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N-nitrosamines in the southern Swedish rubber industries – exposure, health effects, and immunologic markers

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Objectives The aim of this study was twofold: (i) to evaluate the air levels of N-nitrosamines in the Swedish rubber industry and (ii) to estimate the risk of symptoms and changed levels of immunologic markers in relation to these levels.

Methods Using adsorption tubes, we collected samples of N-nitrosamines in the breathing zone of 96 rubber workers and analyzed them with liquid chromatography tandem mass spectrometry. Of these 96 workers, 66 were included in a medical examination and blood analysis together with an additional 106 rubber workers and 118 unexposed subjects. Medical and occupational histories were obtained in structured interviews, symptoms were recorded and immunologic markers analyzed in blood.

Results The sum of N-nitrosamines ranged from less than the limit of detection to 36 µg/m³ and differed with the vulcanization method (ie, curing process) used. Workers vulcanizing with salt baths had the highest levels (median 4.2 µg/m³). Compared to the unexposed subjects, the rubber workers had an increased risk of nosebleeds, eye and throat symptoms, hoarseness, cough, nausea, headache, and changed levels of eosinophils and total immunoglobulin G (IgG). However, we found no clear exposure-response relationship with the symptoms or the immunologic markers studied.

Conclusions High levels of N-nitrosamines were found and must be lowered considerably in order to decrease the risk of cancer. There is a need for an occupational exposure limit for N-nitrosamines in Sweden. The lack of exposure-response relationships with the subacute symptoms examined in this study may be due to a healthy-worker selection or to the possibility that the symptoms are caused by an exposure not co-varying with N-nitrosamines.

Key terms airway symptom; antibody; biomarker; exposure-response relationship.

N-nitrosamines have been found to be associated with increased risks of cancer of the esophagus, lip, oral cavity, and pharynx (1). Therefore, risk assessments for these substances previously have been focused on malignant diseases. There is a continuing need for exposure measurements of N-nitrosamines in order to estimate the risk of cancer in the environment and industry.

In addition, different parameters reflecting humoral and cell-mediated immunity have been shown to be suppressed at subchronic or chronic exposures to N-nitrosodimethylamine in mice (2–4). Thus, N-nitrosamines may be associated with other kinds of health effects, such as airways diseases. However, to our knowledge, no examination has been made of the association

between N-nitrosamines and human symptoms of the eyes, airways, nausea, headaches, or responses of the immune system.

The highest known exposures to N-nitrosamines among humans have occurred in the rubber industry (5, 6). Therefore, studies of the relationship between N-nitrosamine exposure and health effects are preferably performed in this type of industry.

N-nitrosamines are formed during the vulcanization (ie, curing process) of rubber. Accelerators, necessary for the vulcanization process, are decomposed when heat is applied. This gives rise to free secondary amines that can react with nitrosating agents, (eg, molten nitrate, nitrite salts in the salt baths, and NO_x in the air)

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to form N-nitrosamines (6). Thus, in addition to studying the effects of N-nitrosamines, researchers can look for the compounds as a marker of vulcanization fumes. Such fumes are a mixture of hundreds of compounds and have been associated with airways diseases (7–10). In a previous study, we have shown that vulcanization workers have increased urinary levels of 2-thiothiazolidine-4-carboxylic acid (11) and 1-hydroxypyrene (12). We have suggested that these may be used as markers of vulcanization fumes.

This study aimed to monitor the air levels of N-nitrosamines in the Swedish rubber industry. Another objective was to analyze the exposure-response relationships between (i) exposure to N-nitrosamines and vulcanization fumes, and (ii) several symptoms (primarily in the eyes, nose, and lower airways) and the effects on the levels of immunologic markers.

Study population and methods

Description of companies

We selected companies to participate in the study on the grounds of geographical accessibility and diversity of production methods. The companies, and the numbering thereof, are the same as in an earlier study by our group (11). The production profiles and methods of the eight companies participating in the study are shortly described below.

Company 1 produced industrial rubber profiles as well as form-pressed products but also special products like seals for ship doors and lock gates. The rubber goods were produced by vulcanization methods using microwaves and salt baths as well as steam vulcanization in an autoclave. Compression vulcanization was also applied to join rubber profiles into rings or frames.

Company 2 manufactured components for vibration damping of heavy trucks, excavators, and the like. Most of the products consisted of rubber bound to metal. The workers used a number of machines for compression and injection vulcanization, all situated in the same large production hall.

Company 3 comprised two different factories (factory 1 and factory 2 hereafter). Divided into three divisions, factory 1 produced profiles and sealing products for the industrial market. Two divisions were located next to each other and treated as one unit (division A in our study). Division A manufactured industrial profiles; one division used continuous vulcanization in salt baths while the other employed microwaves and fluid-beds. The third division (division B) was separate from division A, but situated in the same building. Here workers produced rubber membranes for water-proofing

applications by steam vulcanization in an autoclave. Several joining operations were also performed. Factory 2 produced sealing strips and profiles for the consumer market; it used continuous vulcanization in salt baths with non-nitrite containing salt.

Company 4 produced primarily industrial profiles in a large production hall with continuous vulcanization in several salt bath lines and one line using hot air. Apart from this, there was a production line that used peroxide as the vulcanization agent, but this was located in a separate building.

Company 5 produced different hoses and components for automotive applications and the white goods industry. The company had a number of injection and compression vulcanization units. Some post-vulcanization was performed in hot-air ovens.

Company 6 manufactured different rubber details such as gaskets and seals for cables in the automotive sector and other industries. The company had both injection and compression vulcanization units.

Company 7 produced industrial rubber products for the food, offshore and automotive industries. Among the products were seals for cables and pipes, and gaskets for plate heat exchangers. The production methods were compression and injection vulcanization.

Company 8 consisted of two different factories. Factory 1 produced sealing profiles, including roof and window applications, in continuous vulcanization lines using hot air or salt baths. Furthermore, a few small compression vulcanization units were situated in an adjacent room where some special products were produced. Factory 2 primarily manufactured gaskets for plate-heat exchangers in a production hall with a large number of compression vulcanization units. Post-vulcanization was done in hot-air ovens.

Levels of N-nitrosamines in the air

We measured N-nitrosamines in the breathing zones of 96 rubber workers from the eight aforementioned companies by collecting samples on Thermosorb/N adsorption tubes (Thermedics Inc, Woburn, MA, USA) with a flow rate of 1.5 l/min (table 1). The work was uniform throughout the 8-hour workshift and therefore we performed the sampling only during 3 hours. The samples were collected on one occasion on a Tuesday, Wednesday, or Thursday and were stored at +8° C until analysis was conducted.

At the time of the analysis, 50 µl of deuterium-labeled N-nitrosodimethylamine (Sigma-Aldrich, St. Louis, MO, USA) in methanol (25 µg/ml) was added to each sampler. The samplers were dried by air suction (1.5 l/min) for two minutes. Thereafter, the N-nitrosamines were eluted using 2.0 ml methanol in a syringe. The eluates were transferred to analytical vials and

Table 1. Levels [median (range) $\mu\text{g}/\text{m}^3$] of six different volatile N-nitrosamines in the breathing zone of 96 workers employed in the companies, and different subdivisions of the same company, included in the study. Categories indicated refer to total levels of N-nitrosamines. N-nitrosomethylethylamine and N-nitrosodi-n-propylamine were always <LOD and are not presented in the table. (LOD = limits of detection, N_{med} = number of participants in the medical examination, N_{air} = number of participants in the personal monitoring of N-nitrosamines, N_{overlap} = number of workers participating in both the medical examination and the personal monitoring of N-nitrosamines, NDMA = N-nitrosodimethylamine, NDEA = N-nitrosodiethylamine, NDBA = N-nitrosodi-n-butylamine, NMor = N-nitrosomorpholine, NPip = N-nitrosopiperidine, NPyr = N-nitrosopyrrolidine)

Company	N_{med}	N_{air}	N_{overlap}	NDMA	NDEA	NDBA	NMor	NPip	NPyr
Company 1 ^a	11	10	7	0.24 (<LOD–0.45)	<LOD	0.14 (0.07–0.21)	0.46 (0.20–0.99)	<LOD	<LOD
Company 2 ^b	18	9	9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Company 3									
Division A ^c	26	13	11	2.8 (1.9–7.5)	<LOD (<LOD–0.16)	0.25 (0.13–0.34)	1.4 (0.88–2.0)	<LOD (<LOD–0.06)	<LOD
Division B ^a	3	5	3	0.83 (0.50–1.1)	0.63 (0.14–0.92)	0.090 (0.04–0.11)	0.32 (0.13–0.46)	<LOD	0.080 (0.07–0.79)
Factory 2 ^b	4	2	0	<LOD	<LOD–0.10	0.04–0.08	<LOD	<LOD	<LOD
Company 4									
Sulfur ^c	21	6	5	8.2 (4.4–28)	1.4 (0.81–4.6)	0.28 (0.18–0.53)	<LOD	1.3 (0.75–2.8)	<LOD
Peroxide ^b	0	1	0	<LOD	<LOD	0.06	<LOD	0.08	<LOD
Company 5 ^a	16	8	8	0.78 (0.47–0.99)	<LOD	<LOD	<LOD	<LOD	0.25 (0.16–1.9)
Company 6 ^b	14	12	6	<LOD	<LOD (<LOD–0.25)	<LOD	<LOD (<LOD–0.24)	<LOD	<LOD
Company 7 ^a	11	8	7	0.87 (0.59–1.2)	<LOD	<LOD	<LOD	<LOD	0.10 (0.07–0.19)
Company 8									
Factory 1 ^a	14	10	8	0.93 (0.30–2.0)	<LOD (<LOD–0.08)	<LOD	<LOD	0.080 (0.04–0.48)	<LOD (<LOD–0.07)
Factory 2 ^b	34	12	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

^a Category intermediate (0.3–3.0 $\mu\text{g}/\text{m}^3$).

^b Category low (<0.3 $\mu\text{g}/\text{m}^3$).

^c Category high (>3.0 $\mu\text{g}/\text{m}^3$).

centrifuged. We performed the analyses of the samples using liquid chromatography tandem mass spectrometry (LC/MS/MS; API 3000, Applied Biosystems, Foster City, CA, USA) and atmospheric pressure chemical ionization at 450° C, coupled with a liquid chromatography system from Perkin Elmer (Norwalk, CT, USA). We injected 10 μl on a C_{18} column (50 $\times$ 4.6 mm; Vydac, Hesperia, CA, USA). The mobile phases were 10 mM ammonium acetate and methanol. The liquid chromatography program was a gradient from 30–100% methanol during 4 minutes. The flow rate was 0.8 ml/min. The LC/MS/MS analyses were carried out using selected reaction monitoring transitions in the positive ion mode (table 2). We performed quantification using standard N-nitrosamine mixes from Sigma-Aldrich (St. Louis, MO, USA). The mixture contained N-nitrosodimethylamine, N-nitrosomorpholine, N-nitrosopiperidine, N-nitrosodi-n-butylamine, N-nitrosodiethylamine, N-nitrosopyrrolidine, N-nitrosomethylethylamine, and N-nitrosodi-n-propylamine.

In order to compare the LC/MS/MS method with the one using a thermo-energy analyzer, which is specific to N-nitrosamines but expensive and not very common, we collected 22 stationary samples. These were gathered in pairs in companies 3 and 4. For each pair, one sample was analyzed with LC/MS/MS and the other was sent to a commercial laboratory (ISCONLAB, Heidelberg, Germany) for analysis of N-nitrosamines

using thermo-energy analyzer. The limit of detection for each N-nitrosamine using the thermo-energy analyzer was 10 ng per sample. A plot of parallel-collected samples analyzed by the LC/MS/MS method and the thermo-energy analyzer method is shown in figure 1.

Study subjects

One hundred and seventy vulcanizers, and two foremen working in the same hall as the vulcanizers, participated in a medical examination and blood analysis ($N=172$). Table 1 shows the number of workers from each company. Only 66 of these 172 workers were included among those whose air levels of N-nitrosamines were monitored. The remaining 30 rubber workers of the 96 workers whose N-nitrosamine level had been monitored were not present at work at the time of the medical examinations or did not want to participate and, thus, were not included in this part of the study. Only a few workers used respiratory protection devices.

In addition, 118 unexposed subjects participated in the medical examination. These individuals were not occupationally exposed to rubber chemicals but had similar socioeconomic status as the exposed workers. The unexposed subjects worked as the following: (i) assemblers of armature at an electric light fittings company ($N=21$), (ii) meat cutters in a butchery ($N=20$), (iii) operators in a dairy plant ($N=11$), (iv) postmen

Table 2. Summary of the selected reaction monitoring transitions used for N-nitrosamines in the liquid chromatography tandem mass spectrometry analysis. The limit of detection (LOD) is given as ng/sample and $\mu\text{g}/\text{m}^3$. The declustering potential (Dp) and collision energy (Ce) in volt (v) for each transition are tabulated.

Analyte	Transitions	LOD (ng/sample)	LOD ^a ($\mu\text{g}/\text{m}^3$)	Precision ^b (%)	Dp (v)	Ce (v)
N-nitrosodimethylamine	74.8→42.8	50	0.19	4	30	25
N-nitrosomethylethylamine	88.8→60.7	5	0.02	8	25	22
N-nitrosopyrrolidine	100.9→54.7	5	0.02	6	25	25
N-nitrosodiethylamine	102.8→74.7	20	0.07	4	60	20
N-nitrosopiperidine	114.8→68.7	5	0.02	4	25	20
N-nitrosomorpholine	116.9→86.2	50	0.19	8	40	20
N-nitrosodi-n-propylamine	130.9→88.9	5	0.02	6	38	17
N-nitroso-n-butylamine	158.9→102.8	5	0.02	6	30	15
[2H6]-N-nitrosodimethylamine ^c	81.7→46.0	—	—	—	30	25

^a At three hours of sampling and a flow 1.5 l/min.

^b The precision (N=10) in the analysis is given as the coefficient of variation at 10 $\mu\text{g}/\text{ml}$.

^c Internal standard used in the analysis.

Figure 1. A plot of the levels of N-nitrosamines in the parallel collected samples analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) and thermo energy analyzer (TEA).

Table 3. The characteristics of the exposed workers and unexposed subjects participating in the medical examination.

	Exposed workers (N=172)				Unexposed subjects (N=118)			
	N	%	Median	Range	N	%	Median	Range
Age (years)	—	..	37	19–65	—	..	41	19–63
Gender								
Male	85	..	..	..	57	..	..	..
Female	87	..	..	..	61	..	..	..
Smoking ^a	55	32	..	..	44	37	..	..
Snuffing ^a	33	19	..	..	22	19	..	..
Atopy	53	32	..	..	31	27	..	..

^a Denoted smoker or snuffer if stopped less than six months previous.

(N=27), or (v) department store workers (N=39). They worked in the same region of Sweden, and in towns of the same size as the exposed workers. Some individual characteristics of the study subjects participating in the medical examination are shown in table 3.

All study subjects gave their informed written consent to participate. The study was approved by the Regional Ethical Committee of Lund University.

Medical examination

Medical and occupational histories were obtained in structured interviews, based on a slightly modified questionnaire adopted from Nielsen et al (13) and used earlier by Littorin et al (14). A physician, who was a trained specialist in occupational medicine, carried out the structured interviews. The same person performed all the interviews. Questions dealt with symptoms occurring during the past 12 months that had affected the eyes (itching, running, and/or burning), nose (bleeding or running, stuffiness, and/or sneezing/itching), or lower airways (severe dry cough and/or dyspnea, wheezing, chest tightness). Furthermore, symptoms such as throat burning or dryness, hoarseness, headache, and nausea were recorded. At the time of the medical examinations, the results of the exposure measurements and immunologic marker analyses were known neither to the workers nor the investigators.

Blood analysis

Venous blood samples were collected during work and handled according to the instructions from the Department of Clinical Chemistry, Lund University Hospital, in order to determine the number per liter of leukocytes, neutrophils and eosinophils, plasma concentrations of α_1 -antitrypsin, C-reactive protein, and total immunoglobulins G, M and A (IgG, IgM and IgA). Serum samples were handled according to instructions from the Department of Microbiology and Immunology at Lund University Hospital and analyzed for total, and specific (in the Phadiatop screening test for airborne allergy), immunoglobulin E antibodies using fluoroimmunoassay. Atopy was defined as positive when at least one of the allergens in the Phadiatop test (dog, cat, horse, timothy, birch, mugwort, mite [*D.pteronyssinus*, *D.farinae*], mould [*cladosporium*], olive, or parietaria) gave a response. The blood analyses were performed on blinded samples.

Statistical analysis

The measured levels from the personal samplings were used to calculate a mean value of N-nitrosamine exposure among workers with similar working tasks at each

company, and to categorize the included workers at the different companies or divisions of the company into one of three categories: (i) low = $<0.3 \mu\text{g}/\text{m}^3$, (ii) intermediate = $0.3\text{--}3 \mu\text{g}/\text{m}^3$, or (iii) high = $>3 \mu\text{g}/\text{m}^3$. There was no statistically significant difference between the exposure groups regarding smoking and snuffing habits or atopy status as estimated by the Kruskal Wallis H test.

In our analyses, we compared the exposed workers to the unexposed subjects with respect to symptoms and immunologic markers. Many of the study subjects were the same as in a previous study (15). Moreover, the exposed workers were trichotomized with respect to N-nitrosamine exposure and each subgroup was compared to the unexposed subjects. We estimated the possible effects of exposure on symptoms with odds ratios calculated using logistic regression. Using analysis of variance, we estimated the differences in the levels of immunologic markers between exposure groups.

A probability-probability plot suggested that the levels of immunologic markers should be transformed (natural logarithm) before being used in the models. For the transformed variables, we used the formula $100(e^{\beta} - 1)$, where β is the effect estimate, to estimate the percentage increase or decrease in the average level of the immunologic marker in the evaluated group compared to the reference group (16).

Total N-nitrosamine levels were considered to be below the limits of detection when N-nitrosodimethylamine was below the limits of detection *and* the sum of the other N-nitrosamines were not higher than the limits of detection of N-nitrosodimethylamine. In that case, the levels of total N-nitrosamines were assigned a value of $0.1 \mu\text{g}/\text{m}^3$. The models regarding symptoms and immunologic markers were adjusted for gender, age, atopy, smoking, and snuffing. For all analyses, two-sided significance was used. Statistical significance refers to $P \leq 0.05$ or, equivalently, a 95% confidence interval (95% CI) for an odds ratio that excludes unity or a difference that excludes zero. We used SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) for all statistical analyses.

Results

Exposure

The levels of N-nitrosamines differed between workplaces (table 1). High levels were found at company 3 (factory 1, division A) and company 4 (sulfur vulcanization). Regarding specific N-nitrosamines, the highest levels were found for N-nitrosodimethylamine. Intermediate levels of (i) N-nitrosomorpholine, (ii) N-nitrosopiperidine, (iii) N-nitrosodi-n-butylamine,

(iv) N-nitrosodiethylamine, and (v) N-nitrosopyrrolidine were also found in some companies. N-nitrosomethylethylamine and N-nitrosodi-n-propylamine were always less than limits of detection.

The levels of N-nitrosamines differed between different vulcanization methods (table 4). Thus, there were differences in the measured N-nitrosamine levels between workers vulcanizing with injection and compression compared to those vulcanizing with salt baths ($P < 0.001$) and those vulcanizing with hot air, microwaves, and fluid-beds ($P < 0.001$). When comparing the salt bath method with those of hot air, microwave, and fluid-bed, the difference was not statistically significant ($P = 0.11$). The number of workers vulcanizing with each method differed between the three exposure groups (table 4).

There was a difference in the duration of employment between the three exposure groups. Workers with low exposure had a median employment time of 54 months; those with an intermediate exposure had worked for 59 months while highly exposed workers had been employed for 110 months.

Symptoms

Compared to the unexposed subjects, the exposed workers had a statistically significant increase in the risk of itching, running or burning eyes, nosebleeds, throat burning and dryness, hoarseness, severe dry cough, nausea, and headache (table 5). No clear exposure-response relationships were observed. Regarding all symptoms other than eye symptoms, nosebleeds and dyspnea, wheezing, and/or chest tightness, workers exposed to the highest levels of N-nitrosamines had the lowest risk. A sensitivity analysis showed the same pattern when comparing the analyses of the 66 workers with both measured N-nitrosamine levels and symptom registration to those of all 172 workers taking part in the medical examination (data not shown).

Immunologic markers

Compared to unexposed subjects, exposed workers had a statistically significant increase of eosinophils and total IgG levels (table 6). No clear exposure-response relationships were observed. The levels of the examined cells (leukocytes, neutrophils, and eosinophils) increased the most among workers exposed to high levels of N-nitrosamines, whereas total IgM levels showed a dose dependent decrease. As with symptoms, a sensitivity analysis showed the same pattern for the 66 workers with both measured levels of N-nitrosamines and immunologic markers, as when analyzing all 172 workers taking part in the blood analysis (data not shown).

Table 4. Exposure levels by vulcanization method for 96 workers from the Swedish rubber industry from whom N-nitrosamines were measured. [LOD = limits of detection]

Vulcanization method ^a	N-nitrosamines (µg/m ³)		Workers in each group					
	Median	Range	Low(<0.3 µg/m ³)		Intermediate(0.3– 3.0 µg/m ³)		High(>3.0 µg/m ³)	
			N	%	N	%	N	%
Compression and injection (N=63)	0.24	<LOD-2.9	33	52	30	48	0	0
Hot air, microwaves, fluid-bed (N=15)	1.3	<LOD-13	2	13	7	47	6	40
Salt bath (N=16)	4.2	<LOD-36	2	13	2	13	12	75

^a Two employees were foremen and not possible to classify.**Table 5.** The risk of symptoms during the past 12 months among all exposed workers (N=172) and among exposed workers with different levels of N-nitrosamines exposure compared to unexposed subjects (N=118). Categories indicated refer to total levels of N-nitrosamines. Models were adjusted for gender, age, atopy, smoking, and snuffing. **Boldface** indicates statistically significant results (P≤0.05). (OR = odds ratio, 95% CI = 95% confidence interval)

Symptom	All exposed		Level of N-nitrosamines					
	OR	95% CI	Low (<0.3 µg/m ³)		Intermediate (0.3–3.0 µg/m ³)		High (>3.0 µg/m ³)	
			OR	95% CI	OR	95% CI	OR	95% CI
Itching, running or burning eyes	3.0	1.7–5.2	3.0	1.5–5.9	2.7	1.3–5.6	3.3	1.6–6.8
Running nose	1.0	0.59–1.8	1.1	0.51–2.2	1.3	0.61–2.6	0.78	0.34–1.8
Nose stuffiness	0.98	0.57–1.7	1.1	0.57–2.3	1.0	0.49–2.1	0.77	0.35–1.7
Sneezing and/or nose itching	0.93	0.56–1.6	0.95	0.49–1.8	1.1	0.54–2.2	0.78	0.38–1.6
One or more of nasal symptoms mentioned above	1.5	0.9–2.5	1.4	0.75–2.8	1.8	0.87–3.7	1.3	0.65–2.7
Nose bleeding	3.9	1.5–9.7	3.8	1.3–11	3.7	1.2–11	4.2	1.4–13
Throat burning and dryness	3.2	1.7–6.1	3.7	1.7–7.7	3.4	1.5–7.4	2.6	1.1–5.9
Hoarseness	2.4	1.1–5.1	2.6	1.1–6.3	2.7	1.1–7.1	1.8	0.63–5.0
Dyspnea, wheezing and/or chest tightness	1.2	0.68–2.2	1.1	0.49–2.3	1.5	0.70–3.3	1.2	0.52–2.8
Severe dry cough	3.9	1.9–7.8	4.0	1.8–9.2	5.1	2.2–12	2.7	1.1–6.7
Nausea	4.5	1.6–12	5.2	1.7–16	6.1	1.9–20	2.1	0.52–8.8
Headache	2.6	1.5–4.5	2.6	1.3–5.2	3.9	1.9–8.2	1.7	0.80–3.6

Table 6. The risk of increased levels of immunologic markers among all exposed workers (N=172) and exposed workers with different levels of N-nitrosamines exposure compared to unexposed subjects (N=118). Categories indicated refer to total levels of N-nitrosamines. Models were adjusted for gender, age, atopy, smoking, and snuffing. Risk estimates represent percentage change in the average value of the outcome in each examined group compared to the unexposed subjects. **Boldface** indicates statistically significant results (P≤0.05). [95% CI = 95% confidence interval]

Marker	All exposed		Level of N-nitrosamines					
	Risk estimate	95% CI	Low (<0.3 µg/m ³)		Intermediate (0.3–3.0 µg/m ³)		High (>3.0 µg/m ³)	
			Risk estimate	95% CI	Risk estimate	95% CI	Risk estimate	95% CI
Leukocytes	4.3	-1.8–11	2.9	-4.7–11	2.6	-5.5–11	8.0	-0.75–18
Neutrophils	4.0	-3.9–13	3.5	-6.4–15	3.2	-7.3–15	5.5	-5.6–18
Eosinophils	14	0.60–30	11	-6.1–30	7.8	-9.7–29	28	6.4–53
α1-antitrypsin	0.36	-3.3–4.1	1.4	-3.3–6.2	-0.37	-5.3–4.8	-0.23	-5.3–5.1
C-Reactive Protein	1.6	-6.0–9.8	0.24	-9.2–11	7.4	-3.4–19	-2.6	-13–8.7
IgG	11	5.4–16	8.3	1.7–15	16	8.8–24	8.1	0.85–16
IgA	-4.5	-14–5.8	-5.2	-17–8.0	2.4	-11–18	-10	-22–3.5
IgM	4.6	-5.9–16	8.1	-5.6–24	3.2	-11–19	1.3	-13–18
IgE	28	-2.5–69	31	-7.6–86	25	-15–82	28	-13–89

Discussion

This study showed that workers in the Swedish rubber industry are exposed to high levels of N-nitrosamines. Thus, there still seems to be a possible risk of cancer. Moreover, the rubber workers had an increased risk of eye and throat symptoms, hoarseness, cough, nosebleeds, nausea, headache, and changed levels of eosinophils and total IgG. However, no clear exposure-response relationships with the examined symptoms or levels of immunologic markers were found.

There has not been a large number of samplings of N-nitrosamines in the rubber industry. One reason is the rare use of the thermo-energy analyzer in the analysis of N-nitrosamines. Recently Zhao et al (17) presented a well validated LC/MS/MS method for the analysis of N-nitrosamines in water. The LC/MS/MS technique used in this study is sensitive, selective, precise and accurate, with results similar to those obtained from commercial laboratories using the thermo-energy analyzer (figure 1).

In a French survey by Oury et al (18), the sum of N-nitrosamines ranged from 0.05–40 $\mu\text{g}/\text{m}^3$. The median concentration in salt bath vulcanization was 11 $\mu\text{g}/\text{m}^3$. In a study by Reh & Fajen (19) looking at the rubber industry in the United States, the sum of volatile N-nitrosamines was measured in different work categories. Concentrations ranged from 3.1–9.4 $\mu\text{g}/\text{m}^3$, 0.81–16 $\mu\text{g}/\text{m}^3$ and 0.76–6.2 $\mu\text{g}/\text{m}^3$ among salt bath line operators, salt bath line feeders and assistants, and molding and finishing operators respectively. Thus, our study is in accordance with both these studies. However, in an Italian survey by Iavicoli & Carelli (20) most of the personally monitored N-nitrosamine levels were lower than limits of detection (0.06 $\mu\text{g}/\text{m}^3$). In a comparative study by de Vocht et al (21), the levels from our study were found to be higher than what was found in the Netherlands, Germany, UK, and Poland.

The highest levels of N-nitrosamines in our study were found among salt bath vulcanizers. This is probably due to the nitrosation of nitrosable chemicals on the surface of the rubber products by nitrite used for heat transfer in the salt baths (6). Such nitrosable chemicals are secondary aliphatic amines formed from accelerators used in the rubber industry. Carbon disulfide is also formed from these accelerators. In accordance, we found a high correlation between N-nitrosamines in personal air samples and 2-thiothiazolidine-4-carboxylic acid in urine from the same workers (data not shown). We found no difference in N-nitrosamine levels when comparing salt bath vulcanization with other types of continuous vulcanization. This is probably due to the diffusion of N-nitrosamines in halls where different types of continuous vulcanization lines are situated close to each other.

The only available standard for occupational N-nitrosamine exposures is the German target value (Technische Richtkonzentration), which is an exposure limit based on what is technically manageable rather than health effects. The target value for the sum of N-nitrosamines present in air in the rubber industry is 0.5 $\mu\text{g}/\text{m}^3$ for vulcanization in the tire industry and 1 $\mu\text{g}/\text{m}^3$ in all other cases in the rubber industry (22). Almost half of the exposed workers (45%, N=77) in our study exceeded this higher level. Upon the introduction of the German target value, de Vocht et al (21) found a reduction in N-nitrosamine levels. A successful strategy to decrease the high N-nitrosamine levels in the Swedish rubber industry, therefore, could be to introduce a similar target value or, even better, a more health-based occupational exposure limit.

Cancer has been the focus of studies on health effects caused by N-nitrosamines. Straif et al (1) found increased risks of several cancers among subjects assumed to be exposed to low levels of N-nitrosamines. In animal studies, N-nitrosodimethylamine has been shown to be an extremely potent inducer of tumors. Klein et al (23) exposed 36 rats to 120 $\mu\text{g}/\text{m}^3$ of N-nitrosodimethylamine and 13 animals developed tumors in the nose. The N-nitrosamine levels found in our study of the Swedish rubber industry were high and the highest levels were only three times lower than those inducing tumors in a large fraction of exposed animals. Furthermore, the highest levels found in our study may have been underestimated since the capacity of the sampler (about 1500 ng) was over-exceeded in these samples. Thus, there is a considerable need to lower these levels substantially.

In the health monitoring part of this study, we focused on airway diseases and some other non-malignant symptoms. The study participants were almost the same as in our previous work on the relationship between 2-thiothiazolidine-4-carboxylic acid in urine and symptoms/immunologic markers (15). In both these studies, we found increased risks of eye and throat symptoms, as well as nosebleeds, hoarseness, severe dry cough, nausea, and headache among exposed workers. Overall, the risks were lowest in the group of workers exposed to high levels of N-nitrosamines. This might be due to a healthy-worker selection within each company and may also be supported by the difference in the length of employment – workers in the high exposure group had on average been employed much longer than the others – suggesting a stable force of invulnerable workers in this exposure group.

Another possibility could have been that the levels of N-nitrosamines measured by personal monitoring among the 96 workers (table 1), are different than the levels assessed among the whole group of 172 workers

participating in the medical examination. However, our sensitivity analysis does not suggest this.

More probably, other substances found in the rubber fumes and not co-varying with N-nitrosamines, caused the symptoms we registered. Rubber fumes are multitudinous and contain hundreds of compounds; the airway symptoms could, for example, be caused by particles or some reactive chemicals. We found increased levels of eosinophils and total IgG among the exposed workers and a dose-dependent decrease regarding total IgM levels. An earlier study has shown that N-nitrosodimethylamine produces dose-related suppression in IgM responses in mice (4), which is in accordance with our results. Furthermore, earlier studies suggested that IgM contributes to the recognition and elimination of pre-cancerous and cancerous lesions (24).

In conclusion, we found high air levels of N-nitrosamines in the Swedish rubber industry. These levels need to be considerably lowered in order to lower the risk of cancer. We found no clear associations between the exposure to N-nitrosamines and the non-malignant symptoms studied.

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References

1. Straif K, Weiland SK, Bungers M, Holthenrich D, Taeger D, Yi S, et al. Exposure to high concentrations of nitrosamines and cancer mortality among a cohort of rubber workers. *Occup Environ Med.* 2000;57(3):180–7.
2. Desjardins R, Fournier M, Denizeau F, Krzystyniak K. Immunosuppression by chronic exposure to N-nitrosodimethylamine (NDMA) in mice. *J Toxicol Environ Health.* 1992;37(3):351–61.
3. Holsapple MP, Bick PH, Duke SS. Effects of N-nitrosodimethylamine on cell-mediated immunity. *J Leukoc Biol.* 1985;37(4):367–81.
4. Holsapple MP, Tucker AN, McNerney PJ, White KL Jr. Effects of N-nitrosodimethylamine on humoral immunity. *J Pharmacol Exp Ther.* 1984;229(2):493–500.
5. Fajen JM, Carson GA, Rounbehler DP, Fan TY, Vita R, Goff UE, et al. N-nitrosamines in the rubber and tire industry. *Science.* 1979;205(4412):1262–4.
6. Spiegelhalter B, Preussmann R. Occupational nitrosamine exposure. 1. Rubber and tyre industry. *Carcinogenesis.* 1983;4(9):1147–52.
7. Fine LJ, Peters JM. Respiratory morbidity in rubber workers: II. Pulmonary function in curing workers. *Arch Environ Health.* 1976;31(1):10–4.
8. Weeks JL, Peters JM, Monson RR. Screening for occupational health hazards in the rubber industry. Part II: health hazards in the curing department. *Am J Ind Med.* 1981;2(2):143–51.
9. Weeks JL, Peters JM, Monson RR. Screening for occupational health hazards in the rubber industry. Part I. *Am J Ind Med.* 1981;2(2):125–41.
10. Zuskin E, Mustajbegovic J, Schachter EN, Doko-Jelinic J, Budak A. Longitudinal study of respiratory findings in rubber workers. *Am J Ind Med.* 1996;30(2):171–9.
11. Jönsson LS, Broberg K, Bergendorf U, Axmon A, Littorin M, Jönsson BAG. Levels of 2-thiothiazolidine-4-carboxylic acid (ITCA) and effect modification of polymorphisms of glutathione-related genes in vulcanization workers in the southern Sweden rubber industries. *Int Arch Occup Environ Health.* 2007;80(7):589–98.
12. Jönsson LS, Broberg K, Axmon A, Bergendorf U, Littorin M, Jönsson BAG. Levels of 1-hydroxypyrene, symptoms and immunologic markers in vulcanization workers in the southern Sweden rubber industries. *Int Arch Occup Environ Health.* 2008;82(1):131–7.
13. Nielsen J, Welinder H, Schütz A, Skerfving S. Specific serum antibodies against phthalic anhydride in occupationally exposed subjects. *J Allergy Clin Immunol.* 1988;82(1):126–33.
14. Littorin M, Rylander L, Skarping G, Dalene M, Welinder H, Strömberg U, et al. Exposure biomarkers and risk from gluing and heating of polyurethane: a cross sectional study of respiratory symptoms. *Occup Environ Med.* 2000;57(6):396–405.
15. Jönsson LS, Broberg K, Axmon A, Jönsson BAG, Littorin M. Symptoms and immunologic markers among vulcanization workers in rubber industries in southern Sweden. *Scand J Work Environ Health.* 2007;33(4):272–9.
16. Vittinghoff E, Shiboski SC, Glidden DV, McCulloch CE. Regression methods in biostatistics. Linear, logistic, survival and repeated measures models. New York (NY): Springer; 2005.
17. Zhao YY, Boyd J, Hrudef SE, Li XF. Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry. *Environ Sci Technol.* 2006;40(24):7636–41.
18. Oury B, Limasset JC, Protois JC. Assessment of exposure to carcinogenic N-nitrosamines in the rubber industry. *Int Arch Occup Environ Health.* 1997;70(4):261–71.
19. Reh BD, Fajen JM. Worker exposures to nitrosamines

- in a rubber vehicle sealing plant. *Am Ind Hyg Assoc J*. 1996;57(10):918–23.
20. Iavicoli I, Carelli G. Evaluation of occupational exposure to N-nitrosamines in a rubber-manufacturing industry. *J Occup Environ Med*. 2006;48(2):195–8.
 21. de Vocht F, Burstyn I, Straif K, Vermeulen R, Jakobsson K, Nichols L, et al. Occupational exposure to N-nitrosodimethylamine and N-nitrosomorpholine in the European rubber industry. *J Environ Monit*. 2007;9(3):253–9.
 22. Technische Regeln für Gefahrstoffe, N-Nitrosamine [Technical Standards for Hazardous Materials, N-nitrosamines] (TRGS 552). Bundesanstalt für Arbeitsschutz und Arbeitsmedizin. Bremerhaven (Germany): NW-Verlag; 2007.
 23. Klein RG, Janowsky I, Pool-Zobel BL, Schmezer P, Hermann R, Amelung F, et al. Effects of long-term inhalation of N-nitrosodimethylamine in rats. Lyon: International Agency for Research on Cancer (IARC); 1991. IARC Scientific Publications, volume 105, p 322–8.
 24. Vollmers HP, Brändlein S. Natural antibodies and cancer. *J Autoimmun*. 2007;29(4):295–302.

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