

Original article

A prospective study of the relationship between exposure and specific antibodies in workers exposed to organic acid anhydrides

Background: The exposure–response relationships for the induction of specific IgE and IgG were evaluated in a prospective study of workers exposed to organic acid anhydrides (OAAs). Special attention was paid to the modifying effects of atopy and smoking.

Methods: The subjects were 163 previously unexposed persons exposed to epoxy resins with hexahydro-, methylhexahydro-, and methyltetrahydrophthalic anhydride as curing agents. The levels of OAAs in air and of specific IgE and IgG in serum were recurrently monitored. The mean observation time was 32 (1–105) months.

Results: The mean combined OAA exposure of the subjects was 15.4 (<1–189) $\mu\text{g}/\text{m}^3$. Positive specific IgE was demonstrated by 21 (13%) subjects with a mean induction time of 8.8 (1–35) months. The incidence of sensitization was 4.1 cases/1000 months at risk. The relative risk (OR) for atopics was 5.4 (1.9–15.3; 95% CI). An exposure–response relationship was demonstrated by an increasing risk of sensitization with increasing exposure.

Conclusions: An association between exposure and atopy, respectively, and the induction of specific antibodies against OAAs were observed. The risk for atopics was comparable with the risk for the subjects in the most exposed group.

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Many proteins and low-molecular-weight chemicals have the potency to induce allergic sensitization of the airways including the formation of specific IgE antibodies (1). However, very little is known about the relationship between the dose of allergen and sensitization (2, 3). Such information is of theoretical interest and important for preventive measures (4). Our present knowledge mainly derives from the results of cross-sectional studies. However, a drawback of the cross-sectional study is that the association between exposure and effects may be obscured by selection bias, as in healthy worker selection. Prospective studies should be more suitable and informative.

High prevalences of IgE have been reported in several studies on workers exposed to organic acid anhydrides (OAAs) in industry (5–9). The frequent use of OAAs in the industry causes sensitization and work-related conjunctivitis, rhinitis, and asthma in exposed subjects (10). Thus, OAAs are good model compounds for studies of relationships between exposure and allergic sensitization of the airways. There have been conflicting results on the modifying effects of atopy and smoking in different studies, perhaps as the result of limitations in cross-sectional studies (11).

In this study, the exposure–response relationships for the induction of specific IgE and IgG have been evaluated

prospectively in workers exposed to some hydrogenated phthalic anhydrides. Special attention has been paid to the modifying effects of atopy and smoking.

Material and methods

Study design

All workers who were employed in departments handling uncured epoxy resins at three different plants were included in the study. A prerequisite was the absence of previous exposure to OAAs. The subjects were observed until a positive specific IgE was induced.

Subjects

The investigation started in 1988, and no new subjects were recruited after 1997. Altogether, 163 persons (66 women and 97 men) were qualified, and all took part in the study. The average age at inclusion in the study was 32.8 (18–64) years, and the mean observation time (time of exposure) was 32 (1–105) months. Forty percent of the group were current smokers, 35 subjects (23%) had a history of atopy, and 27 (19%) demonstrated a positive skin prick test (SPT) to at least one common allergen (see below; Table 1).

Plants

Plant 1. The plant produced electrical capacitors that were mechanically fixed and electrically isolated by epoxy resin with hexahydrophthalic anhydride (HHPA) and methylhexahydrophthalic anhydride (MHHPA) as curing agents. Most workers

investigated were fillers and did repetitive work during all work shifts. The length of the work shifts was 8 h, and the workload was light. The epoxy resin was used in closed systems or ventilated hoods, but some operations included the open handling of mixtures with uncured anhydrides. The main exposure occurred in casting and from leakages in the curing ovens. The study started in June 1989. Thirty-seven subjects took part in the study (Table 1).

Air sampling was performed once a year from 1989 to 1998. In total, 463 samples were collected with a total sampling time of 1245 h. The mean total exposure of HHPA and MHPA was $39 \mu\text{g}/\text{m}^3$ (Table 2).

Plant 2. The plant produced components for ignition systems. The production started in June 1992. The components were isolated in epoxy resin with methyltetrahydrophthalic anhydride (MTHPA) as curing agent. The resin was finally cured in ovens at temperatures of $90\text{--}140^\circ\text{C}$. The length of the work shifts was 8 h, and the workload was light. The main exposure came from leaks in the curing ovens. In February 1996, the epoxy system was replaced by a system with HHPA and MHPA as hardeners, and the study was closed. The plant contributed 81 subjects (Table 1).

Air sampling was performed from 1992 to 1996. In total, 169 samples were collected with a total sampling time of 428 h. The mean exposure level during the observation period was $10 \mu\text{g}$ MTHPA/ m^3 (Table 2).

Plant 3. The plant produced barrels for grenade launchers, epoxy resin with a 1:1 addition of MTHPA being used as a hardener. Rotating cylinders were prepared by hand swabbing, with epoxy resin on glass-fiber fabric. The cylinders were then wound automatically with glass-fiber cords wet by the epoxy resin. The cylinders were cured at temperatures of $90\text{--}120^\circ\text{C}$. After curing, the plastic barrels were processed mechanically. The investigation started in June 1988. Forty-five persons took part in the study.

Air sampling was performed from 1989 to 1997. In total, 116 samples were collected with a total sampling time of 284 h. The mean exposure was $6 \mu\text{g}$ MTHPA/ m^3 (Table 2).

Medical examinations

In plants 1 and 2, a questionnaire was administered and a blood sample was collected from all subjects before the start of exposure. Each year, the subjects were checked for work history and a structured interview and a clinical examination were administered by a physician (J.N.). A blood sample was collected and a skin prick test (SPT) was performed with common allergens (ALK A/S, Copenhagen, Denmark) at the first examination, and with OAA-specific antigens at each examination.

Subjects were defined as atopic by history when they had a history of hay fever, asthma, atopic eczema, or urticaria (when the history of relation to a particular agent was convincing) during childhood or adolescence.

In plant 3, the medical investigations were less frequent. Blood samples for antibody assays were collected each year, but the specific SPTs were performed less frequently.

SPTs

SPTs were performed with 12 common allergens. Persons with one positive reading or more (see below) were defined as atopic.

SPT were also performed with conjugates between OAAs and human serum albumin (HSA) synthesized at our department (12). The hapten-HSA concentration was $3 \text{ mg}/\text{ml}$ in 0.1 M NaHCO_3 . Reaction sizes were recorded in relation to histamine ($3 \text{ mg}/\text{ml}$) response, according to Aas & Belin (13). A positive SPT reaction was scored when the size of the wheal was at least half that induced by histamine.

Antibody determinations

Specific antibodies were monitored by procedures previously

described (12). Specific IgE was assayed by RAST and specific IgG by ELISA. The titers were regarded as positive if they were larger than the highest referent value in a group of controls. The upper limits of the reference values were calculated from a total of 633 analyses of 159 negative controls during 1989–97. IgE in the controls was $\leq 0.3\%$ specific binding for all OAAs. The absorbance values (A) of IgG in the controls were <0.12 A for MHPA and <0.3 A for MTHPA and HHPA, respectively.

Exposure to OAAs

Sampling and analysis. The anhydride levels in air were determined by personal sampling in the breathing zone of the workers or, when the work tasks were very stationary, by area sampling by battery-operated pumps on XAD-2 absorbent tubes. Sampling and analysis were done by procedures described earlier (14, 15). The limit of detection was $0.2 \mu\text{g}/\text{ml}$ toluene for MTHPA and $0.1 \mu\text{g}/\text{ml}$ toluene for HHPA corresponding to $0.5\text{--}1 \mu\text{g}/\text{m}^3$ at a sampling volume of 200 l.

Altogether, 748 samples with a total sampling time of 1957 h were collected.

Calculations of individual exposures. From the work histories and the exposure measurements for different departments and work tasks, individual mean and peak exposure levels were calculated for all subjects. The mean exposures were calculated from start of exposure until a positive reaction to RAST, ELISA, and SPT, respectively, was demonstrated. The date for the positive reaction was considered as the midpoint of the interval between the positive test and the last negative one. For nonsensitized individuals, the mean values were calculated for the whole observation period. The maximum peak values were calculated as the highest value obtained at the specific work tasks at the relevant time period.

Statistics

Each subject's average exposure intensity was calculated from start of work until a positive antibody was obtained or until the end of the study period. The effects of exposure, smoking, and atopy on the development of specific IgE and IgG antibodies, respectively, were evaluated by logistic regression models where odds ratios (OR) and 95% confidence intervals (CI) were obtained. Effect modification was evaluated by including interaction terms in the models.

Results

Exposure levels

The mean combined OAA exposure of the subjects was $15.4 \mu\text{g}/\text{m}^3$ and varied between <1 and $189 \mu\text{g}/\text{m}^3$. The median value was 9.2 (10th percentile, $1 \mu\text{g}/\text{m}^3$; 90th percentile, $26 \mu\text{g}/\text{m}^3$) $\mu\text{g}/\text{m}^3$. The mean peak value was 84 ($<1\text{--}604$) $\mu\text{g}/\text{m}^3$. The exposure levels were highest in plant 1 with mean exposures of $27 \mu\text{g}$ HHPA m^3 and $12 \mu\text{g}$ MHPA/ m^3 (Table 2). The subjects in plants 2 and 3 were exposed to MTHPA, and the highest mean exposure was in plant 2.

Specific IgE antibodies

A positive RAST of specific IgE against OAAs was demonstrated by 21 (13%) subjects in the total group (Table 2), and the incidence was 4.1 cases per 1000 months at risk. The mean induction time for the

Table 1. Descriptive measures of prospective cohort

Plant	All	Plant 1	Plant 2	Plant 3
No. of persons	163	37	81	45
Men	97	34	33	30
Women	66	3	48	15
Exposure time (months)				
Total	5142	1208	2141	1793
Mean (range)	32 (1–105)	33 (1–85)	26 (2–38)	40 (1–105)
Blood samples (n=)				
Total	808	225	381	202
Mean (range)	4.9 (1–14)	6.1 (1–12)	4.7 (2–6)	4.4 (1–14)
Smokers (n=, %)	64 (40)	17 (46)	33 (41)	14 (32)
Atopy by history (n=, %)	35 (23)	8 (22)	20 (25)	7 (20)
Atopy by positive skin prick test (n=, %)	27 (19)	10 (29)	15 (19)	2 (7.7)

Table 2. Exposure levels, specific antibodies to hexahydrophthalic anhydride (HHPA), methylhexahydrophthalic anhydride (MHHPA), and methyltetrahydrophthalic anhydride (MTHPA), and induction time of sensitization in plants 1–3

Plant	All	Plant 1	Plant 2	Plant 3
Mean exposure ($\mu\text{g}/\text{m}^3$)	15.4 (0–189)	38.7 (1–189)	10.1 (6–38)	5.9 (0–50)
MTHPA	6.6 (0–50)	<1	10.1 (9–38)	5.9 (0–50)
HHPA	6.1 (0–187)	26.9 (0–187)	<1	<1
MHHPA	2.7 (0–37)	11.8 (1–37)	<1	<1
Positive IgE by RAST (n,%)	21 (12.9)	10 (27)	7 (8.6)	4 (8.9)
Induction time (months)	8.8 (1–35)	6.8 (1–21)	12.4 (4–35)	7.3 (1–18)
Positive IgG (n,%)	26 (16)	18 (49)	5 (6)	3 (7)
Induction time (months)	15 (1–73)	19 (1–73)	6.8 (1–12)	4.0 (1–9)
Positive IgE by SPT (n,%)	13 (8.0)	8 (21.6)	4 (4.9)	1 (2.2)
Induction time (months)	12.8 (3–31)	12.8 (3–31)	13.3 (4–24)	11.0

Table 3. Effects of exposure, smoking, and atopy on specific IgE and IgG, respectively, obtained from multivariate logistic regressions. Odds ratios (OR) with 95% confidence intervals (CI) are shown

	IgE						IgG		
	RAST			SPT					
	n ^a	OR	95% CI	n ^a	OR	95% CI	n ^a	OR CI	95%
Mean exposure ($\mu\text{g}/\text{m}^3$)									
10	93	1.0		91	1.0		90	1.0	
> 10–15	17	1.7	0.3–8.0	18	1.5	0.2–9.6	20	9.2	1.9–43.4
> 15	29	3.5	1.1–11.2	28	4.9	1.2–19.6	29	31.6	7.9–126
Smoking									
No	82	1.0		81	1.0		Not included		
Yes	57	1.7	0.6–5.0	56	2.1	0.6–7.6			
Atopy									
No	112	1.0		110	1.0		112	1.0	
Yes	27	5.9	2.0–17.3	27	6.5	1.7–24.1	27	2.8	0.8–9.4

^aNumbers of missing values on atopy were IgE-RAST = 24, IgE-SPT = 3, and IgG = 24. These individuals were excluded from multivariate analyses.

development of positive IgE was 9 (1–35) months. The fraction of sensitized workers increased with increasing exposure (Fig. 1). As compared with subjects with a mean exposure of $\leq 10 \mu\text{g}/\text{m}^3$, subjects with a mean exposure of $> 10\text{--}15 \mu\text{g}/\text{m}^3$ had a nonsignificantly increased risk of development of IgE (OR 1.8; 95% CI 0.4–7.1), whereas subjects with a mean exposure of $> 15 \mu\text{g}/\text{m}^3$ had a significantly increased risk (OR 3.4; 95% CI 1.2–9.4). There was no statistically significant difference between peak exposure levels in sensitized and nonsensitized workers.

Among RAST-positive subjects, there were more smokers (52%) than among RAST-negative ones (38%), corresponding to an OR of 1.8 (95% CI 0.7–4.6).

Fifty-three percent of the IgE-positive subjects had a positive SPT to a common allergen compared with 14% in the IgE-negative subjects. The OR for atopics to become IgE-sensitized as compared with nonatopics was 6.0 (95% CI 2.2–16.6). Atopy by history ($n = 35$) was less related to positive specific RAST (OR 1.4; 95% CI 0.5–4.0).

Including exposure, smoking, and atopy simulta-

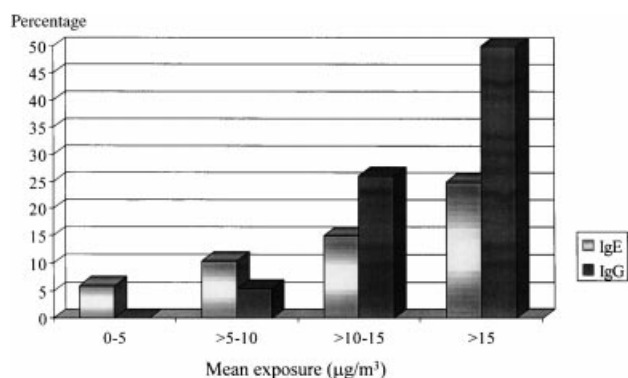


Figure 1. Percentage of subjects with positive RAST against human serum albumin conjugates of hexahydrophthalic-, methylhexahydrophthalic, or methyltetrahydrophthalic anhydride at different mean exposure levels (0–5, >5–10, >10–15, and >15 µg/m³).

neously in a multivariate model changed the univariate estimates only marginally (Table 3). Neither smoking nor atopy modified the effect of exposure. In addition, atopy did not modify the effect of smoking.

A positive SPT reactivity to hapten conjugates of the relevant OAAs was induced in 13 subjects, and the incidence was 3.0 cases per 1000 months at risk. All specific SPT-positives but one were also positive by RAST. The mean induction time for a positive SPT was 13 (3–31) months, which was 4 months longer than the induction time of a positive RAST (Table 2). However, the difference was not statistically significant when only subjects ($n=12$) with a positive specific reaction in SPT as well as RAST were compared ($P=0.16$). The effects of exposure, smoking, and atopy on SPT-positive IgE showed patterns similar to RAST-positive IgE (Table 3).

Specific IgG antibodies

A positive ELISA of specific IgG against OAAs was demonstrated by 26 (16%) subjects in the total group (Table 2), and the incidence was 5.1 cases per 1000 months at risk. The mean induction time for the development of positive IgG was 15 (1–73) months, which was considerably longer than the induction time for RAST-positive IgE. Fifteen out of the 21 RAST-positive subjects developed a positive IgE before a positive IgG reaction was induced.

The highest risk was found in plant 1 in workers exposed to HHPA and MHPA (data not shown). Moreover, a clearly positive association between mean exposure and IgG was observed. Smoking did not show an association with IgG (OR 0.9; 95% CI 0.4–2.2), whereas atopy did (OR 2.7; 95% CI 1.0–7.3). The effect of exposure and smoking changed only marginally when they were both included in a multivariate model (Table 3). Smoking did not modify the effect of exposure.

Eight workers in plant 1 used respiratory protection

devices during periods of their employment. The exposure–response relationships were even more evident when those workers were excluded from the calculations for plant 1 compared with the other plants, as well as in the calculations of exposure–response relationships in all workers.

Symptoms

Work-related symptoms were preferably induced in IgE-sensitized subjects compared with nonsensitized ones, as demonstrated by preliminary results (eyes 30% vs 11%, nose blockage 35% vs 11%, and running nose 48% vs. 9%). The remaining results of the clinical examinations and the questionnaires will be presented in a separate publication (Nielsen et al.; to be published).

Discussion

In this prospective study we found an incidence of IgE sensitization of 4.1 cases/1000 months at risk. The relative risk of sensitization increased with increasing intensity of exposure. No association was found with peak exposure levels. Atopics ran the same risk of sensitization as the subjects with the highest exposure levels.

Prospective studies have many advantages over cross-sectional ones, but may also involve methodological problems. Such a problem may be a drift in analytic and clinical assessments when data are collected over a long time (in this case for more than 10 years). The present study was conducted by the same scientific and technical staff over the whole period with good control of the methods applied.

The induction of specific IgE in workers exposed to OAAs is of clinical significance, as work-related airway symptoms are associated with increased levels of specific IgE (11, 16–19). This was also confirmed in the present work (Nielsen et al.; to be published). The fraction of IgE-positive subjects is lower than we earlier found by cross-sectional studies in workers exposed to MTHPA (18%) (6) or HHPA and MHPA (24%) (7). A much higher prevalence has been reported by Yokota et al. (8), who found a prevalence of 66% in workers exposed to MTHPA. In workers exposed to phthalic anhydride (PA) (20) and tetrachlorophthalic anhydride (THPA) (21), the prevalence was <10%. The incidence in the present study is comparable with findings in bakers (22) or in platinum salt-exposed workers (23). Differences in the incidence of sensitization between different OAAs may be explained by different exposure levels, but there may also be differences in sensitizing capacity. Thus, a wide variation in antibody titers was found in guinea pigs (24) and rats (25) after equimolar intradermal immunization of varying OAAs. For the evaluation of the sensitizing potential of OAAs in man, the assessment of exposure–response relationships for varying OAAs should give valuable information.

We found a statistically significant association between exposure and specific IgE antibodies in the present investigation. Such a relationship was expected, but quantitative confirmatory data are limited (3, 11, 26). For specific IgE, there is often no evident exposure–response relationship in cross-sectional studies (6, 7, 27). One major reason for this may be selection bias, but another important reason is surely the uncertainty of the exposure assessments (9). Although a large number of air samples have been collected in the present study, the time of sampling represents only a very small fraction of the time of exposure. Thus, the exposure assessments are still rather crude, making the exposure–response relationships found even more convincing. Advances in biologic monitoring of long-time exposure will in the future present new opportunities for exposure assessment (28).

IgE was associated with mean exposures, and not with peak exposures. Thus, the results do not give active support to the frequent suggestions that sensitization is induced by high peak exposures.

The analysis of the effects of exposure and predisposing factors was performed in the combined group of subjects due to the low number of sensitized subjects in the single plants. The combined analysis of subjects exposed to MTHPA, HHPA, and MHHPA was justified by the agreement in sensitizing capacity of the different anhydrides in studies of laboratory animals (24, 25) as well as exposed workers (6, 7). The fact that subjects exposed to HHPA and MHHPA demonstrated a higher incidence of positive specific IgE than subjects exposed to MTHPA can be explained by the difference in exposure. As there is cross-reactivity between antibodies against HHPA and MHHPA, respectively, differences in sensitizing capacity between the anhydrides could not be analyzed in the present material.

The often reported indistinct association with exposure has led to discussions about the relative importance of predisposing factors, such as smoking and atopy, for sensitization. In the present study, there was no significant effect of smoking. Contradictory results have earlier been reported for the effect of smoking on the induction of IgE against OAAs. Some studies have reported a positive effect (11, 21) while others have been negative (6, 7, 18, 29, 30). It is very likely that the effects of smoking are rather small.

Atopy is suggested as an important risk factor for the induction of specific IgE against low-molecular weight compounds such as OAAs (21). However, the results have not been concordant, as no such association has been found in some other studies (6, 7). Moreover, in a cross-sectional study at plant 1 in 1994, which involved 154 exposed subjects with a prevalence of RAST-positive specific IgE of 22%, no effect of either atopy or smoking was observed (Nielsen et al.; submitted for publication). A clear-cut, significant effect was found in the present study. The atopics ran the same risk of sensitization as the subjects in the most exposed group. However, the

exposure–response relationship was only moderately modified by adjustment for smoking and atopy. However, it is still possible that there was a difference in the exposure–response relationships between atopic and nonatopic workers, the atopics being sensitized at lower exposure levels. But our material did not have power for such calculations.

The role of specific IgG antibodies induced after exposure is unclear (31). IgG4 is the predominant subclass formed (6). Thus, it has been suggested that IgG4 antibodies may enhance the immediate reaction and cause the late reaction (32). However, IgG4 has also been held merely to reflect exposure and function as a blocking antibody (33). The latter hypothesis is supported by Bernstein et al. (34), while Yokota et al. (19) did not find any blocking function of IgG4. In the present study, IgE was induced earlier or simultaneously with IgG except in one case. IgG was induced with a larger incidence than IgE and showed a weaker correlation with atopy. As every second subject was negative even in the most exposed group, IgG is a poor biomarker of exposure at an individual level.

To our knowledge, there are no occupational limits established for the hydrogenated phthalic anhydrides. The present investigation demonstrated that exposure levels of $10 \mu\text{g}/\text{m}^3$ or even less are enough to cause a positive IgE. Thus, it is not yet possible to indicate a safe exposure level that does not cause IgE sensitization. Moreover, the present equipment and technique for the handling of epoxy resins at elevated temperatures cannot cope with such low limits. However, data from Yokota et al. (27) and Nielsen et al. (submitted for publication) indicate that symptoms even from IgE-sensitized workers are limited to levels below $10 \mu\text{g}/\text{m}^3$. This will be further investigated in the compilation of data on health effects in our prospective study.

An association between exposure and the induction of specific antibodies against MTHPA, HHPA, and MHHPA was observed. The effect was correlated with mean exposure levels, and not with peak exposures. There was a significant effect of atopy on sensitization, while the effect of smoking was meager. Atopy and smoking had a minor modifying effect on the exposure–response relationships. The results of this prospective study diverged from the results of our previous cross-sectional studies in the same or corresponding plants.

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