

Specific antibodies to methyltetrahydrophthalic anhydride in exposed workers

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Summary

A group of 145 workers exposed to methyltetrahydrophthalic anhydride (MTHPA) was investigated. They were working in a plant which, since 1983, handled an epoxy resin with MTHPA as a hardener. Specific IgE antibodies (RAST) to a conjugate between MTHPA and human serum albumin (HSA) were statistically significantly increased ($P=0.001$; 26 subjects = 18% positive) in the exposed group, compared to a non-exposed control group ($n=33$). One positive worker was only exposed for 2 months. Twenty-three exposed subjects were also skin-prick test positive to MTHPA-HSA. The exposed group was divided into three different exposure categories, according to their contact with the epoxy resin. The average exposure levels at the time of the investigation were, in zone I 0.085 mg/m³, in zone II 0.014 mg/m³, and in zone III 0.010 mg/m³, though the exposure probably had been higher earlier. There was an association between exposure intensity and RAST-positive persons ($P=0.0025$, chi-square trend test). Forty-four persons (30%) were smokers, and 16 (11%) atopics. No association between sensitization and either atopy or smoking was found. There was an association between exposure intensity and specific IgG antibodies ($P=0.0003$, chi-square trend test). Specific IgG₄ antibodies were closely related to specific total IgG antibodies ($P=0.0001$). These findings demonstrate that MTHPA is a sensitizing agent at low levels of exposure.

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Introduction

Acid anhydrides are small reactive organic molecules with a wide use in the chemical industry. The acid anhydrides have an irritating effect on mucous membranes, causing lacrimation, rhinorrhoea, and cough. Rhinitis and asthma have been described in association with anhydride exposure [1–5]. Specific serum IgE antibodies and positive skin-prick tests have been found in patients exposed to phthalic anhydride (PA) [6–8], trimellitic anhydride (TMA) [1,7,9,10], tetrachlorophthalic anhydride (TCPA) [11], hexahydrophthalic anhydride (HHPA) [12], maleic anhydride (MA) [13], himic anhydride [14], and methylhexahydrophthalic anhydride [15]. These data indicate an IgE-mediated mechanism, at least in some cases. Conjugates between the anhydrides and human serum albumin

(HSA) have been used as antigens in the immunoglobulin assays.

Specific IgG antibodies have been found in high prevalences in groups of PA-exposed workers. Further, there is an association between specific IgG antibodies and exposure intensity [8,16]. The level of specific IgG antibodies against TMA-HSA were higher in workers with systemic reactions, than in subjects with only asthmatic or irritative symptoms [9,10]. The IgG antibodies are generally not assumed to be involved as a pathogenic factor in occupational asthma. However, Akiyama *et al.* [17] suggested a mechanism for the involvement of IgG antibodies in immediate-type reactions, cases in whom IgE is not presented. Nielsen *et al.* [8] found an association between specific IgG₄ antibodies to PA and symptoms. The hypothesis, that IgG₄ may be a pathogenic factor, is interesting, but controversial [18,19].

Exposure data for anhydrides are scanty and sometimes not reliable. Dose-response investigations

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providing data upon which threshold limit values (TLV) may be based are few.

We have already described a case of sensitization to another anhydride, methyltetrahydrophthalic anhydride (MTHPA) [20]. In the present investigation, the relation between exposure and the levels of total and specific IgE and IgG antibodies in persons exposed to MTHPA were investigated in a cross-sectional study.

Materials and methods

Production and exposure

The plant is producing and manufacturing varying plastic products. A main product is barrels for grenade firearms based on an epoxy resin with a 1:1 addition of MTHPA as a hardener. Rotating cylinders were prepared by hand swabbing, with a mixture of epoxy compound, MTHPA, and methylimidazol on glass fibre. Then the cylinders were wound automatically with glass-fibre cords, which had been running through a bath containing the epoxy resin mixture. The cylinders passed a curing oven at a temperature of 90–120°C. After curing, the plastic barrels were processed mechanically. In total, 54 persons were working with this product on a special line situated at one end of a big workshop. Continuous production started in 1983. The production was doubled in 1986 by introduction of a new production line. There has been continuous efforts to reduce the exposure to vapours from the epoxy resin and especially during July 1987. It was found that air moved from the epoxy line to other parts of the workshop both before and after the rebuilding. Measurements of the levels of MTHPA in air started in November 1987.

In the rest of the workshop, a variety of different thermoplastics were manufactured in forming machines. In other parts of the factory small amounts of other anhydrides (MA, tetrahydrophthalic anhydride, HHPA, and PA) are used in the production.

Subjects

All in the workforce who were employed and available in the workshop were offered an investigation in October–November 1988. No former employees were included. In total, 157 persons were employed in the workshop and 134 (86%) investigated. Only one refused to take part. Twenty-two persons were sick or on leave due to studies, vacation, military service, etc. There was nothing notable about those absent. Another 11 persons, e.g. repairmen and technicians, from other parts of the factory with periodical exposure were included. Thus, 145 exposed workers were studied of which 13 (9%) were females. They were divided into different exposure categories i.e. zones I–III. Twenty-two subjects could not be defined as

belonging to one of these categories; they were designated as 'others'. In zone I, the workers were handling the epoxy system directly; zone II was made up by adjoining departments; and zone III comprised the departments at the other side of the workshop.

As control subjects (group 1), 33 persons, (three females), from a mechanical workshop situated nearby were studied. Their exposure to dust and irritants were low. In addition, for an antibody study, a group of 30 local government employees (group 2) from another area, with no history of anhydride exposure was included.

Medical examination

In each case we recorded an occupational history and smoking habits. Venous blood samples were analysed for total serum levels of IgE, IgG, and IgM, and for specific IgE and IgG antibodies. In all subjects, skin-prick tests with 13 common allergens (Allergologisk Laboratorium, Copenhagen, Denmark) and with a conjugate of MTHPA and HSA (15 mg/ml) were performed. Positive prick-test reactions were scored when the area of the weal was at least half that induced by histamine (1 mg/ml). Reaction sizes were recorded in relation to histamine according to Aas and Belin [21] (1/4 of the histamine weal = +, 1/2 = ++, 1/1 = +++, 2 × = +++++, 4 × = ++++++, 8 × = ++++++). Persons, with one positive or more readings in the standard skin-prick test, were described as atopic.

MTHPA in air

MTHPA levels in air were determined by personal and area sampling on XAD-2 tubes (No. 226–30, SKC Inc., Eighty Four, PA, U.S.A). The sampling rate was ≤ 0.2 l/min and the sampling volume 10–60 l. MTHPA was desorbed by 1 ml of toluene during 1 hr. The anhydride was analysed by gas chromatography, with a flame ionization detector (GC-FID). The limit of detection was 0.2 µg/ml toluene, corresponding to 0.01 mg/m³ at a sampling volume of 20 l. The GC-FID analysis gave 20% higher results, compared to an analysis by GC with a mass selective detector (GC-MS) in 16 samples. The accuracy of the sampling method was checked by parallel sampling in impingers containing 5 ml of 0.1 M NaOH. The MTHPA formed in the impinger was analysed as the dimethyl ester with GC-FID after esterification with BF₃/methanol. In 11 impinger samples we got a recovery of 75% compared to the XAD-2 sampling method. MTHPA could not be detected on glass-fibre filters.

Antibody determinations

The antibody determinations followed, with slight modifications, a procedure that has been described in detail previously (Nielsen *et al.* 1988). Thus, here we only present the fundamental features.

Antigen. A hapten conjugate was prepared from MTHPA (Ciba-Geigy AB, Basel, Switzerland) and HSA (Kabi, Stockholm, Sweden); 85 mg of MTHPA was added to a cooled solution of 300 mg HSA in 100 ml of 0.1 M NaHCO₃. The protein was purified from low molecular weight compounds (< 30 000 daltons) by filtration in an Amicon ultra filtration cell (8200; Amicon Corp., Danvers, Mass., U.S.A.). The protein was lyophilized and reconstituted in 0.1 M NaHCO₃ when used.

Specific IgE antibodies. The MTHPA-HSA conjugate was bound to cyanogen bromide-activated filter paper discs for use in RAST. We used the Phadebas RAST system (Pharmacia Diagnostics AB, Uppsala, Sweden), following the standard procedure, using 50 µl serum and 50 µl anti-IgE ¹²⁵I solution. All samples were analysed twice. Results were expressed as % specific binding (c.p.m. of test disc - c.p.m. of HSA reference disc) of total added radioactivity. A control serum with a total IgE of 3000 kU/l had a RAST value of 0.1, which is within the reference range.

RAST inhibition tests were performed on sera from five persons with IgE-MTHPA-HSA in the range 10.9-27.0% binding. Fifty microlitres of serum were incubated with 50 µl of the MTHPA antigen in different dilutions (3.0, 0.6, 0.12, 0.024, 0.0048 and 0 mg/ml RAST-buffer solution) at 8°C overnight. Then, the RAST assay was performed as described.

Specific IgG antibodies to MTHPA-HSA were analysed by an ELISA assay. Wells of polystyrene microtitre plates (Nunc-Immuno Plate 1, Nunc, Kamstrup, Denmark) were coated by adding 100 µl of the antigen solution (150 µg/ml; 0.005 M PBS, pH 7.2). The wells were washed six times (Titertek Microplate Washer, Flow Laboratories, Irvine, U.K.) with a washing solution, after storage in a refrigerator overnight, and incubated with blocking solution. The plates were stored with blocking solution at -18°C. Analytical procedure: (i) washing six times, (ii) addition of 100 µl of a 1:50 dilution (PBS) of serum, (iii) incubation for 60 min at 20°C, (iv) washing six times, (v) addition of 100 µl of an optimal dilution of alkaline phosphatase conjugated rabbit anti-human IgG (Dakopatts, Copenhagen, Denmark), (vi) incubation for 60 min at 20°C, (vii) washing 10 times, (viii) addition of 100 µl substrate solution (disodium *p*-nitrophenol phosphate),

and (ix) incubation for 120 min at 20°C. The results were read at 405 nm (Titertek-Multiskan, Eflab Oy., Helsinki, Finland). All samples were analysed in triplicate and read against an HSA antigen as a control for non-specific binding to MTHPA-HSA. The results are expressed as the absorbance (optical density, OD) value. All values were corrected for the absorbance of positive controls on each microtitre plate. Subjects and controls were mixed on the plates.

Specific IgG₄ antibodies. The procedure for the subclass assay was mainly the same as described above for the IgG assay: (i) coating of the wells with MTHPA-HSA antigen, (ii) washing six times, (iii) addition of 50 µl serum (1:50), (iv) incubation for 60 min at 20°C, (v) washing six times, (vi) addition of 100 µl of mouse anti-human IgG₄ (diluted 1:1000; Unipath Ltd, Bedford, U.K.), (vii) incubation for 60 min at 37°C, (viii) washing six times, (ix) addition of 100 µl of an optimal dilution (roughly 1:3000) of rabbit anti-mouse IgG conjugated with alkaline phosphatase (Sigma Chemical Co., St Louis, MO., U.S.A.), (x) incubation for 60 min at 20°C, (xi) washing 10 times, (xii) addition of 100 µl substrate solution, and (xiii) incubation for 120 min at 20°C. The results were read as above.

Statistics

For comparison of proportions, the chi-square and Fisher two-tailed test were used. The chi-square trend test was used for test of trends. Spearman's rank-correlation test was used for testing of the association between pairs of variables. For comparisons of distributions between different groups, then Mann-Whitney *U*-test was used. All *P*-values are two-tailed. 'Statistically significant' refers to *P* < 0.05.

Results

MTHPA exposure

The results of the samples relevant for inhalation exposure obtained in the different work zones are presented in Table 1. The exposure to MTHPA varied between <0.001 and 0.38 mg/m³. The average exposure level for the most exposed group, working with wet material in zone I, was 0.10 mg/m³.

Up to 3 mg MTHPA/m³ was measured close to heated, wet material before curing.

Medical examination

There were 44 (30%) smokers, 28 ex-smokers (19%), and 17 (12%) atopics among the exposed workers. In zone I

the subjects were younger (not significant) and there were more non-smokers (72%, $P < 0.01$) than in the other exposure groups. There were no significant differences between the groups with regard to atopy.

Twenty-three (16%) of the exposed subjects were skin-prick test positive to MTHPA-HSA (Table 2). Scores up to + + + + + were recorded. There were more positive persons in zones I-II, compared to zone III, but the differences were not statistically significant. Among the 'other' subjects the prevalence was similar to zones I and II. Nobody in the control group had a positive prick test to MTHPA-HSA. There was a significant rise of the

prevalences over the exposure categories controls/zone III/zone II/zone I (others not included, $P > 0.008$).

Antibody findings

There were no significant differences between the groups with regard to total immunoglobulin levels (Table 2).

The levels of specific IgE antibodies to MTHPA-HSA were significantly ($P = 0.001$) increased in the exposed workers, compared to control group 1 (Fig. 1). The specific IgE levels were significantly increased in zone I ($P = 0.0001$) and zone II ($P = 0.04$), but not in zone III, compared to control group 1 (Fig. 1). There was no statistically significant correlation between serum levels of total IgE and specific IgE to MTHPA-HSA.

The RAST values for the 63 control subjects in the two control groups were in the range 0.0-0.3% binding. Values in the exposed group higher than the highest control value were regarded as positive. There was a significant rise of the number of positives over the exposure categories controls/zone III/zone II/zone I ('others' not included, $P = 0.003$; Table 2). The median exposure time for MTHPA in IgE-MTHPA-HSA positive persons was 5 years (range 0.2-5).

In the five persons tested, 61-78% inhibition of the IgE-MTHPA-HSA binding was reached at the addition of 0.24 µg MTHPA-HSA and 91-95% at 6 µg.

There was an excellent association ($P < 0.001$) between positive specific IgE and positive skin-prick tests to MTHPA-HSA. Everybody with positive skin-prick test

Table 1. The level of exposure to methyltetrahydrophthalic anhydride (MTHPA) in three different parts of the workshop

	Samples (no.)	Sampling (hr)	MTHFA (mg/m ³)
Zone I	40*	148	0.085 (0.007-0.38)
Wet part	32	117	0.10 (0.020-0.38)
Dry part	8	31	0.015 (0.007-0.030)
Zone II	12†	57	0.014 (<0.001-0.030)
Zone III	4‡	17	0.010 (0.007-0.014)

* Personal sampling.

† Personal and area sampling.

‡ Area sampling.

Table 2. Atopy, smokers, specific skin-prick tests and number of persons with specific antibodies to a hapten conjugate of methyltetrahydrophthalic anhydride and human serum albumin (MTHPA-HSA) and total immunoglobulin levels (median; ranges in parentheses) in different exposure categories in workers exposed to MTHPA and a control group

	Exposed workers				Control group (n=33)
	Zone I (n=54)	Zone II (n=46)	Zone III (n=23)	Others (n=22)	
Atopy (no., %)	6 (11)	5 (11)	2 (9)	4 (18)	6 (18)
Smokers (no., %)	6 (11)	21 (46)	9 (39)	8 (36)	8 (24)
Pos. skin-prick test (no., %)	10 (19)	7 (15)	2 (9)	4 (18)	0
Total IgE (kU/l)	32 (2-411)	19 (1-442)	13 (1-311)	38 (3-345)	28 (1-525)
Total IgG (mg/l)	9.8 (6.1-36.7)	9.3 (5.1-20.3)	10.7 (5.7-17.2)	9.6 (6.9-13.1)	10.5 (5.6-13.9)
Total IgM (mg/l)	0.9 (0.2-2.8)	0.9 (0.3-5.9)	1.0 (0.5-2.2)	1.0 (0.4-2.0)	1.0 (0.5-1.7)
Specific IgE (no., %)	12 (22)	8 (17)	2 (9)	4 (18)	
Specific IgG (no., %)	13 (24)	2 (4)	1 (4)	1 (5)	

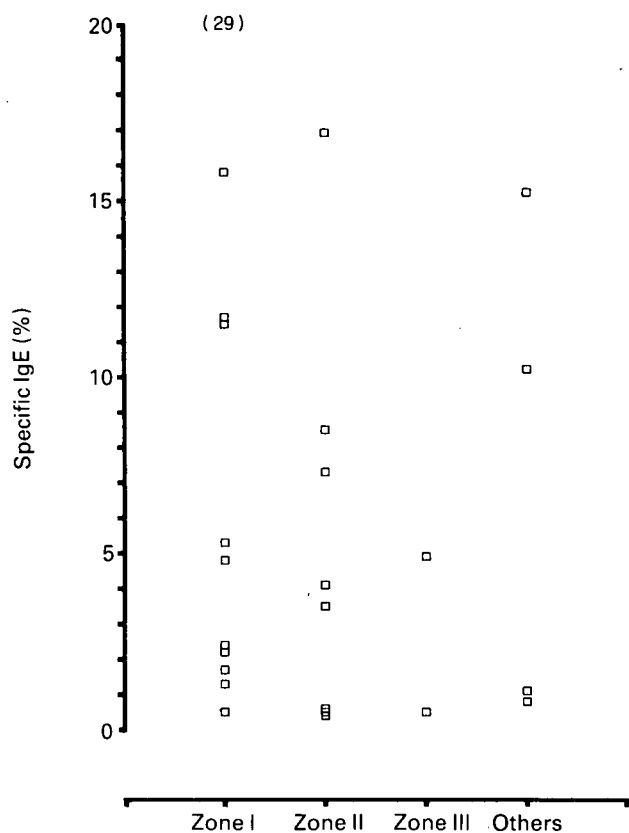


Fig. 1. Serum levels of 26 workers with positive specific IgE antibodies (% binding in RAST test) to methyltetrahydrophthalic anhydride (MTHPA-HSA) in different exposure categories.

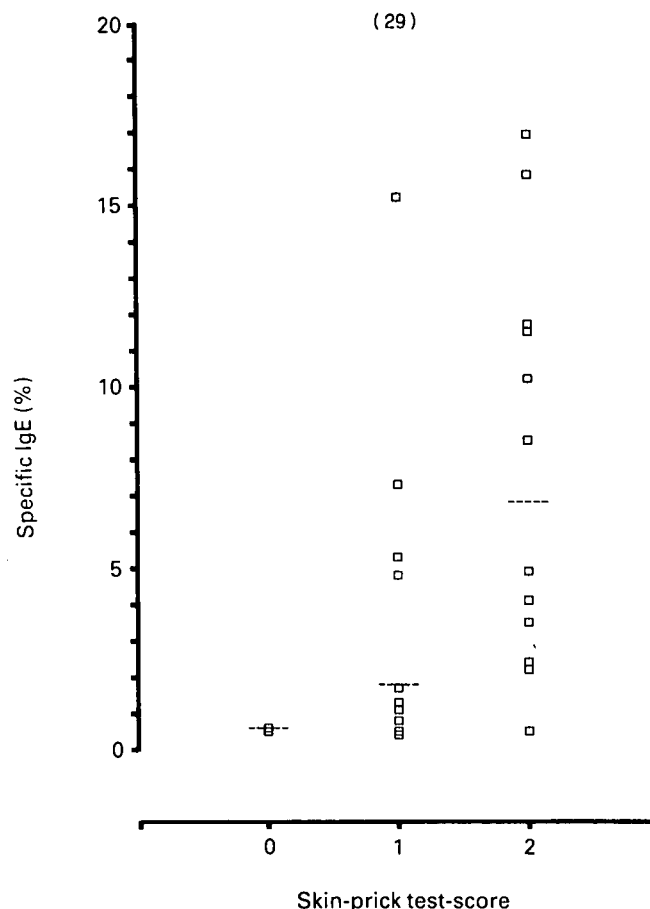


Fig. 2. Relationship between skin-prick tests (scores -, + = 0; ++ to ++++ = 1; +++++ or higher = 2) and serum levels of specific IgE in 26 workers exposed to methyltetrahydrophthalic anhydride and positive to specific IgE.

was RAST positive, whereas three persons with RAST values of 0.5, 0.5, and 0.6% were prick-test negative. Three persons with RAST values of 0.4, 0.5, and 0.5 were prick-test positive (Fig. 2).

The levels of specific IgG antibodies to MTHPA-HSA in the two control groups were in the range 0.00–0.65 OD ($n=63$). There was no significant difference between exposed subjects and control group 1. However, the level was significantly higher in zone I than in the other zones or in the controls ($P<0.02$; Fig. 3).

Significantly more subjects in zone I were positive to specific antibodies of IgG type to MTHPA-HSA than in the other zones (Table 2). There was a significant rise of the number of positives over the exposure categories controls/zone III/zone II/zone I ('others' not included; $P=0.0003$). There was also a significant association between skin-prick test positivity and positive specific IgG antibodies ($P<0.001$).

Specific IgG₄ serum levels were associated with total specific IgG ($r_s=0.7$, $P=0.0001$). Accordingly all IgG positive persons were also positive in IgG₄.

Table 3. Atopy and smoking habits in persons positive in specific antibodies to a hapten conjugate of methyltetrahydrophthalic anhydride (MTHPA) and human serum albumin (HSA)

	Atopy		Smoking	
	No	Yes	No	Yes
Pos. IgE-MTHPA-HSA (no.)	25	1	22	4
Neg. IgE-MTHPA-HSA (no.)	104	15	79	40
Pos. IgG-MTHPA-HSA (no.)	16	1	15	2
Neg. IgG-MTHPA-HSA (no.)	113	15	86	42

No significant associations were found between the presence of specific IgE or IgG to MTHPA on the one hand and atopy of smoking on the other (Table 3).

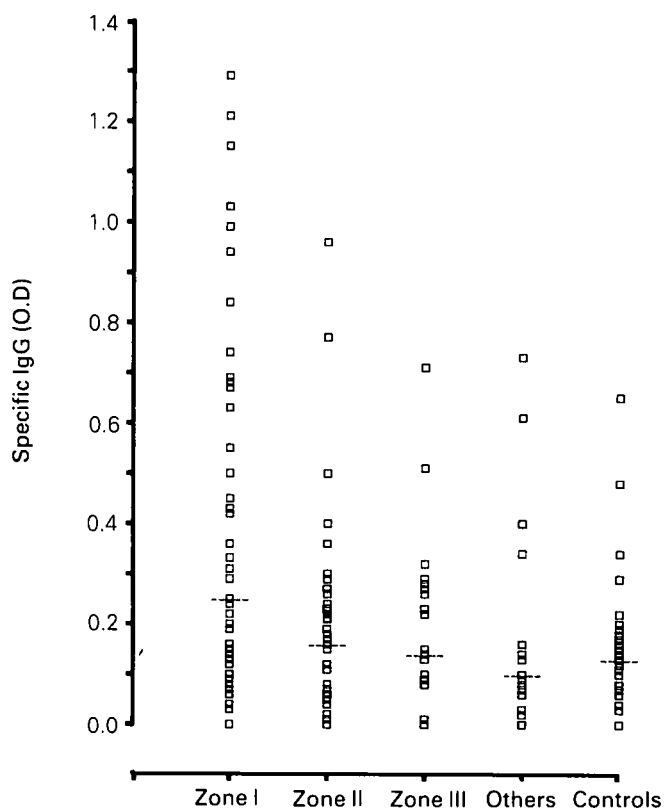


Fig. 3. Serum levels of specific IgG (optical density, OD) to methyltetrahydrophthalic anhydride (MTHPA-HSA) in different exposure categories.

Discussion

A high prevalence (18%) of sensitized workers was found in the factory handling MTHPA. The MTHPA exposure originates mainly from handling of 'wet' material before the curing. A job rotation system did not make it possible to differentiate the workers in zone I in different exposure categories. Zones II and III have similar levels of exposure and could have been combined in the presentation of the results. However, there may still be differences in the exposure which are not obvious from our measurements, for example differences in peak exposures. MTHPA has a relatively high vapour pressure. In accordance with this, the results of the different ways of sample collection indicate an inhalation of MTHPA in vapour form. The level measured reflects the conditions after the changes in the process in the middle of 1987 and the medical examination was done in 1988. Before 1987 the exposure may have been higher. Estimations of how much higher the exposures may have been, can only be very loose guesses in our opinion. Thus, the prevalence of antibodies may partly reflect exposures other than these measured. However, three of the sensitized workers were

employed after the elimination measures were taken. One of these had been employed for only 2 months, during a period when there were probably no peaks.

The average exposure level was surprisingly low (<0.1 mg/m³), compared to the TLV [22] for PA and MA (6 and 0.3 mg/m³ time-weighted average level, respectively). The levels are comparable with the TLV value for TMA (0.04 mg/m³).

An excellent sensitivity and specificity of the RAST test of IgE-MTHPA was proven by good correlation with skin-prick tests, and by the RAST inhibition tests. The persons in zones I-III were not exposed to other anhydrides. Thus, MTHPA is the responsible compound.

The prevalence of specific IgE antibodies to MTHPA is high compared to most small organic reactive compounds including other acid anhydrides and other compounds, with the exception of proteins. Moreover, it must be stressed that our study is cross-sectional; a healthy worker selection may thus have occurred. This may also have 'flattened' the exposure-response relationships and exposure categories/antibody levels. In these analyses the group 'others' was excluded, because many of the subjects moved in all areas, and the group also contained subjects removed from exposure because of symptoms.

The prevalence of IgE antibodies is much higher in the present study than in our findings in workers exposed to much higher levels of PA [8]. The prevalence is comparable to the findings by Moller *et al.* [12] for HHFA, but the exposure levels for these workers were much higher (0.6-8 mg/m³). Newman Taylor *et al.* [4] reported that 24 out of 300 TCPA-exposed workers were positive in specific IgE antibodies. The latter authors also found a high correlation between antibodies on one hand and a combination of smoking and exposure on the other. This was not seen in our study, as there was not a higher frequency of smokers among the sensitized subjects than among the non-sensitized ones, indicating that smoking did not facilitate sensitization. This is in accordance with a previous study of PA-exposed workers [8]. However, it is interesting to note that the fraction of smokers was lower in zone I than in other areas, which might indicate a selection.

In the present study, as in the study of PA-exposed workers, we found that the workers in the most exposed group often had specific IgG antibodies. However, the present study did show a closer association between specific IgG₄ and total specific IgG. This may be an effect of the different compounds and/or the different nature of the exposure (vapour vs. dust).

Some studies have indicated that atopy may run a particularly high risk to be sensitized to acid anhydrides [5]. In the present study we did not find that atopic subjects were more likely to develop specific antibodies

than non-atopics. In fact, we found specific antibodies (IgE as well as IgG) less frequently among the atopics. As there are only 11% atopics in the exposed workers compared to 18% in the controls, we may again have an indication of a healthy worker selection. We also know that the company physician has recommended the company not to employ atopics. More definite conclusions from the relation between sensitization and smoking/atopy can be made after an investigation of the leavers.

Work-related symptoms and lung function in the workers will be reported separately.

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