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Exposure to hexahydrophthalic and methylhexahydrophthalic anhydrides — dose-response for sensitization and airway effects

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Objectives This study clarified the exposure-response relationships for the organic acid anhydrides (OAA) hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) anhydrides and the development of specific immunoglobulin (Ig) E and G antibodies and work-related symptoms.

Methods In an epoxy resin-using factory, air levels of OAA were determined by gas chromatography-mass spectrometry. Occupational, smoking, and medical histories (questionnaire) were obtained for 154 exposed workers and 57 referents. Work-related symptoms of the eyes and airways were recorded, and OAA metabolites were analyzed in urine. A skin-prick test with common allergens and conjugates of OAA were performed. Specific IgE (radioallergosorbent test) and IgG (enzyme-linked immunosorbent assay) antibodies were determined in serum, and spirometry was performed.

Results Air levels of the OAA were low (HHPA <1 to 94, MHHPA <3 to 77 µg/m³) and associated with the concentrations of the OAA metabolites in urine. Furthermore, for the exposed workers, there were high prevalences of sensitization (IgE 22%, IgG 21%), which correlated with the exposure. Neither atopy nor smoking increased this risk significantly. Furthermore, work-related symptoms were more prevalent among the exposed workers than among the referents (eyes 23% versus 14%, nose 28% versus 16%, nose bleeding 8% versus 0%, lower airways 10% versus 4%), and they were related to the exposure [adjusted prevalence odds ratios (POR) in the highest group 7.7, 3.6 and 17, respectively) and the IgE levels (POR 4.9, 3.1 and 5.6, respectively).

Conclusions In spite of the very low OAA levels in the air and metabolites in the urine, there were high and exposure-related risks of specific IgE and IgG sensitization and of work-related symptoms for the eyes, nose (especially bleeding), and lower airways.

Key terms air, biological monitoring, epistaxis, immunoglobulin E, immunoglobulin G, metabolites, organic acid anhydrides, work-related symptoms.

Dicarboxylic acid anhydrides are a group of organic acid anhydrides (OAA) widely used in the chemical and pharmaceutical industries (1, 2). Some of them, such as hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) anhydrides are used as hardeners in epoxy resins (3).

OAA-exposed workers are frequently sensitized and complain of symptoms of the eyes and upper and lower airways (2, 4). Different OAA have varying sensitizing potentials (3, 5, 6). In particular, HHPA causes high frequencies of symptoms (6–10).

However, as for other allergens, exposure-response data for sensitization and symptoms caused by OAA are scanty. Such knowledge is important for preventive work. It is a basis for permissible exposure limits (PEL), technical measures to reduce exposure, and surveillance programs for exposed workers.

The establishment of reliable exposure-response relations for OAA requires adequate measurements of exposure, either from the sampling of air or from biological samples followed by accurate determinations of OAA or their metabolites and careful examination for

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the presence of specific antibodies and symptoms in a sufficient number of workers. In this paper we present such data.

Subjects and methods

The plant

The plant produces capacitors, fixed and isolated with epoxy resin with HHPA and MHHPA as hardeners. Measurements of HHPA in air have been performed since 1989, and for MHHPA similar measurements have been made since 1991. The exposure originates from casting, leaks in curing ovens, and hot components.

Subjects

In 1994, altogether 154 workers (98%) exposed to HHPA and MHHPA were examined (table 1). As a reference group 57 subjects (86%) were recruited from two mechanical industries in the same area, with no heavy exposure to sensitizing or irritating agents.

Exposure assessment

Hexahydrophthalic and methylhexahydrophthalic anhydrides in air. Air samples were collected on 10 different days in 1994 by battery-operated pumps (0.2–1.0 l/min) on Amberlite XAD-2 tubes (no 226–30, SKC Inc, Eighty Four, PA, USA), by personal sampling in the breathing zone of the workers (N=122), as well as by area sampling (N=97). The total sampling time for the personal samples was 427 hours.

The OAA were analyzed by capillary gas chromatography with flame-ionization detection or by gas-chromatography mass-spectrometry (GC-MS) (6, 11, 12). The limit of detection corresponded to 0.2 µg HHPA/m³.

Hexahydrophthalic acid and methylhexahydrophthalic acid in urine. Urine was collected during the last 4 hours of the workshift for 140 workers and frozen. HHP and MHHP acids were determined by GC-MS as previously described (13).

Medical examinations

Extensive occupational and medical histories were obtained by a questionnaire. Current and previous work tasks in the present workplace were recorded, as were symptoms of the eyes (lacrimation, itching, scratching, smarting, or burning eyes), nose (blocked, itchy, or running or attacks of sneezing or bleeding), and lower airways (dyspnea, wheezing, chest tightness, or dry cough) during the last 12 months. The symptoms were denoted "work-related" if they appeared in relation to special

worktasks or if they improved during weekends or holidays.

Skin-prick test

A skin-prick test (SPT) was made with six common allergens (ALK laboratories, Copenhagen, Denmark) and conjugates between HHPA and MHHPA and human serum albumin (HSA) (6, 14).

A positive reaction was recorded if the weal was > 2 mm, and the negative control was < 1 mm. Furthermore, for positive reactions, the ratio between the mean of the widest diameter and that of 90 degrees from the mid-point and the corresponding value of the histamine reaction was calculated ("SPT ratio").

Lung function tests

Forced expiratory volume in 1 second (FEV_{1.0}) and forced vital capacity (FVC) were determined for all the subjects (Vitalograph S spirometer, Vitalograph Ltd, Buckingham, England) (15).

Antibody determinations

Antigens. Hapten conjugates were prepared from HHPA (Ciba-Geigy, Basel, Switzerland) or MHHPA (Janssen Chimica, Geel, Belgium) and HSA (Kabi, Stockholm, Sweden) (6, 16).

Specific IgE antibodies were determined by the radioallergosorbent test (RAST) (Phadebas RAST system, Pharmacia Diagnostics AB, Uppsala, Sweden) (6, 16). All the samples were analyzed in duplicate. The results were expressed as the percentage of specific binding [counts per minute (cpm) of the test disc – cpm of the HSA reference disc] of the total added radioactivity.

Specific IgG antibodies were analyzed by an enzyme-linked immunosorbent assay (ELISA) (6, 16). The results were expressed as the absorbency values (A). For both IgE and IgG, positivity was defined as a reading above that of the highest reference subject.

Statistics

The model used for the analysis of the data is shown in figure 1. Univariate associations between the parametric values were assessed by Spearman's rank correlation (rS). Trends for the exposure parameters were evaluated by the Jonckheere-Terpsta test or test for trend. Differences between the groups were tested by the chi-square test or the Mann-Whitney U test. The effects of exposure on the immunologic parameters, symptoms and lung function were analyzed by linear or logistic regression, with consideration of possible confounders or effect modifiers. "Statistically significant" denotes P<0.05 (two-tailed).

Results

Exposure

The OAA levels in the air ranged up to 94 $\mu\text{g}/\text{m}^3$ for HHPA and 77 $\mu\text{g}/\text{m}^3$ for MHHPA in the personal samples, the corresponding values for the area samples being 510 and 150 $\mu\text{g}/\text{m}^3$.

On the basis of the estimated current exposure (both HHPA and MHHPA), the workers were divided into three exposure categories (< 10, 10–50, and > 50 $\mu\text{g}/\text{m}^3$) (table 1). In the multivariate analyses, the concentrations were assumed to be 5, 30, and 70 $\mu\text{g}/\text{m}^3$, respectively.

The HHP and MHHP acid concentrations of the urine rose with increasing air levels (table 1). There was no association between the levels of the two acids (table 2).

Figure 1. Model for the relationship between inhalation exposure to organic acid anhydrides (OAA), metabolites in urine, specific immunoglobulin (Ig) E and G antibodies in serum, and work-related symptoms and lung function. In addition, the factors considered to be confounding or effect-modifying are indicated.

Table 1. Gender, age, smoking habits, atopy (positive skin-prick test to ≥ 1 of six common antigens) and anhydride-derived organic acids in the urine of workers exposed to hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) anhydrides, classified according to exposure status, and of the referents (whose exposure was 0 $\mu\text{g}/\text{m}^3$).

							Organic acids in urine (μmol/mol creatinine)					
Exposure (μg/m³)	Number of workers	Women (%)	Age (years)		Smokers (%)	Atopy (%)	HHP acid		MHHP acid		Sum	
			Median	Range			Median	Range	Median	Range	Median	Range
0	57	40	39	21–61	26	19	.	.	.	.	.	.
< 10	53	85	36	20–64	44	17	18	6– 320	20	6– 120	43	14– 340
10–50	72	19	29	22–61	25	26	17	7– 590	88	8–1100	150	15–1100
> 50	29	0	32	21–64	38	24	220	36–3100	43	11–1700	500	92–3100
Test for trend (P)		<0.001	0.003		0.85	0.30	<0.001		<0.001		<0.001	

Table 2. Associations (rS) between the concentrations of metabolites in urine [hexahydrophthalic (HHP) and methylhexahydrophthalic (MHHP) N = 124–126], skin-prick tests (SPT) (sum of HHPA and MHHPA SPT ratios, N = 152–153), specific antibodies against HHP anhydrides, and MHHP anhydrides in workers exposed to HHPA and MHHPA (N = 152) and lung function [forced vital capacity (FVC), forced expiratory volume in 1 second ($\text{FEV}_{1.0}$), N = 153]. (U = urinary, S = serum, Ig = immunoglobulin)

	U-MHHP acid	SPT ratio	S-IgE		S-IgG		Lung function	
			HHPA	MHHPA	HHPA	MHHPA	FVC	$\text{FEV}_{1.0}$
U-HHP acid								
rS	-0.042	-0.034	0.12	0.11	0.37	0.28	-0.22	-0.18
P-value	0.6	0.7	0.2	0.2	<0.0001	0.002	0.01	0.04
U-MHHP acid								
rS	-	0.093	0.019	0.078	-0.047	0.043	-0.14	-0.079
P-value		0.3	0.8	0.4	0.6	0.6	0.1	0.4
SPT ratio								
rS	-	-	0.22	0.22	0.14	0.16	-0.19	-0.02
P-value			0.008	0.007	0.08	0.05	0.02	0.8
S-IgE-HHPA								
rS	-	-	-	0.75	0.45	0.44	-0.11	0.02
P-value				<0.0001	<0.0001	<0.0001	0.2	0.8
S-IgE-MHHPA								
rS	-	-	-	-	0.54	0.55	-0.06	-0.05
P-value					<0.000	<0.0001	0.4	0.5
S-IgG-HHPA								
rS	-	-	-	-	-	0.85	-0.14	-0.03
P-value						<0.0001	0.08	0.7
S-IgG-MHHPA								
rS	-	-	-	-	-	-	-0.11	-0.01
P-value							0.2	0.9

Sensitization versus exposure

Positive SPT against any of the OAA (table 3) were found for 26 (17%) of the exposed workers; most of those sensitized were sensitized to both HHPA and MHHPA. The prevalence of positivity rose with increasing air concentrations, but there was no association with the urinary metabolites (table 2).

Specific serum antibodies. IgE against any of the OAA (table 3) was found in the serum (S) of 34 (22%) of the exposed group. There was a close association between the HHPA and MHHPA values (table 2).

Specific S-IgG antibodies against any of the OAA-HSA (table 3) were found in 33 (21%) of the exposed group, again with a close association between the two OAA species (table 2). The S-IgE and S-IgG values for the respective OAA were fairly closely associated.

There were significant associations between the OAA SPT ratios on one hand and the S-IgE-HHPA and S-IgE-MHHPA on the other, to some degree also with S-IgG-MHHPA and S-IgG-HHPA (table 2).

The frequency of positive values for both S-IgG increased as the air levels increased (table 3). For both S-IgE, the highest exposure groups had lower prevalences than the second highest ones. The S-IgG against both HHPA and MHHPA correlated with the urinary HHP acid concentration, while MHHP acid displayed no such association (table 2). Furthermore, there were no associations between either type of IgE and the urinary metabolites.

The "background" factors atopy and smoking were entered into the multivariate models describing the relations between the immunologic parameters and the air levels. Neither atopy nor smoking modified the response significantly (data not presented).

Symptoms versus exposure

When the exposed group was compared with the referents, the exposed workers had more complaints of work-related

symptoms of the eyes, nose (bleeding), and lower airways, which correlated with the air levels of OAA (table 3).

The women had higher prevalences of eye and nose symptoms than the men, and the elderly had more eye symptoms than the younger workers ($P < 0.20$). Furthermore, associations were found ($P < 0.20$) between gender and age on one hand and air levels of OAA, as well as total urinary metabolites, on the other (table 1). Thus these factors were considered possible confounders and were adjusted for in the rest of the multivariate analyses.

With such an approach, there were significant associations between the eye, nose, and lower airway symptoms on one hand and air levels on the other (table 4). Any interaction between the air levels and positivity for the specific antibodies, as regards risk of symptoms, was assessed for the exposed workers. There was no consistent and significant interaction for either specific IgE or specific IgG.

Furthermore, there were significant associations between work-related complaints of the eyes, nose, and lower airways on one hand and urinary levels of OAA metabolites on the other (table 4).

Symptoms versus sensitization

Nose bleeding was much more common among the exposed workers who had a positive SPT result with the OAA than among those who were negative (36% versus 3%, $P < 0.001$, not shown in table). Associations were also seen for the total number of symptoms of the nose, eyes, and lower airways, although they were not that close.

The risk of symptoms of the eyes, nose (particularly bleeding), and lower airways increased as the values for the sums of the OAA-specific S-IgE and S-IgG increased (table 4).

Among the subjects with symptoms, between one-third and one-half had specific IgE or IgG antibodies (table 5). The exception occurred for the workers who reported nose bleeding, among whom as many as 77–85% were positive.

Table 3. Skin-prick tests and specific serum antibodies against hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) anhydride and the work-related symptoms of workers exposed to various air levels of HHPA and MHHPA and of the referents (whose exposure was 0 $\mu\text{g}/\text{m}^3$). (Ig = immunoglobulin)

Exposure ($\mu\text{g}/\text{m}^3$)	Number of workers	Skin-prick test (% positive)		Specific antibodies (% positive)				Work-related symptoms (%)			
		HHPA	MHHPA	IgE		IgG		Eyes	Nose		Lower airways
				HHPA	MHHPA	HHPA	MHHPA		Total	Bleeding	
0	57	0	0	0	0	0	0	14	16	0	4
<10	53	13	11	13	15	2	4	15	30	4	8 ^a
10–50	72	19	18	26	26	22	26	25	26	13	14
>50	29	14	14	21	17	41	38	34	28	7	17
Test for trend (P)		0.005	0.004	<0.001	<0.001	<0.001	<0.001	0.01	0.22	0.01	0.02

^a One observation missing.

Table 4. Risk (adjusted for gender and age) of work-related symptoms for 154 persons exposed to hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) anhydrides and 57 referents in relation to air levels and the urinary metabolites (sum of HHP and MHHP acids) and specific serum IgE and IgG (sums of values for HHPA and MHHPA). According to the metabolite and antibody values, the exposed workers were divided into quartiles. (POR = prevalence odds ratio, 95% CI = 95% confidence interval, Ig = immunoglobulin)

Group	Eye symptoms		Nasal symptoms		Lower airway symptoms	
	POR	95% CI	POR	95% CI	POR	95% CI
Referents	1.0	.	1.0	.	1.0	.
Exposed workers						
Air levels ($\mu\text{g}/\text{m}^3$)						
<10	0.6	0.2– 1.7	1.4	0.5– 3.8	1.2	0.2– 7.2
10–50	4.1	1.4–12	2.8	1.1– 7.3	8.3	1.5– 44
>50	8.7	2.4–32	3.9	1.2–13	21	2.7–170
P-value	0.004	.	0.02	.	0.007	.
Urinary metabolites (quartiles)						
1st	1.0	0.3– 3.4	1.3	0.4–4.0	2.1	0.3–14
2nd	1.7	0.5– 5.6	1.5	0.5–4.7	6.5	1.2–35
3rd	2.3	0.7– 8.1	1.9	0.6–6.2	1.2	0.1–14
4th	4.1	1.2–13	3.0	1.0–9.4	8.1	1.3–50
P-value	0.01	.	0.005	.	0.06	.
Serum IgE (quartiles)						
1st & 2nd	1.4	0.5– 3.7	1.6	0.6–4.0	2.8	0.6–14
3rd	0.5	0.1– 2.6	2.3	0.8–7.1	4.9	0.8–29
4th	5.2	2.0–14	3.3	1.3–8.4	5.6	1.1–28
P-value	0.01	.	0.04	.	0.01	.
Serum IgG (quartiles)						
1st	1.8	0.6– 5.4	4.1	1.5–11	2.8	0.5–16
2nd	1.1	0.3– 3.7	0.3	0.1– 1.7	2.8	0.4–18
3rd	1.3	0.4– 4.5	2.0	0.7– 6.1	4.3	0.7–25
4th	3.9	1.5–10	3.0	1.1– 7.7	6.1	1.2–31
P-value ^a	0.001	.	0.02	.	0.02	.

^a P = test for trend.

Table 5. Sensitization [skin-prick test or specific serum immunoglobulin (Ig) E and G antibodies] against hexahydrophthalic (HHPA) and methylhexahydrophthalic (MHHPA) acids and the work-related symptoms of 154 anhydride-exposed workers.

Parameter	Eye symptoms		Nasal symptoms				Lower airway symptoms	
	Yes (N = 36)	No (N = 118)	Total		Bleeding		Yes (N = 19)	No (N = 135)
			Yes (N = 43)	No (N = 111)	Yes (N = 13)	No (N = 141)		
Skin-prick test positive (%)								
HHPA	44	8	35	9	69	11	32	14
MHHPA	42	7	30	9	62	11	32	13
Specific antibodies positive (%)								
IgE								
HHPA	47	13	37	14	85	15	37	19
MHHPA	47	13	37	14	85	15	37	19
IgG								
HHPA	39	13	30	14	77	13	37	16
MHHPA	42	14	33	16	77	16	37	19

Lung function and exposure

In the univariate analyses, a decrease in FVC was associated with an increase in urinary HHP acid (table 2). Furthermore, the FVC declined as the SPT ratio against OAA increased.

Current smoking was associated with a significant decrease in the $\text{FEV}_{1.0}$, but not for a decrease in FVC

(not shown in table). In the additional multivariate analyses of the relations between lung function and air levels, adjustments were made for smoking. A decrease in FVC was found ($P=0.04$, not shown in table) as the air levels increased, while $\text{FEV}_{1.0}$ was not significantly associated with the exposure.

There was no statistically significant association between lower airway symptoms and the two lung function variables (not shown in table).

Discussion

The most important findings were the fairly close association between the low levels of OAA in air and the metabolites in urine, the high risks of developing specific S-IgE and S-IgG against the OAA, and work-related symptoms of the eyes, nose (especially nose bleeding), and lower airways, even at very low exposure levels, and the associations between specific S-IgE and S-IgG and such symptoms.

Some methodological aspects should be commented upon. This was a cross-sectional study. Thus a healthy worker selection (into or out of the factory) may have occurred, as seen earlier in OAA-exposing settings (9). Hence the true risks may have been even higher than those observed by us.

Since it is possible that different OAA have different sensitizing potencies (17), we tried to use the air levels of the specific OAA in the estimates of the exposure-response relation, which, however, was not fruitful; hence we employed the sum air levels of both OAA.

The workers in the highest exposure category used protective respiratory devices. Thus the true exposure behind the mask may have been lower than indicated by the air levels, and therefore exposure-response relations may have been obscured. Hence, as an alternative to air levels, we used the specific OAA metabolites in urine as exposure indices. Still, even the mask-wearing workers had significant levels of metabolites, and this finding may indicate infrequent use of the protectors or overloading of the filters.

Biomarkers have several advantages. A limitation of the ones used by us is their fairly rapid turnover (18). Hence, day-to-day variations in exposure may have caused a misclassification. This possibility may explain why the metabolite levels showed only occasional associations with the effect parameters, which are more long-term.

There were very close associations between the results of the testing with HHPA and MHHPA, both for SPT and antibody determinations. This finding may have been due to the simultaneous exposure, but it is more likely to be due to cross-sensitivity.

Of course, it is difficult to obtain valid replies regarding the work-relatedness of common symptoms. However, uncertainty in this regard would probably obscure any relations with exposure and immunologic parameters. Rather high prevalences of eye and nose symptoms (except nose bleeding) were found in the ref-

erence group of industrial workers. This occurrence was probably due to occasional exposures to non-OAA irritants or exposure to dust; and therefore the exposure-response relations may have been less steep than they should have been.

In the multiple regression models, we used prevalence odds ratios (POR) to describe the risks. Sometimes, the POR values were rather high (> 2). Therefore, it should be noted that such figures are higher than the relative risk and cannot be directly interpreted as such.

We found clear exposure-response relations for exposure, both as measured in air and as biomarkers on one hand and the development of specific antibodies and symptoms on the other. Detailed information on such relations is not available for any low-molecular-weight chemical. There is also very limited information for high-molecular-weight antigens, although nice exposure-response data have recently been published for proteins in rat urine (19).

The specific S-IgE increased already at a low air concentration, while the S-IgG rose mainly at high concentrations. It is unlikely that this finding is due to workers who developed IgE antibodies having symptoms and being therefore moved to sites with lower exposure. Instead, the finding probably indicates that, in predisposed subjects, even low exposure causes sensitization. Atopy has, in some studies (20, 21), though not in all (22), been associated with sensitization to OAA; however, although there was such a tendency in our study, the finding was not statistically significant. Selection may explain this result. Furthermore, smoking did not increase the risk of sensitization, as claimed in some other studies on workers exposed to other OAA (23), although not in all (22).

The levels of S-IgE and S-IgG antibodies were highly correlated and were both closely associated with eye and airway symptoms. We have earlier shown that IgE antibodies have a pathomechanistic role (24). As to IgG, the antibodies probably mainly reflect exposure (16).

Among the effects, the rather high prevalence of work-related epistaxis is of special interest. The bleeding was not profuse, but neither was it only a staining of the mucus when the nose was blown. Almost all of the affected workers had specific IgE antibodies. Interestingly, after the inhalation of labeled OAA, the nasal mucosa was loaded (25). Furthermore, hemorrhagic rhinitis has earlier been described in a case report of six men exposed to a heated epoxy resin, which contained HHPA (exposure level not stated) (26). These men were also sensitized to HHPA. It is interesting that exposure to trimellitic anhydride may cause hemoptysis (2). Such was not the case for our workers.

There were also close associations between exposure and sensitization on one hand and lower airway symptoms on the other. However, as indicated by the spiro-

metric recordings, only minor bronchial obstruction was represented. An association between bronchial hyperresponsiveness and OAA sensitization has recently been reported (20).

As seen earlier in other factories (27), more than half of the workers with eye and airway symptoms (except nose bleeding) were negative for specific S-IgE. The pathomechanism in these cases is unknown. OAA are irritants, but fairly weak as such (21). In the factory of our study, there was exposure to N,N-dimethylbenzylamine, which may have been partly responsible (28).

The high risk of sensitization and symptoms among our workers is remarkable when the low air concentrations are considered. The levels were probably not higher before the study. The exposure levels, as well as the prevalences of sensitization and work-related symptoms, were lower than found in an earlier study of workers exposed to HHPA (7), but similar to those found in other factories, where the exposure was comparable (8–10).

The prevalences were also lower than for workers exposed to methyltetrahydrophthalic anhydride (22, 24, 29, 30), in spite of the fact that the exposure levels were similar. This finding may have been caused by different OAA having varying sensitizing properties, as mentioned earlier, but it is possibly also due to healthy worker selection in our study. Yokota et al (22) proposed that the permissible exposure limit for MTHPA should not exceed 10–20 µg/m³; our data indicate that this limit would prevent symptoms, but not sensitization to HHPA.

These OAA are among the most potent nonprotein sensitizers known. And the risk is high, in spite of extended control of industries using OAA on the part of the Swedish labor inspectorate. The use of some of them, including HHPA and MHHPA, even requires special permission and the health surveillance of exposed workers.

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