

# Symptoms and immunologic markers induced by exposure to methyltetrahydrophthalic anhydride

Nielsen J, Welinder H, Bensryd I, Andersson P, Skerfving S. Symptoms and immunologic markers induced by exposure to methyltetrahydrophthalic anhydride.

Allergy 1994; 49: 281–286. © Munksgaard 1994.

A study was performed in 43 workers exposed to methyltetrahydrophthalic anhydride (MTHPA) used as a hardener in an epoxy resin system. Ten workers sensitized to MTHPA (group SS; presence of serum IgE antibodies against a conjugate of MTHPA and human serum albumin (HSA) detected by RAST) had significantly higher levels of tryptase in nasal lavage fluid than 19 nonsensitized workers with work-related nasal symptoms (group NS) and 14 nonsensitized workers without nasal symptoms (group NN). This suggests an ongoing mast-cell-mediated reaction in the sensitized group. No statistically significant differences were found in the three groups concerning eosinophil cationic protein (ECP) and TAME-activity in lavage fluid. However, there was a significant increase in serum ECP in the SS group, as compared with a group of unexposed controls. Nasal challenge with MTHPA-HSA, performed in a subsequent study in seven workers from the SS group, six from the NS group, and seven from the NN, caused a larger increase of symptom score and a more pronounced decrease in nasal inspiratory peak flow in the SS group than in the other two groups. No significant rise was recorded for tryptase and ECP in lavage fluid in any of the three groups after challenge. The combined results of the two studies indicate that specific IgE antibodies play a pathogenetic role in at least some of the cases of work-related nasal symptoms associated with MTHPA exposure.

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Key words: challenge; eosinophil cationic protein; methyl histamine; nasal lavage; organic acid anhydride; specific antibodies; TAME.

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Accepted for publication 12 July 1993

Organic acid anhydrides such as methyltetrahydrophthalic anhydride (MTHPA) are used in the production of alkyd and polyester resins and as curing agents for epoxy resins. Studies of effects from exposure to acid anhydrides have mainly been concerned with symptoms from the lower airways. However, in earlier studies from our group, symptoms from eyes and nose are much more frequently reported than asthma by workers exposed to phthalic anhydride (10, 20) or MTHPA (11). Even if nasal symptoms are not considered to be as serious as asthma, they can be disabling for many patients. Moreover, nasal symptoms may precede occupational asthma (20); thus, studies of such symptoms are important.

The mechanism behind symptoms related to organic acid anhydride exposure has not been totally elucidated. The organic acid anhydrides have irritant as well as sensitizing properties. Specific serum anti-

bodies of IgE, IgG, IgA, and IgM class against several acid anhydrides have been described (15). It is generally claimed that specific IgE is the class of antibodies which best distinguishes symptomatic from nonsymptomatic organic acid anhydride-exposed workers (15). However, several studies present a more complex picture of the association between symptoms and specific antibodies of IgE and IgG class (10, 16, 21).

We have earlier noted that nonsensitized MTHPA-exposed workers suffer from symptoms nearly as frequently as IgE-sensitized workers (11). Thus, the clinical consequences of IgE sensitization to organic acid anhydrides are not obvious. To clarify this matter, we have now studied the clinical effects, as well as the immunologic markers in nasal lavage fluid, in relation to challenge with a conjugate of MTHPA and human serum albumin (HSA) in sensitized and nonsensitized subjects, the latter with or

without work-related nasal symptoms. Furthermore, we have studied whether immunologic markers in serum, in fluid from nasal lavage, and in urine obtained in the workplace differ in these three groups of workers. To our knowledge, such studies have not before been undertaken in acid anhydride-exposed subjects.

## Material and methods

### *Subjects and medical examinations*

In the study of *immunologic markers* in serum, in nasal fluid, and in urine *obtained in the workplace*, 43 subjects working in a plant producing barrels for rocket guns, based on an epoxy resin system with MTHPA as a hardener and methylimidazol (0.3%) as an accelerator, participated (17). Their exposure to MTHPA ranged from 20 to 100  $\mu\text{g}/\text{m}^3$ ; time-weighted average. In relation to the study, a medical and occupational history was obtained, and the atopic status was assessed by history and a skin-prick test against 13 common allergens (11). Furthermore, a skin prick test with MTHPA-HSA was performed, as earlier described (17). A physical examination was done, and venous blood samples were collected for analysis of specific IgE- and IgG-antibodies against MTHPA-HSA and eosinophil cationic protein (ECP). Urine was sampled for analyses of methyl histamine.

Ten of the workers were sensitized to MTHPA (group SS; presence of specific serum IgE in a radioallergosorbent test (RAST) to a conjugate of MTHPA and HSA). Eight subjects in this group had specific serum IgG antibodies against MTHPA-HSA. Because of symptoms and sensitization, seven subjects had been taken out of exposure for 2–3 weeks. Before the study, they had been reexposed for from 0.5 to 1.5 working-days. The other 33 workers showed no signs of "IgE sensitization". Nineteen of them suffered from work-related nasal symptoms (group NS) and only one of them had specific serum IgG antibodies against MTHPA-HSA. The remaining 14 subjects did not complain of work-related nasal symptoms (group NN), and three of them had specific serum IgG antibodies.

As control subjects, 27 workers from a nearby mechanical workshop were studied. They were not exposed to organic acid anhydrides, and their exposure to potentially irritant/sensitizing aerosols and vapors was low.

Later, after a large MTHPA-exposure reduction (17), it was possible to perform a *nasal challenge study* in the group. At that time, seven subjects in the SS group were available for testing. Two of them had a history of atopy, and a third was positive in the standard skin prick test. None were smokers. All had experienced work-related nasal symptoms.

Seven workers from each of the other two groups (matched with regard to age) were asked to participate. One had a common cold on the test day; thus, group NS comprised six subjects, one of whom had a history of atopy. None were smokers. In the NN group, none of the seven subjects were atopics. One subject was a smoker. All 20 workers were medically reexamined before the challenge, as described above.

### *Nasal challenge procedure*

The challenge was performed according to the procedure (slightly modified) described by Andersson et al. (2). With a De Vilbiss sprayer (atomizer No. 15), 0.1 ml of a 1:100 solution in isotone saline of the MTHPA-HSA conjugate used for skin prick tests was sprayed into each nasal cavity (challenge 1). Before and 10–12 min after each challenge, registration of symptoms and nasal inspiratory peak flow were performed. If no serious symptoms were registered, the challenge was repeated, with the conjugate in a solution of 1:10 (challenge 2) and later undiluted (challenge 3). Nasal lavage was performed before and 12–15 min after the last challenge.

Nasal symptoms were recorded according to Malm et al. (9). Blockage and secretion were each rated on a scale of 0–3 (no symptoms = 0, mild = 1, moderate = 2, and severe = 3). The rating was performed for each nose cavity, and the average was obtained. The sneezes were counted. Furthermore, a composite nasal symptoms score was calculated by adding the score for blockage, secretion, and sneezing (no sneeze attacks = 0, 1–5 = 1, 6–15 = 2, > 15 = 3).

Immediately after registration of symptoms, nasal inspiratory peak flow was performed with a Youlten peak nasal inspiratory flow meter (Clement Clarke International Ltd, London, UK). The best of three trials was noted.

Nasal lavage was performed before and 12–15 min after the last challenge, by the method described by Greiff et al. (5). A compressible plastic container with a nasal adapter, filled with 25 ml isotone saline solution, was inserted into the nasal opening of the subject, who was sitting in a forward-flexed neck position. By compression of the container, the solution was blown into one of the nasal cavities. The transmission was stopped when the solution appeared in the opposite nostril. By the vacuum generated in the container when compression was reduced, the solution was sucked back into the container and then again blown into the cavity. This procedure was repeated three times. The lavage fluid obtained was immediately chilled and later frozen at  $-70^\circ\text{C}$  for subsequent analysis without previous centrifugation.

### Laboratory analyses

**Specific serum IgE antibodies.** HSA conjugates were prepared and used in RAST (Phadebas RAST system, Pharmacia Diagnostics AB, Uppsala, Sweden) to detect specific serum IgE antibodies against MTHPA, as earlier described (19).

**Specific serum IgG antibodies** were analyzed, the MTHPA-HSA conjugate being used to coat the wells of polystyrene microtiter plates (Nunc-Immuno Plate 1, Nunc, Kamstrup Denmark) in an ELISA assay, as earlier described (19).

**Tryptase** was analyzed in lavage fluid by a commercial assay (Pharmacia tryptase RIACT, Pharmacia Diagnostics AB, Uppsala, Sweden). In the assay, the tryptase in the sample (50 µl lavage fluid) reacts simultaneously with solid-phase antitryptase, bound to the wall of the test tube, and added  $^{125}\text{I}$ -labeled-antitryptase. After incubation for 16 h at room temperature, unbound  $^{125}\text{I}$ -labeled-antitryptase was removed by washing three times, and the remaining radioactivity was determined. The human tryptase standards included in the kits (2–50 U/l) were diluted 1:3 with sample diluent. The limit of detection was 0.5 U/l, and the precision within assay was 2.5%.

**Eosinophil cationic protein (ECP)**, a marker of eosinophil activation, was analyzed in lavage fluid and serum with a commercial assay (Pharmacia ECP RIA, Pharmacia Diagnostics AB, Uppsala, Sweden). The ECP RIA is a double antibody radioimmunoassay. ECP in the sample (50 µl) competes with  $^{125}\text{I}$ -labeled ECP for the binding sites of specific antibodies. Bound ECP is separated by addition of a second antibody immunosorbent, followed by centrifugation and decanting. The radioactivity in the tube is then measured. The detection limit of the method is  $< 2 \mu\text{g/l}$ , and the precision within the assay is 8%.

**N- $\alpha$ -tosyl-L-arginine-methyl esterase (TAME)**, a nonspecific marker of inflammation in lavage fluid, was analyzed according to Andersson et al. (2). The TAME assay is a radiochemical method based on liberation of  $^3\text{H}$ -labeled methanol from synthetic substrate [ $^3\text{H}$ ] TAM-ester. A volume of 1 µl of lavage fluid was added to a propylene microtube containing 10 µl of a Tris buffer. The reaction was initiated by addition of 10 ml [ $^3\text{H}$ ] TAM-ester. The microtube was immediately suspended in econofluor and in stop solution in a closed vial. The microtube was incubated for 1 h at room temperature in the closed vial before the reaction was terminated by shaking. The vial was then counted in a liquid scintillation spectrometer twice for 1 min, and the result was expressed as counts/min (cmp).

**Methyl histamine** was analyzed in urine by a commercial assay (Pharmacia Histamine RIA 50, Phar-

macia Diagnostics AB, Uppsala, Sweden). The assay is a double antibody radioimmunoassay. Methyl histamine in the sample competes with a fixed amount of  $^{125}\text{I}$ -labeled histamine albumin conjugate for the binding sites of specific monoclonal antibodies. Antibody-bound material is then separated by the addition of a second antibody immunosorbent, followed by incubation, centrifugation, and decanting. The radioactivity is then measured. The detection limit of the method is  $< 0.1 \text{ ng/ml}$ , and the coefficient of variation within an assay is 6%.

### Statistical methods

The equality of medians between groups was tested by the Mann-Whitney U-test. Individual changes within the groups were tested by the Wilcoxon matched-pairs, signed-rank test. Differences were considered to be statistically significant at two-sided  $P$  values  $< 0.05$ .

## Results

### Study in the workplace

The SS group, but not the NS and NN groups, had a significantly higher level of ECP in serum than the control group, whereas no statistically significant difference between the SS and the two nonsensitized groups was found (Table 1). When mediators were studied in nasal lavage fluid (Table 1), the SS group had higher levels of tryptase than the two nonsensitized groups, with or without work-related nasal symptoms. No difference was found between the two latter groups. The SS group also had higher levels of tryptase than the control group, which did not differ from the other groups. No differences between the groups were found for ECP or TAME.

There was an association between serum ECP and TAME level in lavage fluid in the sensitized group ( $r_s = 0.82$ ;  $P = 0.004$ ), but not in the other three groups.

No differences were found between the groups for methyl histamine in urine.

### Nasal challenge

No significant differences in the composite symptoms score were found among the groups before nasal challenge with MTHPA-HSA (Table 2). Because of severe symptoms after challenge 1, challenge 2 was performed in only four and challenge 3 only in one of the workers from the SS group. In the nonsensitized symptomatic (NS) group, only one person did not pass through the last challenge, and in the nonsensitized nonsymptomatic (NN) group, everybody participated in the whole challenge procedure. Group SS had the largest rise of symptoms per challenge

Table 1. Numbers of smokers and skin-prick-test-positive subjects against panel of common allergens, and levels (medians; ranges) of eosinophil cationic protein (ECP) in serum and nasal lavage fluid, and tryptase and TAME in nasal lavage fluid in subjects sensitized to methyltetrahydrophthalic anhydride (MTHPA), in nonsensitized subjects with and without work-related symptoms exposed to MTHPA, and in control subjects not exposed to MTHPA

|                                     | MTHPA-exposed workers             |                                |                                   |                          |
|-------------------------------------|-----------------------------------|--------------------------------|-----------------------------------|--------------------------|
|                                     |                                   | Nonsensitized                  |                                   |                          |
| Marker                              | Sensitized (SS)<br><i>n</i> = 10  | Symptoms (NS)<br><i>n</i> = 19 | No symptoms (NN)<br><i>n</i> = 14 | Controls<br><i>n</i> =27 |
| Smokers ( <i>n</i> , %)             | 1 (10)                            | 4 (21)                         | 1 (7)                             | 5 (19)                   |
| Prick test positive ( <i>n</i> , %) | 1 (10)                            | 2 (11)                         | 1 (7)                             | 5 (19)                   |
| ECP (μg/l)                          |                                   |                                |                                   |                          |
| Serum                               | 9 <sup>cc</sup><br>(3–53)         | 7<br>(1–12)                    | 6.5<br>(3–11)                     | 5<br>(2–12)              |
| Lavage fluid                        | 1<br>(1–10)                       | 1<br>(1–24)                    | 1<br>(1–60)                       | 1<br>(1–13)              |
| Tryptase (U/l)                      | 1.2 <sup>aabbc</sup><br>(1.1–1.4) | 1.1<br>(0.6–1.6)               | 1.1<br>(0.5–1.5)                  | 1.1<br>(0.5–1.6)         |
| TAME (cpm)                          | 1600<br>(2.2–5600)                | 2000<br>(670–20 000)           | 2200<br>(340–21 500)              | 1500<br>(180–8000)       |

<sup>a</sup> P values in Mann-Whitney U-test between sensitized (SS) and nonsensitized subjects with symptoms (NS): aa<0.01.

<sup>b</sup> P values in tests between sensitized (SS) and nonsensitized subjects without symptoms (NN): bb≤0.01.

<sup>c</sup> P values in tests between sensitized (SS) and control subjects: c<0.05; cc<0.01.

step of the three groups (Table 2; Fig. 1). The rise was significant, as compared with the two other groups.

*Nasal inspiratory peak flow* before challenge did not differ among the three groups (Table 2). Group SS had by far the highest decrease per challenge step; the drop was significantly different from the two other groups.

*Tryptase in lavage fluid* did not differ among the three groups before challenge (Table 2). After challenge, a rise was seen in the SS group, though it was not statistically significant. No increase was observed in the NS or NN groups.

The level of *ECP in lavage fluid* was low in all three groups and without any differences among the groups before challenge. After challenge, no increase was recorded (Table 2).

## Discussion

In the study of immunologic/inflammatory markers in samples, *obtained in the workplace*, a slight, but significantly elevated level of tryptase in lavage fluid was recorded in the sensitized group, as compared with the two nonsensitized groups and the controls.

Table 2. Number of challenge steps performed, and composite nasal symptoms score, nasal inspiratory peak flow, and tryptase and eosinophil cationic protein (ECP) in nasal lavage fluid, before and after challenge with a conjugate of methyltetrahydrophthalic anhydride (MTHPA) and human serum albumin in MTHPA-sensitized and nonsensitized subjects with or without symptoms (medians; ranges). For nasal symptoms score and inspiratory peak flow, the results after challenge are given as "change/challenge step", calculated as the value after the last challenge subtracted from the reading "before", and divided by the number of challenges performed

| Symptom/marker                 | Time/challenge        | Sensitized                         | Nonsensitized        |                         |
|--------------------------------|-----------------------|------------------------------------|----------------------|-------------------------|
|                                |                       | Symptoms (SS)<br>n=7               | Symptoms (NS)<br>n=6 | No symptoms (NN)<br>n=7 |
| Number of challenge steps      |                       | 2 (1–3)                            | 3 (2–3)              | 3 (3–3)                 |
| Symptoms score                 | Before                | 0.5 (0–2)                          | 1.3 (0.5–2)          | 0.5 (0–1.5)             |
|                                | Change/challenge step | 2.3 (0.3–8) <sup>abb</sup>         | 0.4 (0–0.8)          | 0 (0–0.8)               |
| Peak flow (1 s <sup>-1</sup> ) | Before                | 110 (80–145)                       | 143 (68–170)         | 130 (68–257)            |
|                                | Change/challenge step | -50 [(-3) – (-78)] <sup>aabb</sup> | -1 [15 – (-41)]      | -1 [12 – (-10)]         |
| Tryptase U/l                   | Before                | 1.4 (1.2–1.6)                      | 1.4 (0.8–1.7)        | 1.2 (1–1.6)             |
|                                | After                 | 2.9 (0.6–7.8)                      | 1.3 (0.9–1.6)        | 0.9 (0.6–1.6)           |
| ECP $\mu\text{g/l}$            | Before                | 2 (1–7)                            | 1 (1–4)              | 2 (1–6)                 |
|                                | After                 | 2(1–5) <sup>b</sup>                | 1 (1–15)             | 1 (1–1)                 |

<sup>a</sup> P values in Mann-Whitney U-tests between the sensitized (SS) and the nonsensitized symptomatic group (NS): a<0.05; aa<0.01.

<sup>b</sup> P values in tests between sensitized (SS) and nonsensitized groups without symptoms (NN): b<0.05; bb<0.01.

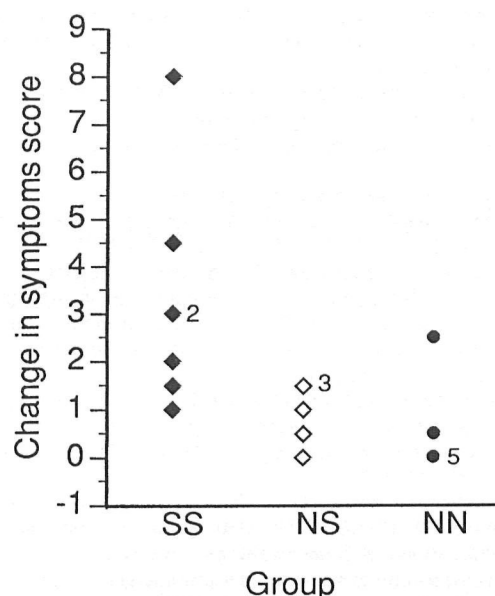


Fig. 1. Changes in composite nasal symptoms scores from pre- to postchallenge in three groups of workers: sensitized with symptoms (SS), nonsensitized with symptoms (NS), and nonsensitized without symptoms (NN).

Tryptase is considered to be a marker of mast-cell-mediated response (1, 4), and should thus, in accordance with the present findings, be supposed to be increased in an IgE-sensitized group exposed to the allergen. The serum ECP finding was also in accordance with an inflammatory reaction, possibly as a consequence of the allergic response. Elevated serum ECP was also found during the season in pollen-allergic patients (13).

Surprisingly, no rise of the ECP or TAME levels in the lavage fluid was recorded in the sensitized group. This contrasts with the finding of increased levels of ECP and TAME in nasal lavage fluid in studies of pollen-allergic patients with rhinitis during the pollen season (2, 14).

However, in the present study, because of symptoms and sensitization, seven of the sensitized workers had been out of exposure for 2–3 weeks before the short reexposure in the lavage study. They had been exposed, at most, 36 h before the lavage. Hence, the time for induction of an elevated level of ECP and TAME in the lavage fluid may have been too short at this low exposure level. In fact, a lag phase for ECP in nasal lavage fluid in relation to pollen exposure in pollen-allergic patients is described by Svensson et al. (14).

In the *nasal challenge study*, the group of IgE-sensitized subjects showed much more severe symptoms after exposure to MTHPA-HSA than the two nonsensitized groups. Moreover, a marked decrease in nasal inspiratory peak flow was recorded in the

former group, but not in the other two. These findings indicate a clinical significance of the sensitization, possibly mediated by a mast cell response, even though the increase of tryptase in nasal lavage did not reach statistical significance.

In the present study, ECP did not rise after challenge. In nasal challenge with natural allergens, increase of ECP has been observed as early as 30 min after challenge (8). However, early increase of ECP after challenge is not a consistent finding, as shown by Klementsson et al. (7) and Bisgaard et al. (3). In the present study, for practical reasons, it was not possible to perform the last lavage later than 12–15 min after the last challenge step, i.e., up to 45 min after the start of the challenge. This might have been too early to record an effect.

The present conclusion on sensitization as the background of symptoms and signs in some workers exposed to MTHPA agrees with findings in parallel studies of hexahydrophthalic anhydride-exposed subjects (12). In the latter study, it was shown that the reaction was associated with IgE rather than IgG. The present study did not allow as firm conclusions on this point, because of limited material and the intercorrelation between the two classes of antibodies. However, it seems reasonable to assume that the conditions are similar for the two anhydrides.

The mechanism behind the symptoms in the non-sensitized group with work-related symptoms remains unclear. The irritant effect of MTHPA is reduced by the conjugation to HSA. Thus, the symptoms in the workplace may have been caused by an irritant effect, or by some other reaction to the unconjugated compound. In addition, confounding exposures must be considered: the epoxy resin has a low irritating effect, but Kanerva et al. (6) showed that an epoxy resin caused IgE sensitization. However, in the present study, nobody had a positive reaction in skin prick testing with diglycidylether of bisphenol A, indicating that sensitization to the epoxy resin had not occurred. Furthermore, amines can irritate mucous membranes. Whether the amine used in the present plant is an irritant of the upper airways is not known to the authors, nor is it known whether it has a sensitizing effect. Further studies, including challenge tests with the substances involved, are necessary to clarify these matters.

A remarkable feature of the respiratory disorder caused by MTHPA is the extremely low exposure, on average less than  $100 \mu\text{g}/\text{m}^3$ , which causes a high frequency of IgE sensitization. The same is true for HHPA (18). This, in addition to the occupational risk, makes the organic acid anhydrides interesting as model compounds in the study of allergic diseases.

## Acknowledgments

This study was supported by the Swedish Work Environment Fund and the Medical Faculty, Lund University, Sweden. We thank the company and the personnel involved for help and for commitment to the study. We also thank Piteå Hospital for "housing" during the study and Dr Frede Mortensen, MD, and Ms Gård Åhlund, RN, for cooperation. A special thanks to Prof. Lars Malm for valuable advice during the study and to Dr Morgan Andersson, MD, for advice on the nasal lavage procedure. Skillful technical assistance was given by Ms Pia Aprea, Ms Birgitta Björk, and Ms Helene Ottosson.

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