to Sunday was observed for the total population (*t*-test, p=0.08). The increase in urinary mutagenicity was most pronounced among technical engineers (+4196 rev./g. creat.; *t*-test, p=0.08) and subjects with potential high TSPM and surface mutagenic exposure levels (+3206 rev./g. creat. ;*t*-test, p=0.12). Subsequent multivariate regression analyses revealed statistically significant associations between the mutagenic activity of potential contact surfaces, inhalable particulate exposure, skin aberrations and slow acetylation phenotype and weekday urinary mutagenicity. Similar trends were observed with Sunday urinary mutagenicity levels except for slow-acetylation phenotype that only resulted in increased weekday urinary mutagenicity levels. Surface mutagenic exposure levels were estimated to increase weekday urinary mutagenicity with about 67%, while inhalable particulate exposure resulted in an increment in weekday urinary mutagenicity levels of about 23%. These results suggested that the dermal exposure route contributed more to urinary mutagens of rubber workers than the inhalable route. Especially since subjects with skin aberrations revealed an additional increase in weekday urinary mutagenicity of about 40%.

In section 4.5, we investigated the occurrence of DNA adducts in exfoliated urothelial bladder cells of currently exposed, non-smoking rubber workers (n=52) and their relationship with occupational exposure estimates and biotransformation phenotypes. Four DNA adducts were identified with the proportion of positive samples ranging from

3.8% to 79%. The highest proportion of positive samples and the highest relative adduct labeling (RAL) levels were in workers involved in the production functions 'mixing' and 'curing', areas with potential for substantial exposure to a wide range of compounds used in rubber manufacturing ($p < 0.05$ for adducts #2 and/or #3, compared to all other departments). However, no significant relationships were found between identified DNA adducts and non-specific personal inhalable and dermal CSM exposure estimates or urinary mutagenicity. Interestingly, subjects with a fast NAT2 acetylation phenotype tended to have higher levels of DNA adducts. Aryldiamines, common in mixing and curing, might represent a potential exposure consistent with these data. This study suggested that workers in mixing and curing are exposed to compounds that can adduct DNA in urothelial cells.

In conclusion, results of this study provided evidence of genotoxic exposures in the rubber manufacturing industry and biological effects among rubber workers. Four possibly, work-related DNA adducts were identified in exfoliated urothelial bladder cells in certain groups of non-smoking rubber manufacturing workers. The identified DNA adducts were prevalent and were observed for all production functions but occurred predominantly in the production functions 'mixing' and 'curing'. The presence of genotoxic compounds in the air and on contact surfaces was demonstrated by the detection of substantial mutagenic activity in TSPM and in surface wipe samples. Subsequently, elevated levels of mutagens were detected in urine samples of rubber workers. These elevated levels of urinary mutagenicity were related to the mutagenicity of skin contact surfaces and airborne particulate exposure levels. In addition, increased levels of urinary mutagens were observed for rubber workers with an impaired skin barrier function and slow-acetylation status. Prevalence rates of these unfavorable skin and biotransformation conditions are high in this population, 40% and 60%, respectively. Therefore increase in urinary mutagenicity levels due to these factors are of real significance for a large group of rubber workers. The potential ambiguous role of *N*-acetylation activity in relation to urinary mutagenicity and urothelial DNA adduct levels, however, needs more clarification.

Samenvatting

De Nederlandse samenvatting is geschreven voor een ieder die geïnteresseerd is in het onderzoek beschreven in dit proefschrift.

In buitenlands epidemiologisch onderzoek onder werknemers in de rubberverwerkende industrie is meerdere malen een oversterfte geconstateerd aan diverse vormen van kanker. Welke stoffen in deze bedrijfstak verantwoordelijk zijn voor het verhoogde risico op kanker van onder andere de blaas en longen is niet met zekerheid te zeggen. De IARC gaat vooralsnog (veiligheidshalve) dan ook niet verder dan de gehele bedrijfstak als kankerverwekkend te kwalificeren.

Opname van chemische stoffen gebeurt in het algemeen via de ademhalingswegen, ingestie en door de huid. Het is tot nu toe onbekend of de opname via de huid (dermale route) van mogelijke kankerverwekkende stoffen een werkelijke risicofactor is voor werknemers in de rubberverwerkende industrie. In het onderzoek beschreven in dit proefschrift is gekeken naar de relatieve bijdrage van de dermale route voor de opname van genotoxische stoffen en mogelijk biologische effecten. Genotoxische stoffen zijn stoffen die in staat zijn mutaties in het erfelijk materiaal (DNA) te veroorzaken. Om dit te kunnen bestuderen is in 1995 begonnen met een vooronderzoek in twee rubberbandenfabrieken waarna in 1997 een grootschalig onderzoek is gestart onder 225 mensen werkzaam in 9 verschillende Nederlandse rubberverwerkende bedrijven.

Werknemers in de rubberverwerkende industrie worden blootgesteld aan een complex mengsel van bekende en onbekende chemische stoffen. Bij het onderzoek naar blootstelling aan een complex mengsel van chemische stoffen, waaronder mogelijke kankerverwekkende stoffen wordt vaak gebruik gemaakt van zogenaamde niet-selectieve bepalingen. Met behulp van deze bepalingen tracht men een schatting te maken van de blootstelling aan of opname van een groep van chemische stoffen met overeenkomstige eigenschappen. Voor de niet-selectieve bepalingen, die in dit onderzoek gehanteerd zijn, is gebruik gemaakt van het feit dat één van de stappen in de ontwikkeling van kanker beschadiging van of mutatie in het DNA-materiaal is. Mutagene stoffen dienen om die reden dan ook als mogelijk genotoxisch te worden beschouwd. Als een vroegtijdig biologische effect is gezocht naar stoffen die binden aan lichaamseigen moleculen (adduct-vorming). Uitkomsten van dergelijke metingen geven niet alleen inzicht in de hoeveelheid stof die in het lichaam aanwezig is, maar ook in de effecten die ze teweeg brengen.

Het onderzoek in dit proefschrift kan beschreven worden aan de hand van drie primaire onderzoeksdoelen:

- het bepalen van de huidige inhaleerbaarstof en dermale blootstellingsconcentraties in de Nederlandse rubberverwerkende industrie (Sectie 3.1 & 3.2);

- het in kaart brengen van de mutagene blootstellingsconcentraties in de Nederlandse rubberverwerkende industrie (Sectie 4.2 & 4.3);
- Het bestuderen van de relatie tussen de inhaleerbaarstof, dermale blootstellingsconcentraties en mutagene stoffen in de urine enerzijds en bindingsproducten met DNA-moleculen (DNA-adducten) in blaascellen anderzijds (Sectie 4.4 & 4.5).

Op basis van deze drie onderzoeksdoelen is uiteindelijk de relatieve bijdrage van de dermale route voor de opname van genotoxische stoffen en biologische effecten afgeleid. Aangezien een verminderde barrièrefunctie van de huid mogelijk tot een verhoogde opname van chemische stoffen kan leiden is tevens gekeken naar het voorkomen van huidaandoeningen in de geselecteerde onderzoekspopulatie.

Bij het stellen van de diagnose 'handeczeem' kan gebruik gemaakt worden van verschillende onderzoeksmethodieken. In sectie 2.1 is de diagnose 'handeczeem' gebaseerd op een gestandaardiseerde vragenlijst vergeleken met de diagnose zoals die is gesteld door een dermatoloog. Helaas bleek de diagnose op basis van de vragenlijst slechts een beperkte validiteit te hebben (sensitiviteit 71.4% en specificiteit 76.1%). In het vervolg van het onderzoek is daarom alleen gebruik gemaakt van de diagnose zoals die is gesteld door de dermatoloog. Bij 7% van de werknemers in de rubberverwerkende industrie is ten tijde van de studie handeczeem ('major' dermatitis) geconstateerd. Schattingen van de prevalentie van handeczeem in de algemene bevolking variëren van 2 tot 10%. Dit is vergelijkbaar met het percentage werknemers met handeczeem in de rubberindustrie. Geringe huidaandoeningen ('minor' dermatitis), die beschouwd kunnen worden als een voorstadium van eczeem, en verwondingen, meestal van geringe omvang, zijn regelmatig bij de onderzochte populatie geconstateerd, respectievelijk bij 28 en 17% van de onderzoekspopulatie (n=202) (Sectie 2.2). Het voorkomen van deze geringe huidaandoeningen is sterk gerelateerd aan het regelmatig of frequent wassen van de handen. Dit wordt versterkt als men hierbij gebruik maakt van zogenaamde schuurzepen. Deze zepen bevatten naast zeepbestanddelen harde stukjes om een extra schurende 'schoonmakende' werking te krijgen.

Van de 9 bedrijven die aan het onderzoek deelnamen hadden 7 bedrijven al in een eerder onderzoek (eind jaren tachtig) naar verbeteringen van arbeidsomstandigheden in de Nederlandse rubberverwerkende industrie geparticipeerd. Op basis van deze twee onderzoeken is een schatting gemaakt van de historische blootstellingsdaling in deze 7 bedrijven. Inhaleerbaarstof- en dermale blootstellingsconcentraties zijn in de 9 tussenliggende jaren met ongeveer de helft teruggebracht (Sectie 3.1). Opmerkelijk is dat bedrijven en productiefuncties met de hoogste blootstellingsconcentraties in 1988 en werknemers met meer werkervaring een nog sterkere daling in de blootstellingsconcentraties vertonen. Doordat ook veranderingen in het productieproces en de getroffen beheersmaatregelen bekend zijn, is het mogelijk om de effectiviteit van deze maatregelen (in het totaal 57 beheersmaatregelen) te bestuderen. Op basis van deze informatie is 80% van de historische daling in inhaleerbaarstof concentraties verklaard.

Voor de huidblootstelling kon echter slechts 30% van de daling op basis van de getroffen beheersmaatregelen worden verklaard. Verwijderen van de blootstellingsbron is de meest effectieve manier om zowel de inhaleerbaarstof en dermale blootstelling te verlagen, respectievelijk met 67 en 66%. Beheersmaatregelen gericht op het controleren van reeds ontstane emissies zijn minder effectief, maar nog steeds gerelateerd aan een significante daling in zowel de inhaleerbaarstof - als de dermale blootstellingsconcentraties (respectievelijk 34 en 49%). Op basis van de gevonden trends en de relaties met de getroffen beheersmaatregelen kan geconcludeerd worden dat pogingen om de blootstelling aan chemische stoffen in de Nederlandse rubberindustrie te reduceren geresulteerd hebben in een verlaging van zowel de inhaleerbaarstof als dermale blootstellingconcentraties.

Huidblootstelling kan het gevolg zijn van verschillende processen zoals bijvoorbeeld direct contact met chemicaliën of door depositie van stoffen op de huid. In sectie 3.2 is op basis van een conceptueel model (zie bijlage) geprobeerd inzicht te krijgen in de bijdrage van de verschillende massatransportprocessen (depositie, direct contact etc.) aan de huidblootstelling in de rubberverwerkende industrie. Aangezien de blootstelling op de huid vaak niet gelijkmatig is verdeeld over het gehele lichaam is tevens gekeken naar de verdeling van deze blootstelling over het lichaamsoppervlak. De hoogste huidblootstellingsconcentraties zijn gevonden op meerdere delen van het bovenlichaam. Bovendien blijkt de huidblootstelling aan de onderarm sterk gerelateerd te zijn aan de geschatte totale huidblootstelling. Hierdoor is het mogelijk om voor deze blootstellingssituatie op basis van slechts één meting aan de pols een inschatting van de totale huidblootstelling te maken. De kwalitatieve en kwantitatieve evaluatie van de relevante massatransportprocessen onderstrepen het belang van zowel depositie van deeltjesvormige blootstellingen op de huid als het belang van direct of indirect huidcontact met de bron of besmette oppervlakken. De bijdrage en relevantie van de massatransportprocessen verschillen echter aanzienlijk tussen de onderzochte productiefuncties.

Persoonlijke leefgewoonten, zoals bijvoorbeeld roken, dragen bij aan de aanwezigheid van mutagene stoffen in de urine. Daarom moet bij het kwantificeren van de beroepsmatige bijdrage aan mutagene stoffen in urine rekening worden gehouden met verschillen in persoonlijke leefgewoonten en met mogelijke veranderingen van deze leefgewoonten in de tijd. In sectie 4.1 is de dag-tot-dag variantie in actieve en passieve rookgewoonten bestudeerd door het bepalen van de cotinineconcentraties in zowel zondag- als woensdag- en donderdagurine (verzameld na werktijd) van 105 werknemers. Cotinine is een van de belangrijkste afbraakproducten van nicotine, een bestandsdeel van sigarettenrook. Een sterke daling in cotinineconcentraties gedurende de werkweek ten opzichte van de zondag is waar te nemen voor zowel rokers (-39%) als niet-rokers (-23%). Tevens is een duidelijk relatie gevonden tussen de cotinineconcentraties van zowel de zondag- als het samengevoegde weekmonster (Wo. en Do.) en de mutageniteit van deze urinemonsters. Op basis van deze statistische relatie is vervolgens de mutageniteit van de urine gecorrigeerd voor de blootstelling aan sigarettenrook. Gecorrigeerde concentraties

aan mutagene stoffen in urine zijn vergelijkbaar voor rokers en niet-rokers. Tevens is een vergelijkbare stijging in de concentratie aan mutagene stoffen in de urine van de werkweek ten opzichte van de zondagurine gevonden voor zowel rokers als niet-rokers (respectievelijk +39 en +34%). Deze stijging in mutageniteit van de urine is waarschijnlijk het gevolg van beroepsmatige blootstelling aan mutagene stoffen op de werkplek.

Mutageniteit van rubberstof en dampblootstelling in de mengerij en vulkanisatieafdeling van twee rubberbandenfabrieken, één in Nederland en één in Zweden, staat beschreven in sectie 4.2. Bij het bepalen van de mutageniteit is gebruik gemaakt van verschillende bacteriestammen in de *Salmonella*/microsome test ('Ames' test), namelijk *S. typhimurium* YG1021, YG1024 en YG1041. Door gebruik te maken van bacteriestammen met een specifieke gevoeligheid voor een bepaalde groep mutagene stoffen kan een inschatting gemaakt worden van het soort mutagene stoffen waaraan werknemers mogelijk zijn blootgesteld. De bacteriestammen, die in deze studie gebruikt zijn hebben een specifieke gevoeligheid voor mutagene nitroarenen en/of aromatische amines. Een duidelijk verschil in mutageniteit van de blootstelling, gemeten met *S. typhimurium* YG1041, is zichtbaar tussen de Nederlandse en Zweedse fabriek voor zowel de mengerij (430 rev/m^3 versus 18 rev/m^3) als voor de vulkanisatieafdeling (279 rev/m^3 versus 54 rev/m^3). Mutagene stoffen in rubberstof en dampmonsters zijn alleen gedetecteerd met behulp van de *S. typhimurium*-stammen met een verhoogde activiteit van het enzym *O*-acetyltransferase (YG1041 en YG1024) in de aanwezigheid van een metabolisch activeringssysteem (S9-fractie). Deze resultaten wijzen op de aanwezigheid van aromatische amines met een indirecte mutagene werking in de gemeten rubberstof en dampblootstelling in deze twee rubberbandenfabrieken.

De aanwezigheid van mutagene stoffen in de lucht en op oppervlakken waarmee werknemers direct in aanraking komen tijdens normale werkzaamheden, is vervolgens onderzocht voor alle productiefuncties (n=8) in de negen deelnemende bedrijven van het hoofdonderzoek (Sectie 4.3). Een grote spreiding in de mutageniteit van het rubberstof en damp is gevonden tussen de verschillende bedrijven (spreiding 49 – 1056 rev/m^3) en in mindere mate tussen de verschillende afdelingen (spreiding 129 – 402 rev/m^3). Mutageniteit van de verschillende bemeten contactoppervlakken laten eveneens een grote spreiding zien (spreiding 26 – 665 rev/cm^2). De mutageniteitsconcentraties in de lucht en op oppervlakken, gemiddeld per afdeling per fabriek zijn slechts zwak aan elkaar gerelateerd ($r=0.28$, $p=0.05$). Bedrijf, productiefunctie en blootstelling aan de totaal oplosbare fractie verklaren respectievelijk 79 en 81% van de variabiliteit in de concentratie aan mutagene stoffen in de lucht en op mogelijke contact oppervlakken. Hierbij is vooral het bedrijf waarvan de monsters afkomstig zijn de meest bepalende factor. Dit duidt op het belang van bedrijfsspecifieke determinanten zoals productievolume en het gebruik van specifieke rubberchemicaliën voor de mutageniteit in de lucht en op mogelijke contact oppervlakken. Het detecteren van mutagene stoffen

op oppervlakken, waarmee de werknemers direct of indirect met de huid in contact komen, versterkt tevens het vermoeden dat de dermale blootstellingsroute van belang is in de rubberverwerkende industrie.

In sectie 4.4 is de mogelijke relatie tussen de blootstellingen aan mutagene stoffen en de aanwezigheid van deze stoffen in de urine van werknemers (n=105) in de rubberindustrie bestudeerd. Daarnaast is de mogelijke invloed van een verminderde huidkwaliteit en een snelle of langzame enzymatische omzettingscapaciteit (acetylering en *N*-oxidatie capaciteit) op de aanwezigheid van mutagene stoffen in de urine bekeken. Gemiddeld is voor alle werknemers een toename in de concentratie aan mutagene stoffen in de urine gevonden gedurende de werkweek ten opzichte van zondag (+1605 rev/g. creat.). Deze toename is het sterkst voor werknemers van de technische dienst (+4196 rev/g. creat.) en voor werknemers die blootgesteld zijn aan hoge concentraties aan mutagene stoffen in de lucht en op mogelijke contact oppervlakken (+3206 rev/g. creat.). Mutageniteit van de weekurine is sterk gerelateerd aan de mutageniteit van contactoppervlakken, inhaleerbaar-stofblootstelling, het hebben van huidaandoeningen en een langzame acetyleringscapaciteit. Voor de mutageniteit van de zondagurine zijn vergelijkbare relaties gevonden, alleen minder sterk dan voor de mutageniteit van de weekurine. Vermoedelijk worden de opgenomen stoffen slechts langzaam via de urine uitgescheiden. Dit is belangrijk aangezien gifstoffen meer schade kunnen aanrichten naarmate ze langer in het lichaam verblijven. Direct contact met mutagene stoffen resulteert in een toename van 67% in mutageniteit van de urine. Blootstelling aan mutagene stofdeeltjes resulteert in een toename van 23% in mutageniteit van de urine. Hieruit is afgeleid dat de dermale route waarschijnlijk de meest belangrijke blootstellingsroute is. Dit vermoeden wordt nog eens versterkt doordat werknemers met een slechtere huidkwaliteit een additionele toename in de mutageniteit van de urine van 40% vertonen.

In sectie 4.5 is de aanwezigheid van bindingsproducten met DNA (DNA-adducten) in blaascellen, die in de urine worden uitgescheiden onderzocht voor 52 niet-rokende werknemers. Vier DNA-bindingsproducten konden worden geïdentificeerd. Het hoogste percentages werknemers met DNA-adducten in blaascellen en de hoogste adductniveaus zijn gevonden voor de productiefuncties 'mengen' en 'vulkanisatie'. Er zijn echter geen relaties gevonden tussen het voorkomen van deze DNA-adducten en persoonlijke inhalatoire en dermale blootstellingen. De hoge prevalentie aan DNA-adducten, variërend van 3.8 tot 79% voor de verschillende DNA-adducten, is een aanwijzing dat de blootstelling algemeen voorkomt in de rubberverwerkende industrie, maar dat deze adducten zich voornamelijk manifesteren in de mengerij en vulkanisatieafdeling. Opmerkelijk is dat werknemers met een snelle acetyleringscapaciteit vaker hogere DNA-adductniveaus hebben dan werknemers met een langzame acetyleringscapaciteit.

Op basis van de resultaten van het totale onderzoek kan geconcludeerd worden dat werknemers in de rubberverwerkende industrie in Nederland blootgesteld zijn aan genotoxische stoffen. Ten eerste is de aanwezigheid van genotoxische stoffen in de werkomgeving aangetoond door de detectie van mutagene stoffen in rubberstof en damp

en op mogelijke contactoppervlakken. Daarenboven zijn verhoogde niveaus aan mutagene stoffen in de urine van werknemers gevonden. Ten tweede zijn vier mogelijk werkgerelateerde DNA-adducten geïdentificeerd in blaascellen van werknemers uit de rubberverwerkende industrie.

De relatieve bijdrage van de dermale route voor de opname van genotoxische stoffen en biologische effecten is afgeleid aan de hand van relaties die gevonden zijn met de mutageniteit in de urine. De verhoogde mutageniteitsniveaus in urine zijn gerelateerd aan de mutageniteit van contactoppervlakken en inhaleerbaarstofconcentraties. Gebaseerd op de gevonden relaties is geschat dat huidcontact met mutagene stoffen resulteert in een 3 maal hogere bijdrage aan de mutageniteit in urine dan blootstelling aan inhaleerbaarstof. Bovendien blijken werknemers met een verminderde huidkwaliteit een additionele toename van de mutageniteit in de urine te vertonen. Deze laatste bevinding is een extra aanwijzing voor het belang van opname van genotoxische stoffen via de huid in de rubberverwerkende industrie.

List of abbreviations

137X	caffeine
17X	paraxanthine
1X	1-methylxanthine
2-AF	2-aminofluorene
2-NF	2-nitrofluorene
95% CI	95% confidence interval
ACD	allergic contact dermatitis
AFMU	5-acetylamino-6-formylamino-3-methyluracil
ALARA	as low as reasonably achievable
AM	arithmetic mean
ANOVA	analysis of variance
ATP	adenosine 5′-triphosphate
B_g	James-stein estimator
BP	benzopyrene
CPM	counts per minute
Creat	creatinine
CSM	cyclohexane soluble matter
CYP	cytochrome P_{450}
CYP1A2	cytochrome P_{450} isoform CYP1A2
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acids
EIA	enzyme immunosorbent assay
ETS	environmental tobacco smoke
GM	geometric mean
GSD	geometric standard deviation
GST	gluthathione *S*-transferase
IARC	international agency for research on cancer
ICD	irritant contact dermatitis
IgE	immunoglobulin class E
Ln	natural logarithm
LOD	limit of detection
MBS	2-(morpholinothio) benzothiazole
MBTS	2,2-dibenzothiazyl disulphide
MS	mainstream tobacco smoke
NAT	*N*-acetyltransferase
NAT1	*N*-acetyltransferase type 1
NAT2	*N*-acetyltransferase type 2
NIOSH	National Institute for Occupational Safety and Health
NPV	negative predictive value
NR	nitroreductase
NRL	natural rubber latex
OAT	*O*-acetyltransferase
OD	optical density
OEL	occupational exposure limit
OPD	*O*-phenylenediamine
OR	odds ratio

OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbons
PAS6	Personal inhalable dust sampler with an inlet diameter of 6 mm
PBS	phosphate buffered saline
PPV	positive predictive value
r	correlation coefficient
r^2	explained proportion of total variation
RAL	relative adduct labeling
Range	minimum and maximum value
RE	relative exposure
rev	revertants
RPM	rounds per minute
S	*Salmonella*
S_9-mix	microsomal preparation of liver homogenate
S^2_{bw}	between-worker variance
S^2_{ww}	within-worker variance
SAS	Statistical Analysis System
SCE	sister chromatide exchange
SE	standard error
SMR	standard mortality rate
SD	standard deviation
TLC	thin-layer chromatography
TSM	total soluble matter
TSPM	total suspended particulate matter
TWA	time-weighted average
XAD-2	nonionic polymeric adsorbent
λ	ratio of within- to between-worker variance in log-transformed exposure

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Epilogue

The study described in this thesis is the result of 5 years of research in the rubber manufacturing industry in The Netherlands. Many persons have contributed to the project and without their valuable input the whole project would certainly have been less thriving.

First of all I would like to thank Hans Kromhout for giving me the opportunity to embark on this interesting research topic. Hans, you have been not only the best and most inspiring supervisor I could have wished for but next to that a good friend. Together we have had to take the necessary administrative hurdles that due to several reorganizations came on our path. In spite these difficulties we have managed to keep the project alive and made it to what it is. Next, I would like to thank Rob Bos for his enthusiasm and scientific advice during all this time and for giving me the opportunity to develop my interest in occupational toxicology. Bert Brunekreef is greatly acknowledged for his valuable comments on the various parts of this thesis.

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I am furthermore greatly indebted to the employers and employees of the rubber companies for their close co-operation in this study. I hope the results of this study will help to further improve working circumstances in the rubber manufacturing industry in The Netherlands in order to prevent possible negative health effects in the near future.

As mentioned before, this study was potentially threatened by several reorganizations during the course of the project. Starting of as a collaborative project of the Department of Air Quality at the Wageningen Agricultural University and the Department of Toxicology, Faculty of medical sciences at the University of Nijmegen, it ended as a

concerted project of the Environmental and Occupational Health Group, Institute of Risk Assessment Sciences, Utrecht University and the Department of Pharmacology & Toxicology, UMC St. Radboud, University of Nijmegen. This process included three reorganizations and the loss of two of the initial supervisors. It has become harshly apparent in the last few years that even within academia the process of continuous reorganization has found its way. Often these reorganizational decisions are made on a purely 'political' basis and not on scientific merits or social importance. Although, more flexibility within academia itself is to be welcomed, the influence of such administrative political decisions has to be critically watched.

Finally, I would like to thank Wieteke, my parents, Gert-Jan and Laura, friends and colleagues for their support during these years but more importantly for all the things but work.

About the author

Roel Vermeulen was born in Made, The Netherlands, on October 14th, 1970. After graduating from secondary school (Dongemond College in Raamsdonksveer) in 1989, he started with the M.Sc. program of Environmental Sciences at the Wageningen Agricultural University, Wageningen, The Netherlands. In 1995 he graduated with honors in Occupational and Environmental Health and subsequently started as a research assistant at the former department of Air Quality. Later that year he was appointed as a Ph.D.-fellow at the department of Occupational and Environmental Health, Wageningen Agricultural University (Since May 2000 located at Utrecht University). Besides the Ph.D.-fellowship he continued working as a research assistant in several national and international projects. In this capacity he was involved in subjective assessment of occupational exposures in several epidemiological studies, developed models for the evaluation of health risks due to occupational exposures, participated in the development of a statistical program for the evaluation of exposure data (SPEED) and organized two international short courses on 'Dermal Exposure and Risk Assessment'. From 1996 to 1999 he was a member of the Dermal Exposure Network (DEN), which was a network of identified experts from the European Union working in areas related to dermal exposure of workers to chemical substances.

In October 2000, he started as a research fellow at the Occupational Epidemiology Branch of the National Cancer Institute, Bethesda, Maryland, USA. His research activities will focus on the retrospective assessment of occupational exposures, with special reference to the dermal exposure route and susceptibility parameters in epidemiological cancer research.

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Appendix
Conceptual model for assessment of dermal exposure

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Dermal exposure, primarily to pesticides, has been measured for almost half a century. Compared with exposure by inhalation, limited progress has been made towards standardization of methods of measurement and development of biologically relevant exposure measures. It is suggested that the absence of a consistent terminology and a theoretical model has been an important cause of this lack of progress.

Therefore, a consistent terminology based on a multicompartment model for assessment of dermal exposure is proposed that describes the transport of contaminant mass from the source of the hazardous substance to the surface of the skin. Six compartments and two barriers together with eight mass transport processes are described. With the model structure, examples are given of what some existing methods actually measure and where there are limited, or no, methods for measuring mass in a compartment or transport of mass. The importance of measuring the concentration of contaminant and not mass per area in the skin contaminant layer is stressed, as it is the concentration difference between the skin contamination layer and the perfused tissue that drives uptake. Methods for measuring uptake are currently not available.

Measurement of mass, concentration and transport processes must be based on a theoretical model. Standardization of methods for dermal exposure measurement is strongly recommended.

Occupational & Environmental Medicine, 1999, 56, 765-773

Introduction

Exposure to hazardous substances most commonly occurs either by inhalation, ingestion, dermal contact, or some combination of these routes. Occupational hygiene has traditionally focused on exposure by inhalation because it was almost invariably considered to be the most important pathway. Many methods have been developed to measure exposure levels from inhalation and there is a clear understanding of how such levels should be interpreted to help reduce risk. The situation is less clear for the dermal route of exposure. Practical methods of measurement have been developed to assess dermal exposure [1] and proposals have been made to develop dermal exposure limits in an analogous way to those for inhalation exposure [2; 3]. However, there has been criticism of the existing methods of measurement of dermal exposure because they determine the mass of contaminant either depositing on the skin or retained on the skin at the end of the exposure period [1; 4].

Hazardous substances on the dermal surface will be taken up continuously into the body through the stratum corneum and the epidermis towards the dermis where they or their dermal metabolites will be removed by the blood flow. The transport process is driven by

the concentration gradient between the dermal surface and the perfused tissue. The risk arising from dermal exposure is thus firstly related to the time dependent concentration of a substance on the dermal surface rather than the mass of material on that surface at any given time. Mass is nevertheless important when there is a little material available for uptake.

Contamination of the skin may arise in many different ways. It is possible for hazardous substances to land on or be adsorbed into the skin directly from the air. They may be transferred to the skin from contact with contaminated surfaces or by submersion of part of the body into the substance. Also, the contaminant may be lost from the skin either by evaporation or some other mechanisms such as washing or abrasion, without being taken up into the body. Finally, the presence of clothing or protective garments may modify the rate at which hazardous substances come into contact with the skin. All of these processes are important to consider when making an assessment of dermal exposure and a complete understanding of these complex processes will help in developing an appropriate control strategy.

In this paper we have attempted to produce a consistent terminology for assessment of dermal exposure. The terminology is based on a conceptual model of the processes leading to exposure (from the source of a hazardous substance to the surface of the skin). We have also defined several terms related to exposure, which provide a valid basis for investigating the risks posed by dermal exposure. It has not been our intention to consider the process of uptake into the body or the derivation of dose estimates from dermal exposure, although others have developed models that could be used in such contexts [5; 6].

Conceptual model of dermal exposure

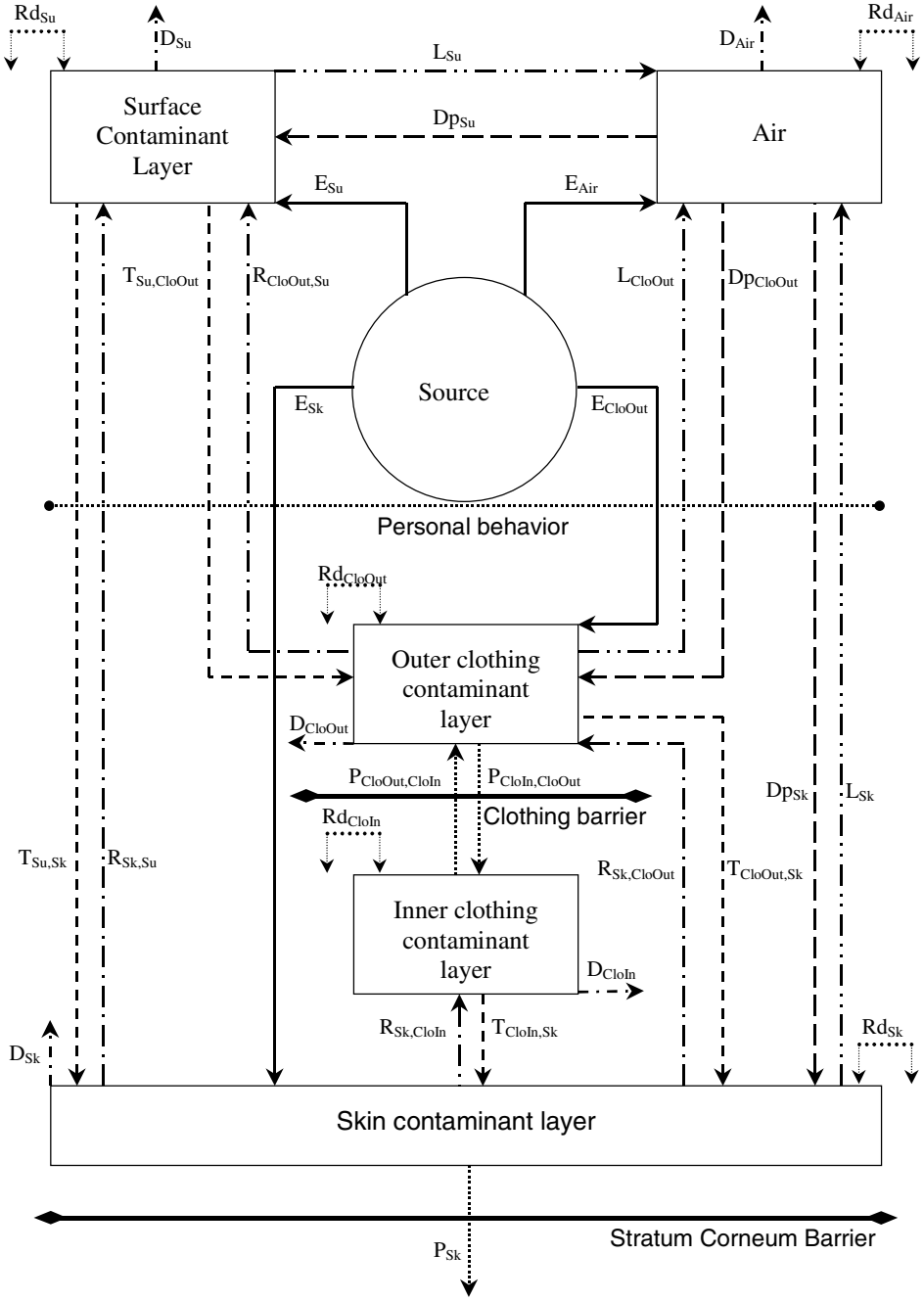
A consistent terminology has to be based on a coherent and systematic description of dermal exposure scenarios. A multicompartment model is an appropriate basis for a terminology as it comprises distinct physical objects or compartments connected by mass transport processes. Models of this type are concerned with what happens - for example, where fingers get contaminated by touching a surface and not why it happens – for example, particle adhesion.

Compartments

All compartments are assumed to be well and instantaneously mixed. As a result the concentration in a compartment is described by the amount of mass and distribution volume of the compartment. Six principle compartments are being distinguished in the model (Figure A-1):

Source (S) - Processes or activities, from which a mass is being introduced into any of the compartments, will be considered as sources.

Figure A-1 Overview of the conceptual model, compartments and rate constants.
(E=emission (———), Dp=deposition (– – – –), L=resuspension or evaporation (—·—·—),
T=transfer (- - - - -), R=removal (—·—·—), Rd=redistribution (·····),
D=decontamination (-·-·-·-), P=penetration and permeation (·····)



Air - The air compartment contains vapors and dispersed particles, which are assumed to be homogeneously distributed in the compartment. The total mass of a given substance in the air compartment is well defined and can, in principle, be measured. The compartment volume is given by the size of room or other boundaries, either physical or virtual.

Surface contaminant layer (Su) - Contaminants on a surface form a layer, which delineates the compartment called surface contaminant layer. The compartment is assumed to be homogeneous. In principle, all substances belonging to the surface contaminant layer can be identified and thus mass in this compartment can be assessed. The compartment volume is given by the three dimensional volume of this layer. For many practical purposes, a two dimensional representation of this layer will suffice.

Outer and inner clothing contaminant layer (CloOut, CloIn) - Solid or liquid contaminants at the boundary between outside and the surface of the outer clothing are modeled as the compartment called outer clothing contaminant layer. The fabric separates this compartment from the inner clothing contaminant layer. For simplicity of the model the fabric is described as a barrier (mass transfer rate limiting) having the property to retain mass ($M_{Reservoir}$). If the mass of the hazardous substance in the outer clothing contaminant compartment is M_{CloOut} then some of this material will be transported through the clothing to the compartment called inner clothing contaminant layer. The mass in the inner clothing compartment is $M_{CloIn} = M_{CloOut} - M_{Reservoir}$. For many practical purposes a two dimensional representation of these compartments will suffice.

Skin contaminant layer (Sk) - On the skin, contaminants, sweat, skin oil, and barrier cream (if applied) form a layer. This layer constitutes the skin contaminant layer. The compartment is assumed to be homogeneous. In principle, all substances belonging to the skin contaminant layer can be identified and thus the mass in this compartment can be identified and measured. The compartment volume is given by the three dimensional volume of this layer. The conventional two dimensional representation of this layer is an oversimplification, which has contributed to the confusion about the principles involved in the choice of measurement principles for and interpretation of dermal contamination in terms of dermal uptake.

Mass transport processes

The mass transport from the source to the compartments and sinks in the system is shown in Figure A-1. Below the horizontal dotted line in the figure a person's movement begins to influence the transport processes. Two units are used to measure transport of mass; $g.s^{-1}$ and $g.event^{-1}$. For processes called events, it is important to describe the number of events within a reference time period, typically 8 hours. The mass transport can be divided into eight distinct processes as described later and in Table A-2.

Emission (E) - Emission (E) is the transport of substances into the air, onto surfaces, outer clothing, and the skin contaminant layer from all primary sources. Evaporation of liquids or emission of droplets or particles into the air gives rise to emission of contami-

nant mass to the air. For aerosols we restrict this emission pathway to those with aerodynamic diameter <100 µm so that sedimentation is relatively unimportant. Emission to the different surfaces in the model can arise from splashing, spilling, immersion and impaction of large particles. Splashing is the emission of large droplets the trajectory of which towards the surfaces is unaffected by air movement, whereas spilling is the event by which a liquid or powder is spilled on a surface. Immersion is an event whereby a part of the body is submerged into a liquid or a powder. Impaction is the process by which large particles are generated at the source and ejected from that source to impact onto surfaces. The emission rate is given as either $g.s^{-1}$ or as $g.event^{-1}$.

Deposition (Dp) - Deposition (Dp) is the transport of substances from the air to surfaces, outer clothing, and the skin contaminant layer. Deposition can be of mass as either solid, liquid, or vapor. The time dependent mass deposition rate Dp(t), in units of $g.s^{-1}$, can be represented by the deposition velocity v(t) in units of $cm.s^{-1}$ as follows:

$$Dp(t) = v(t) \cdot C(t) \cdot A_X \qquad (1)$$

where C(t) is concentration in air outside the boundary layer and A_X is the area of the two-dimensional representation of the compartment, where X is either Su for surface, Clo for clothing or Sk for skin).

Resuspension or evaporation (L) - Resuspension or evaporation (L) is the transport of substances from surfaces, outer clothing, and the skin contaminant layer to the air, as particulate (resuspension), vapors or both. Evaporation is a continuous process driven by diffusion. Mechanical forces cause resuspension. If resuspension is caused by a single mechanical impact then the transport of mass is conveniently modeled as a transfer of mass per single event. If resuspension is caused by a sudden flow of air along a surface, there is a short initial peak of resuspended mass followed by a decay of resuspended mass per time [7]. Thus resuspension can, in general, be measured as a transport of mass per single event.

Transfer (T) - Transfer (T) is the transport of substances by direct contact between surface, skin, and outer and inner clothing contaminant layers in a direction towards the worker. Transfer from the surface to the skin contaminant layer is event based and takes place from a small area of actual contact. That area could be considerably smaller than the surface area of the body part involved in the contact. The actual surface area of the skin that will be contaminated, as well as the efficiency of mass transfer, will depend on the actual contact, - for example, single pressure, or movements of the skin along the surface.

Removal (R) - Removal (R) is the transport of substances by direct contact between skin, inner and outer clothing, and surface contaminant layer in a direction away from a worker. Removal thus is defined as an event based transport in the opposite direction of transfer.

Redistribution (Rd) - If contaminants in the air, on the surface, clothing, or skin are not homogeneously distributed, or different parts of the body such as palm, neck, and trunk need to be distinguished, the compartments can be subdivided initially into subcompartments. Redistribution then is the transport of substances from a subcompartment to another subcompartment of the same type. Redistribution of contaminants from one part of the skin contaminant layer to another can occur as a result of touching the face with contaminated fingers. Also, fabric wetting can redistribute liquid contaminants.

Decontamination (D) - Decontamination (D) is the deliberate transport of contamination from the system – for example, ventilation of room air, cleaning of room surfaces and outer clothing or washing material off the skin. The air compartment is decontaminated by the combined effect of natural and mechanical ventilation. Cleaning of contaminated surfaces, changing of clothing, and cleaning of skin all result in permanent loss of mass from the system and thus are decontamination processes. By contrast, brushing dust off clothing transports particulate mass to the room air and thus is resuspension, not decontamination.

Penetration and permeation (P) - Penetration and permeation (P) both involve transport of substances through a rate limiting barrier - such as clothing or the stratum corneum. Penetration is transport caused by external pressure, capillary penetration, and evaporation-condensation. Permeation always involves diffusion.

Transport of contaminants through permeable clothing occurs by aerosol penetration and liquid transport. External air pressure can be considered to be the driving forces for penetration of aerosols through fabric [8], whereas the mechanisms of liquid transport are capillary penetration, pressure penetration, impact penetration, and evaporation-condensation [9]. Mass transport through non-permeable clothing is a diffusion process driven by concentration. The rate of mass transport through the stratum corneum $P_{Sk}(t)$ can be represented by a permeability coefficient K(t), the concentration difference over the stratum corneum $C_{Sk}(t)$ and skin contaminant layer area A(t) as:

$$P_{Sk}(t) = K(t) \cdot C_{Sk}(t) \cdot A(t) \qquad (2)$$

The area of the skin contaminant layer can be time dependent, which for example is the case for a drop that dries while on the skin. Roed et al. [10] have shown that very small particles may penetrate into the stratum corneum but their fate is not known.

Concentration

The model describes transport of mass of a given substance, which is a conserved quantity provided we neglect chemical reactions. However, several transport processes are driven by the compartment concentration. For the air compartment concentration is readily obtained from mass because an air compartment can be defined such that it has a constant volume.

For the surface and skin contaminant layers, volume is defined by the total amount of material present (Table A-1). If the skin contaminant layer compartment only contains liquids the concentration C_{Sk} of a hazardous substance of mass M_{Sk} is given by the ratio

$$C_{Sk} = \frac{M_{Sk}}{M_{Sk} + M_{Sk,other}} \qquad (3)$$

where $M_{Sk,\ other}$ is the mass of all other liquid substances. This concentration can be readily transformed into the molar concentration, which is more relevant for the skin contaminant layer. As a first approximation and for substances of low volatility C_{Sk} can be considered to be the concentration in the bulk liquid.

The concept of concentration is more complicated for particles as they are discrete entities and solid substances must dissolve to diffuse through the stratum corneum. For soluble or leachable substances it is the concentration in the wet layer around the individual particle that has to be used for C_{Sk} in equation 2. This may mean that uptake will be limited by the rate of dissolution rather than diffusion through the stratum corneum. Furthermore, if uniform deposition takes place a monolayer of particles may be built up until the entire surface is covered. As a multilayer forms, the additional mass will have less and less influence on uptake and may be likely to dislodge and fall off. For this reason the mass of particles in the skin contaminant layer may only have limited relevance to uptake.

Measurement methods of skin and surface contamination

Depending on the perspective from which the exposure scenario is investigated a range of measurement approaches have been developed from the 1950's onwards [11]. In Table A-3 methods in common use for skin and surface contamination are listed. Several of these methods are used to assess both compartment mass – for example, total mass in surface layer and mass transport processes – for example, dislodgeable mass. A clear distinction is not always made and this can create confusion about determination and interpretation of sampling efficiencies. As an example, measurement methods should aim to have 100% sampling efficiency where they intend to assess compartment mass. On the other hand, a 100% sampling efficiency for a wipe test to measure the transfer to skin upon contact with a surface is not necessarily desirable [1]. The amount that can be transported from surfaces to the skin or outer clothing contaminant compartments depends on the type of surface, the contaminant, and on the forces acting on the contaminant layer, rather than how much mass is present in the compartment. Measurement of transport must therefore be based on a model. Several routes may be followed. The transport process from surfaces could be simulated with, for example, using a standardized instrumental sampling method or standardized events. Transport can also be measured during actual field conditions for an exposed population, with the result summarized in the form of a distribution of mass transport. Such reported distributions have been used for risk assessment, - for example, transfer from soil giving the results as soil to skin adherence [12].

Table A-1 Compartment descriptors.

Compartment	Definition of metric	Symbol	Relation	Units
Source	Mass of hazardous substance available for emission	M_s		g
	Concentration of a hazardous substance in the source	C_s		$g.g^{-1}$, $g.m^{-3}$ $g.l^{-1}$
Air	Mass of substance in the air compartment	M_{Air}		g
	Volume of the air compartment	V_{Air}		m^3
	Concentration of hazardous substance in the air	C_{Air}		g/m^3
Surface contaminant layer	Mass of a hazardous substance in the surface contaminant layer	$M_{Su,}$		g
	Concentration of a hazardous substance on the surface	C_{Su}	$M_{Su}/(M_{Su}+M_{other})$ [a]	$g.kg^{-1}$,$Mol.cm^{-3}$
	Area of surface which is contaminated with hazardous substance	A_{su}		cm^2
Outer clothing contaminant layer	Mass of hazardous substance in the outer clothing contaminant layer	M_{CloOut}		g
	Concentration of a hazardous substance in the outer clothing compartment	C_{CloOut}	$M_{CloOut}/(M_{CloOut}+M_{otherOut})$	$g.kg^{-1}$
	Area of the outer clothing which is contaminated with hazardous substance	A_{CloOut}		cm^2
Inner clothing contaminant layer	Mass of hazardous substance in the inner clothing contaminant layer	M_{CloIn}		g
	Concentration of a hazardous substance in the inner clothing compartment	C_{CloIn}	$M_{CloIn}/(M_{CloIn}+M_{otherIn})$	$g.kg^{-1}$
	Area of the inner clothing which is contaminated with hazardous substance	A_{CloIn}		cm^2
Skin contaminant layer	Mass of hazardous substance on the skin surface	M_{Sk}		g
	Concentration of hazardous substance in the skin contaminant layer	C_{Sk}	$M_{Sk}/(M_{Sk}+M_{other})$	$g.kg^{-1}$
	Area of the skin which is contaminated with hazardous substance	A_{Sk}		cm^2

a) M_{other}=mass of all other substances in a particular compartment.

Deposition from air is more predictable and Schneider and Stokholm [13] have proposed a theoretical model dependent on particle size for the transport of airborne dust onto the ocular surface in terms of relative deposition velocities. This could be called the ocular deposition fraction, by analogy with the inhalable fraction. Roed, and others [10] have given experimental data for mean deposition velocities on arms and other body parts for people seated in a test room, which likewise could form a basis for defining other dermal deposition fractions.

There is a close analogy to requirements for sampling inhalable airborne particles. Historically, fractions with biologically relevant particle size have been based on experimental data that reflect mean deposition efficiencies in the airways. An alternative approach has been to define the fraction as that obtained by a given sampling instrument. Initially there were different proposals for the size fractions, and samplers were not available which could measure according to these criteria. However, better experimental data on deposition efficiencies has resulted in the adoption of an international standard for the definition of the respirable, thoracic, and inhalable fractions [14]. Recent development has resulted in samplers, which have aspiration efficiencies that match the definition of the fractions [15].

In the following section some strategies for measuring with the model compartment mass and mass transport processes will be discussed, highlighting several problems in conventional approaches to measurement and possible routes forward.

Compartments

The mass in a compartment at a given time can in principle be measured by sampling all of the contaminant present at that time in the compartment. A strategy often used in monitoring is to estimate the entire compartment mass by sampling a small proportion of the whole [16; 17].

Air - Methods for measurement of concentration of hazardous substances in the air compartment are well established relative to estimation of inhalation risk. For aerosols specifications are available for sampling fractions of biologically relevant inhalable size [14], but methods for determining the concentration of non-inhalable particles, which are relevant for estimating surface deposition, have not yet been developed.

Surface contaminant layer - For surfaces, several in situ methods are available, but they can only measure a limited range of substances, and never total mass. Portable x-ray fluorescence analysis is one example of this type of analysis [18]. Methods based on removal - such as adhesive tape sampling - can have a high sampling efficiency. The efficiency in field use is usually estimated with consecutive sampling of the same area. However, this is based on a circular argument, as only the contaminant that the tape can actually remove will ever be sampled [19].

Clothing contaminant layers - In the model clothing was divided into an outer and inner contaminant layer, with a buffer to represent the mass residing inside the clothing which

does not come into contact with surfaces or skin, respectively. Measurement of the entire mass in the clothing would in principle be no problem and if this were done for non-permeable fabrics theoretically the outer contaminant layer would be measured. To our knowledge a method which measures the inner contaminant layer for permeable layers is not available.

Skin contaminant layer - The skin rinse method [20] has been used to assess the mass in the skin contaminant layer compartment. The method typically recovers 40%-90% of contaminants spiked onto the skin. Recoveries can be determined experimentally. However, spiking experiments have inherent problems as it is unclear whether this result should be interpreted as a 40%-90% sampling efficiency for the skin contaminant layer or if it reflects partitioning between this compartment and the stratum corneum or perfused tissue. Measurement methods based on UV fluorescence of tracer compounds mixed with the contaminant at source [21-25] indirectly measure the mass of the contaminant in the skin contaminant layer. However, fluorescent tracers have the ability to bind with the cell proteins in the stratum corneum and the method is not able to differentiate between the skin contaminant layer and the stratum corneum.

Mass transport processes

Transport can in principle be measured with a mass balance calculation for the change in mass in all relevant compartments per event or per time. Transport can more directly be determined by methods, which standardize a set of external factors considered to be important determinants of the transport process (Table A-3).

Deposition - Adhesive tape can measure the particle deposition unaffected by loss. This has been done for particle deposition onto the face [26], but the method does not work for parts of the body that get into contact with other surfaces. Charcoal cloth has been used to measure skin deposition of volatile compounds. A problem is that the method does not differentiate between vapors from air and liquid splashes [27; 28].

Resuspension or evaporation - Resuspension is usually best described as a series of single events. If events are repeated, say at a frequency n, and the ratio n/T tends to infinity, where T is a suitable reference time interval, resuspension can be treated as a continuous process. In this case it is customary to define a resuspension rate R by the equation where the mass of substance lost from the surface per unit time (L) (Table A-2) is:

$$L(g\ s^{-1}) = R(s^{-1}) \cdot M_{Su}(g) \qquad (4)$$

This definition assumes that R is independent of M_{Su} (see also discussion for transfer). An approach to assess resuspension based on events would be to collect all dust resuspended as the result of a standardized mechanical impact [29] have developed the surface total emission potential of particles (STEPP) tester, which simulates resuspension caused by a walking person from a carpet. Resuspension caused by air movements is simulated in the SMAIR tester [30]. In this instrument a well defined air jet is directed at a surface contami-

nant layer and the resuspended particles collected on a filter. Vacuuming techniques could be used to simulate resuspension caused by strong air velocities, provided the nozzle does not touch the surface. Evaporation is driven by diffusion and thus it is simpler to specify external factors. Wolkoff [31] has developed a field and laboratory emission cell (FLEC), which in principle is a small air compartment that is placed on a surface. A well defined flow of conditioned air is passed through the compartment and the exhaust air is analyzed for evaporated substances. The diffusive transport of water vapor from the skin (Transepidermal water loss) can be measured with simply instruments [32]. A similar principle could be used for other vapors lost from the skin.

Transfer - The increase in mass over time in the skin contaminant layer measures is the measured net balance between transport to and from the compartment and thus is affected by deposition, resuspension or evaporation, removal, and uptake. Surrogate skin methods such as pads, gloves, and coveralls are based on the assumption that they are estimates of the transport of mass to the skin contaminant layer from surfaces. However, the materials used do not have the roughness, stickiness and other properties of human skin and so may not meet this assumption. Dislodgeable foliar residue (DFR), a procedure first described by Iwata *et al.* [33], determines the pesticide remaining on foliage after spraying. It involves destructive sampling of foliage by punching or removing leaves to provide a sample leaf area of about 100 cm^2 and subsequent, partly mechanical partly chemical, removal of pesticide residue by shaking the leaves in water with some drops of surfactant. This process is intended to mimic transfer to skin. Then pesticide is extracted from the water and analyzed chemically. Sometimes an organic solvent is used in the first step, but this then measures the mass in the compartment rather than the DFR. Both methods, however, have been used to indicate the strength to transfer by direct contact from the source, which may be relevant to predict exposure by contact. A more general form of DFR is transferable residue (TR). If TR is measured simultaneously with increase per event of mass per area M_{Sk}/A_{Sk} in the skin contamination layer, a dermal transfer coefficient, DTC, can be defined as (see also Fenske [1] and Zweig *et al.* [34]):

$$DTC(cm^2 event^{-1}) = \frac{M_{Sk}(g \cdot event^{-1})}{A_{Sk} TR(g \cdot cm^{-2})} \qquad (5)$$

For this equation to be useful it has to be assumed that TR is independent of M_{Sk}/A_{Sk}. However, Brouwer *et al.* [35] have shown that TR depends non-linearly on the mass per area in the surface contaminant layer, M_{Su}/A_{Su}. A better approximation would be to assume a functional relationship:

$$TR=TR(M_{Su}/A_{Su}) \qquad (6)$$

determined by non-linear regression. In reality a stochastic relation must be expected, and thus for given M_{Su}/A_{Su} , TR has a given distribution with variables (mean and variance) being functions of M_{Su}/A_{Su}. It is to be expected that liquid contaminants can be assessed with the simple relation, grease or other paste-like contaminants an intermediate

Table A-2 Mass transport process descriptions.

Process	Definition	Symbol[a]	Units
Emission	• Mass of hazardous substance emitted into air from primary sources per unit time	E_{Air}	$g.s^{-1}$
	• Mass of hazardous substance emitted to surface contaminant layer by splashing, spilling and ejection of particles from primary sources per unit time per event	E_{Su}	$g.s^{-1}$ $g.event^{-1}$
	• Mass of hazardous substance emitted to the outer clothing contaminant layer for a particular worker by splashing, spilling and ejection of particles from primary sources per unit time per event	E_{CloOut}	$g.s^{-1}$ $g.event^{-1}$
	• Mass of hazardous substance emitted to skin contaminant layer for a particular worker by splashing, spilling and ejection of particles from primary sources per unit time per event	E_{Sk}	$g.s^{-1}$ $g.event^{-1}$
Deposition	• Mass of hazardous substance deposited from air compartment to the surfaces per unit time	Dp_{Su}	$g.s^{-1}$
	• Mass of hazardous substance deposited from the air compartment to the outer clothing contaminant layer for a particular worker per unit time	Dp_{CloOut}	$g.s^{-1}$
	• Mass of hazardous substance deposited from the air compartment to the skin contaminant layer for a particular worker per unit time	Dp_{Sk}	$g.s^{-1}$
Resuspension or Evaporation	• Mass of hazardous substance lost from the surface contaminant layer to the air compartment by evaporation per unit time or by resuspension/evaporation per event	L_{Su}	$g.s^{-1}$ $g.event^{-1}$
	• Mass of hazardous substance lost from the outer clothing contaminant layer of a particular worker to the air compartment by or evaporation per unit time or by resuspension/evaporation per event	L_{CloOut}	$g.s^{-1}$ $g.event^{-1}$
	• Mass of hazardous substance lost from the skin contaminant layer of a particular worker to the air compartment by evaporation per unit time or by resuspension/evaporation per event	L_{Sk}	$g.s^{-1}$ g $event^{-1}$
Transfer	• Mass of hazardous substance transferred from the surface contaminant layer to the skin contaminant layer for a particular worker by direct contact per event	$T_{Su, Sk}$	$g.event^{-1}$
	• Mass of hazardous substance transferred from the surface contaminant layer to the outer clothing contaminant layer for a particular worker by direct contact per event	$T_{Su, CloOut}$	$g.event^{-1}$
	• Mass of hazardous substance transferred from the inner clothing contaminant layer to the skin contaminant layer for a particular worker by direct contact per event	$T_{CloIn,Sk}$	$g.event^{-1}$
	• Mass of hazardous substance transferred from the outer clothing contaminant layer to the skin contaminant layer for a particular worker by direct contact per event	$T_{CloOut,Sk}$	$g.event^{-1}$

Table A-2 (Continued)

Process	Definition	Symbol[a]	Units
Removal	• Mass of hazardous substance removed from the skin contaminant layer of a particular worker and transferred to the surface contaminant layer by direct contact per event	$R_{Sk,Su}$	$g.event^{-1}$
	• Mass of hazardous substance removed from the skin contaminant layer and transferred to the inner clothing contaminant layer of a particular worker by direct contact per event	$R_{Sk,CloIn}$	$g.event^{-1}$
	• Mass of hazardous substance removed from the skin contaminant layer and transferred to the outer clothing contaminant layer of a particular worker by direct contact per event	$R_{Sk,CloOut}$	$g.event^{-1}$
	• Mass of hazardous substance removed from the outer clothing contaminant layer of a particular worker and transferred to the surface contaminant layer by direct contact per event	$R_{CloOut,Su}$	$g.event^{-1}$
Redistribution	• Mass of hazardous substance transferred from one part of air compartment to other part per event	Rd_{Air}	$g.event^{-1}$
	• Mass of hazardous substance transferred from one part of contaminant layer to other part per event	Rd_{Su}	$g.event^{-1}$
	• Mass of hazardous substance transferred from one part of inner clothing contaminant layer to other part per event	Rd_{cloIn}	$g.event^{-1}$
	• Mass of hazardous substance transferred from one part of outer clothing contaminant layer to other part per event	Rd_{cloOut}	$g.event^{-1}$
	• Mass of hazardous substance transferred from one part of skin contaminant layer to other part per event	Rd_{Sk}	$g.event^{-1}$
Decontamination	• Mass of hazardous substance removed from the air compartment by ventilation per unit of time	D_{Air}	$g.s^{-1}$
	• Mass of hazardous substance removed from the surface contaminant layer by deliberate decontamination per event	D_{Su}	$g.event^{-1}$
	• Mass of hazardous substance removed from the outer clothing contaminant layer by deliberate decontamination per event	D_{CloOut}	$g.event^{-1}$
	• Mass of hazardous substance removed from the inner clothing contaminant layer by deliberate decontamination per event	D_{CloIn}	$g.event^{-1}$
	• Mass of hazardous substance removed from the skin contaminant layer by deliberate decontamination per event	D_{Sk}	$g.event^{-1}$
Penetration and permeation	• Mass of hazardous substance transported through stratum corneum	P_{Sk}	$g.s^{-1}$
	• Mass of hazardous substance transported from the outer clothing contaminant layer to the inner clothing contaminant layer per unit time	P_{CloIn}, P_{CloOut}	$g.s^{-1}$
	• Mass of hazardous substance transported from the inner clothing contaminant layer to the outer clothing contaminant layer per unit time	P_{CloOut}, P_{CloIn}	$g.s^{-1}$

a) Air= air compartment, Su= surface contaminant layer, Sk= skin contaminant layer, CloOut= outer clothing contaminant layer, CloIn= inner clothing contaminant layer

relation, and particulate contaminants with the most complex relation. Transfer of particles will depend on contaminant properties - such as particle size distribution, shape, and humidity or cohesion. In all cases it must be remembered that TR will depend on the method used for its measurement. There are several versions of samplers designed to exert a constant force on a defined area in a wiping action. These methods have potential for simulating the transfer of mass and are non-destructive. One such monitor is a fixed pressure dislodgeable residue sampler such as a polyurethane foam roller [36]. Ross, and others [37] used standardized movements (aerobic dance routines following an instructor and music) to measure transfer of pesticides from fogged carpets onto the skin. Transfer from the inner clothing contaminant layer to the skin contaminant layer is usually estimated with patches or underwear. No other method seems to have been developed specifically for measuring this transfer.

Removal - Dissipation of contaminants from the clothing compartment has been subject of many studies, however, the studies were focussed on the transport of mass from the clothing compartment to the skin compartment – that is, the opposite direction to removal. Yang and Li [38] reported a frictional transport of pesticides of 1% to 5 % from contaminated clothing to underwear. As mechanical or frictional transport may be an important mechanism of removal, these data indicate the range of mass transport from clothing in the opposite direction. Removal from skin to surface has been studied by Brouwer *et al.* [35]. They reported a mean removal efficiency of 38 % during a single pressure contact of a contaminated hand with an uncontaminated flat surface.

Redistribution - Information on the area exposed and the redistribution of contaminant could be obtained by observation. However, the method of choice is the fluorescent tracer technique with a quantitative image analysis system [21-25]. These methods have high spatial resolution.

Decontamination - Decontamination of the air compartment is the removal of mass from the air compartment by either local or general ventilation. Methods to assess the efficiency of ventilation in reducing the concentration of gaseous and particulate contaminants are well developed [39]. Decontamination of surfaces can be assessed by measuring the mass removed – for example, the quantity of dust in a vacuum cleaner filter bag - or by the decrease in mass of the surface contaminant layer. A measurement strategy for decontamination of surface dust in offices has been described by Schneider *et al.* [40] and a standard procedure to assess the efficiency of hand washing has been developed by Fenske and Lu [41].

Penetration and permeation - Penetration and permeation stand for transport of mass through either the clothing barrier or the stratum corneum. These mass transport pathways have been studied in great detail relative to the effectiveness of protective clothing [42] and percutaneous penetration [43]. In both cases a test cell design is used to measure permeation. However, several different methods are used in the assessment of percutaneuos penetration ranging from in vitro models with static diffusion cells and flow through diffusion cells with animal or human skin (viable and non-viable).

General discussion and conclusions

In this paper we propose a conceptual model of both the important pathways leading to dermal exposure and the intermediate compartments where the hazardous substances may reside. This model has allowed us to define a consistent terminology, which should form the basis for improved comparability between studies of dermal exposure or surface contamination. Consistent use of the model will ensure that most appropriate variables are measured in any situation. The model has been constructed at a conceptual level and omits much of the detail, which is evident in real situations. It is possible to extend the model as a series of interlinked compartments for the skin, surfaces, etc. In this way compartments for hands, arms, torso etc., could represent the skin surface; or if greater resolution is required fingers, palms, etc, could represent the skin surface. In this way the existing model encompasses sufficient detail to enable terminology to be developed, it could be generalized for any particular need.

The model can cope with special exposure scenarios, such as immersion of body parts in liquid or powder. In this case the liquid or powder constitutes the skin contaminant layer compartment and has infinite volume. For interpretation of contaminated substances derived from soil and paste-like substances in the skin contaminant layer a refinement of the model is necessary. The skin contaminant layer could be subdivided into an outer layer and an inner layer in intimate contact with the stratum corneum. The possibility exists that it is the supply of hazardous substance from the outer layer to inner layer, which limits the rate of uptake. This identifies a need to refine existing methods of measuring mass in the skin contamination layer compartment.

We have not considered the stratum corneum in much detail, as it is generally not available for surface sampling methods. Skin stripping with adhesive tape is a possible exception. Consecutive stripping allows semi-quantitative depth profiling, but if the skin contaminant layer compartment is not empty, the mass in this compartment and in the stratum corneum will be partially mixed up. The finite thickness of the stratum corneum constitutes a buffer capacity and introduces a lag time for the transport process to reach equilibrium after a step change in concentration [44]. The lag time may vary from a few minutes up to days and its significance for risk assessment would be determined by duration of exposure as compared with lag time.

The inventory of existing methods for skin and surface contamination measurement (Table A-3) is only illustrative. Compilation of an exhaustive inventory and discussion relative to the model is beyond the scope of the present paper. However, with the model structure, limited or non-existing methods for measuring relevant compartment mass or transport processes have been identified:

- The model stresses the importance of measuring concentration in the skin contaminant layer but methods relevant for uptake are lacking;
- The mass of particles in the skin contaminant layer may only have limited relevance for uptake. Particles are less likely to result in uptake than liquids and

Table A-3 Measurement methods (for a review see Carson *et al.* [45]).

Method	Principle of Sampling	Sampling area definition	Measured compartment mass [a]	Measured transport process [b]	Ref.
UV fluorescence	In situ	0.1-2 m^2	M_{Sk}, M_{Su}, M_{CloOut}, M_{CloIn}	All processes	21-25
Portable X-ray fluorescence monitor	In situ	Instrument defined	M_{Su}	All processes related to the surface contaminant layer	18
Wet wipe	Manual wiping	None or template	M_{Su}, M_{Sk}	$T_{Su,Sk}$, $T_{Su,CloOut}$, $R_{Sk,Su}$, $R_{Sk,CloIn}$, $R_{Sk,CloOut}$	46
Wet wipe	Mechanized wiping	Instrument defined	M_{Su}	$T_{Su,Sk}$, $T_{Su,CloOut}$	19
Gelatine foil	Surface dust lifting	1:1 transfer of dust layer	M_{Su}	$T_{Su,Sk}$, $T_{Su,CloOut}$	40
Fixed pressure dislodgeable residue sampler	Mechanical transfer in situ	10-20cm x length sampled	M_{Su}	$T_{Su,Sk}$, $T_{Su,CloOut}$	36
Dislodgeable foliar residue sampling	Surface removal	Punches 0.1-2.5 cm^2, total 100 cm^2	M_{Su}	$T_{Su,Sk}$, $T_{Su,CloOut}$	33
Adhesive tape	Skin stripping	1:1 transfer of dust layer	M_{Sk}	Dp_{Sk}, $R_{Sk,Su}$, $R_{Sk,CloIn}$, $R_{Sk,CloOut}$	44
Hand wash	Wash with water or alcohol	Total hand surface	M_{Sk}	$R_{Sk,Su}$, $R_{Sk,CloIn}$, $R_{Sk,CloOut}$, D_{Sk}	20
Patch	Passive	Sampler defined	M_{Sk}	$T_{Su,Sk}$, $T_{CloIn,Sk}$, $T_{cloOut,Sk}$, Dp_{Sk}	16
Whole body	Passive	Body parts	M_{Sk}	$T_{Su,Sk}$, $T_{CloIn,Sk}$, $T_{cloOut,Sk}$, Dp_{Sk}	16
SMAIR	Resuspension by air jet	Instrument defined	---	L_{Su}	30
STEPP	Resuspension by impact	Instrument defined	---	L_{Su}	29
Microvacuuming	Resuspension by suction	Instrument defined	M_{Su}	L_{Su}	47
FLEC	Evaporation by airflow	Instrument defined	---	L_{Su}	31
TEWL	Evaporation	Instrument defined	---	L_{Sk}	32

a) Definition of symbol see Table A1
b) Mass transport process descriptions see Table A2

solubility of particles is an important qualifier which should be specified along with results of measurements of the mass and composition of skin contaminant layer;

- A clear distinction must be made between mass in compartment and transport of mass. Direct measurement of mass transport must be based on an appropriate theoretical model.

We envision that our paper will stimulate discussions and help in the development of more appropriate methodologies for the assessment and control of dermal exposure.

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