Detection of IgE-mediated respiratory sensitization in workers exposed to hexahydrophthalic anhydride

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Twenty-seven workers with occupational exposure to hexahydrophthalic anhydride (HHPA) from an epoxy resin molding system were studied to evaluate the nature of their reported respiratory complaints. The workers were evaluated by questionnaire, pulmonary function tests, and serologic investigations. The presence of serum-specific IgE and IgG to an HHPA–human serum albumin (HSA) conjugate was measured by use of RAST and ELISA assays. Estimates of exposure to HHPA were made for each worker on the basis of job description and environmental sampling. Seven workers reported symptoms of asthma and rhinitis; four workers had symptoms consistent with occupational asthma. Fourteen of the remaining 20 workers reported nasal or ocular symptoms while they were at work. No worker demonstrated a significant (>20%) pre- to postshift decrement in FEV_1. Twelve workers had significant levels of specific IgE to HHPA-HSA; 11 had elevated levels of specific IgG to HHPA-HSA. A group of workers estimated to have higher exposures to HHPA had a significantly higher mean total IgE level ($p < 0.05$) and significant titers of HHPA-HSA–specific IgE or IgG, or both ($p = 0.048$) as compared to a group with lower exposure to the anhydride. All four workers with occupational asthma/rhinitis had significant levels of specific IgE to HHPA-HSA (ranging from 8.7% to 23.4% RAST binding); three workers did not work directly in the HHPA area but were located in nearby sections of the plant with lower exposures to HHPA. Three workers with symptoms of asthma not clearly associated with the workplace did not have significantly elevated specific IgE levels. Another radioimmunoassay with the use of beads coated with mouse monoclonal antihuman IgE was used to quantitate the amount of specific anti-HHPA-HSA binding (range 1.0 ng to 32.6 ng/ml) present in workers' sera. The solid-phase bead radioimmunoassay was inhibited by the homologous HHPA-HSA conjugate but not by HHPA hapten alone in two workers, suggesting that these workers were sensitized to new antigenic determinants. We conclude that HHPA is a potent industrial sensitizer and is capable of inducing IgE-mediated disease. Prospective investigations are required to define the incidence and severity of clinical sensitivity. (J ALLERGY CLIN IMMUNOL 75:663-72, 1985.)

Acid anhydrides are widely used in the plastics industry as curing agents, adhesives, and plastic reinforcers. These compounds are highly reactive and act to cross-link polymers in epoxy resin systems.[1] PA was the first of several anhydride compounds demonstrated to cause occupational rhinitis, asthma, and direct skin test sensitivity.[2] Passive transfer of skin sensitivity by reaginic antibody in this case implicated an IgE-mediated mechanism. In 1976 Maccia et al.[3]

Abbreviations used

HHPA:	Hexahydrophthalic anhydride
HSA:	Human serum albumin
PA:	Phthalic anhydride
SPBRIA:	Solid-phase bead radioimmunoassay
BSA:	Bovine serum albumin
PBS:	Phosphate-buffered saline
NAD:	New antigenic determinant
OD:	Optical density
RIA:	Radioimmunoassay

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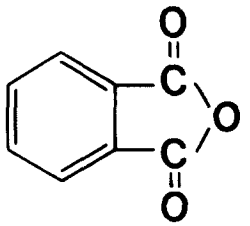
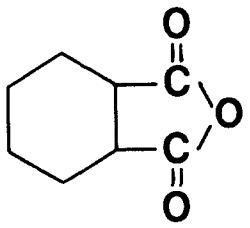


Phthalic (PA)



Hexahydrophthalic (HHPA)

FIG. 1. Chemical structures of hexahydrophthalic anhydride and PA.

first demonstrated serum-specific IgE to PA-HSA conjugate by the in vitro RAST technique in a worker with documented PA–induced asthma.

Since these initial studies trimellitic anhydride,[4, 5] tetrachlorophthalic anhydride[6] and himic anhydride[7] have all been demonstrated to cause IgE-mediated bronchial sensitization in exposed workers. HHPA is a saturated analog of PA (Fig. 1). This article describes a hazards investigation of a group of HHPA–exposed workers, some of whom developed occupational asthma and rhinitis. Serologic investigations were performed to investigate the type and frequency of humoral immune responses associated with HHPA exposure.

METHODS

Study group

Twenty-seven workers in a plant manufacturing bushings for electrical transformers were studied by questionnaire, pulmonary function tests, and serologic investigations. An epoxy resin system with HHPA as a reagent was located in one section of the plant where crystalline HHPA was liquified by heating. Most molds were made by use of an enclosed epoxy mixing and pouring system. However, the production of some molds required that HHPA be mixed in a small nonenclosed mixer, and the resin-containing HHPA was hand carried to the molds in open buckets. Respiratory protection had been recommended to these workers who carried and poured the open buckets of liquid HHPA. Employees involved in the resin mixing and mold pouring procedures were designated "loaders," "mixers," and "molders." The epoxy resin products were further machined, cut, and abraded in another section of the same area by workers called "finishers," "assemblers," or "strippers."

Industrial monitoring

An initial on-site evaluation of the plant was performed to assess pertinent airborne exposures. During this survey area monitoring was performed for HHPA, 1,1,1-trichloroethane, and dust particulates. No other phthalyl-like compounds or other suspected sensitizing agents were used in the area. Air samples for HHPA were collected for time periods from 15 min to 8 hr by use of a specially designed impinger developed by National Institute for Occupational Safety and Health for detection of HHPA vapors, mists, and dust. Concentrations were determined and reported in milligrams per cubic meters and parts per million. A total of 56 samplings for HHPA were performed on two occasions in locations best suited to estimate the workers' exposure to HHPA. Trichloroethane samples were collected separately by use of charcoal absorbent tubes and results were recorded in parts per million. Additional tubes were obtained for qualitative analysis. Total particulate concentrations were collected by use of standard filter techniques. Ventilation measurements were obtained at selected areas.

Questionnaire

An occupational questionnaire was used to evaluate workers' respiratory and ocular complaints. Questionnaire diagnoses were made by use of predetermined criteria. The diagnosis of a history of asthma was made on the basis of symptoms of shortness of breath, wheezing, or coughing. Specification of occupational asthma required additional criteria of unequivocal exacerbation at work and/or nocturnal symptoms, improvement away from the workplace, and a negative history of asthma before occupational exposure. A diagnosis of rhinitis was made if a worker noted rhinorrhea, nasal congestion, and/or sneezing. Conjunctivitis was determined by the presence of ocular itching, burning, or tearing of the eyes. Occupational rhinitis and/or conjunctivitis required the presence of symptoms only at work. Atopic status was assessed by questionnaire regarding prior personal and/or family history of common allergic diseases (hay fever, asthma, and eczema).

Pulmonary function tests

Pre- and post-shift pulmonary function testing was performed on all participants on the first workday of the week. Measurement of FEV_1 and FVC was obtained with standard spirometric techniques by use of a Vitalograph wedge spirometer (Vitalograph Medical Instrumentation, Lenexa, Kan.) and a positive test defined as more than 20% decrement in post-shift FEV_1 as compared to pre-shift FEV_1. These pulmonary function tests were repeated in an identical fashion 1 mo later except that there was an additional day of testing on the morning of the second workday.

Immunologic studies

Immunologic evaluation in this study consisted of RIAs and an ELISA. HSA conjugates of various anhydrides were used in both RIAs and the enzyme-linked assay. Skin testing was not permitted in the study protocol.

A sample of solid HHPA used at the plant was obtained for use in our studies. Reagent grade PA was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis., lot no. 071947. Purity of HHPA and PA samples was determined by gas chromatography. Both were found to be free of contamination with other phthalyl or anhydride compounds. ^{125}I was purchased from Amersham Corp., Arlington Heights, Ill., in 1 mCi quantities with specific activity of 100 mCi/ml. Tandem IgE beads coated with mouse hybridoma anti-human IgE were kindly donated by Hybritech, La Jolla, Calif. (lot 2J6147).

Conjugates of HHPA and PA for the SPBRIA were made by use of a solution of 100 mg of HSA in 20 ml of 7% $NaHCO_3$ (HSA-$NaHCO_3$). One hundred mg of HHPA or PA was solubilized in 1.0 ml of 1,4 dioxane and added dropwise to HSA-$NaHCO_3$. This solution was stirred at 37° C for 1 hr, dialyzed overnight against 0.1 mol/L $NaHCO_3$ at 4° C, and redialyzed three times with distilled water at 4° C. The retentate was adjusted to pH 7.6 with Tris-buffered saline. The molar substitution of HSA with HHPA or PA ligands was determined by gas chromatography. Hapten-carrier molar ratios for HHPA-HSA and PA-HSA conjugates were 8.5:1 and 11.6:1, respectively. Sodium salts of HHPA and PA were dissolved in 7% $NaHCO_3$.

Conjugates were prepared for the RAST system by reacting 100 mg of either HHPA or PA with 100 mg of HSA dissolved in 5 ml borate-buffered solution, pH 8.0. This mixture was stirred overnight at room temperature, and the resultant phthalyl-protein conjugate was separated by sequential centrifugation and dialysis against PBS. The HHPA and PA substitution ratios, as determined by gas chromatography, were 9.4:1 for the HHPA-HSA conjugate and 11.6:1 for the PA-HSA conjugate.

Radioiodination of the anhydride protein conjugate was performed by a modification of Greenwood and Hunter.[8] Fifty micrograms of conjugate was added to 1 mCi Na ^{125}I and radioiodinated by timed, sequential addition of 50 μg of chloramine T, 100 μg of sodium metabisulphite, and 0.5 mg of potassium iodide. Five percent BSA was used to elute the conjugate on a G-25 Sephadex column. The radioactivity was 95% precipitable by addition of 10% trichloroacetic acid. The specific activity of ^{125}I-labeled HSA-HHPA conjugate was 24,500 cpm/ng.

RAST was performed as previously described.[9] Significant levels were considered to be at least 5% of total added radioactivity or twice that of the mean of control subjects not exposed to HHPA.

A modification of a SPBRIA described by Grammer et al.[10] was used to quantify specific IgE against HHPA-HSA conjugate. One Tandem IgE bead coated with mouse hybridoma anti-human IgE was placed in a polystyrene tube followed by 0.2 ml of 5% BSA and .05 ml of test sera. The tube was incubated overnight at 37° C and washed three times with PBS. Five hundred thousand counts per minute of radiolabeled HHPA-HSA was added to each tube and allowed to incubate at 4° C for 48 hr, washed three times with PBS, and counted. The quantity of radiolabeled HHPA-HSA bound in exposed workers was calculated as follows:

[cpm of tube with test sera − mean cpm of four tubes from nonexposed volunteers] ÷ 24,500 cpm (specific activity) × 20 (dilution factor)

Significant antibody concentrations were defined as 1 ng/ml or more of bound radiolabeled HHPA-HSA, which was determined to be 2 SD more than the mean concentration of the control group.

Competitive inhibition studies were performed by addition of various molar concentrations of unlabeled HHPA-HSA, PA-HSA, NaHHPA, or NaPA to respective test tubes together with the radiolabeled HHPA-HSA. Percent inhibition was calculated by the formula:

% inhibition = [1 − cpm of the inhibition tube/cpm of the tube without inhibition reagents] × 100

In all inhibition experiments, the molar concentrations of anhydride ligands were determined directly from the sodium salts of these compounds or by measurement of the respective hapten substitutions of these conjugates.

Total IgE was measured with a Quantitope Kit, Kallestad Lab., Inc., Austin, Texas. Normal levels in an equivalent study population are less than 100 IU/ml.[11]

A modification of an ELISA procedure described by Voller et al.[12] was used to estimate specific IgG antibody to anhydride conjugates. Aliquots of 0.2 ml of 200 μg of HHPA-HSA diluted in 0.1 mol/L NaOH were incubated overnight at 4° C in micro-ELISA plates. After washing with PBS, 0.2 ml of test sera diluted 1:100 in 1% of BSA were incubated, washed, and incubated with goat anti-human IgG conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) diluted 1:100. After further washing with PBS, 0.2 cc of 100 mg *p*-nitrophenyl diphosphate disodium (Sigma Chemical Co.) enzyme substrate dissolved in 50 ml of a 1:1 dilution of alkaline glycine buffer solution (0.05 mol/L of glycine and 0.5 mmol/L of magnesium chloride, pH 10.4) was added. The substrate was hydrolyzed in the presence of alkaline phosphatase for 10 min until the reaction was stopped by addition of 0.05 ml 2 mol/L NaOH. The OD was measured at 405 nm with a PR-50 EIA analyzer (Gilford Instrument Lab., Inc., Oberlin, Ohio). A positive IgG ELISA was defined as an OD 405 nm reading three times the highest level in four nonallergic subjects never exposed to HHPA or other anhydrides.

RESULTS

Environmental sampling

Area monitoring of the work area revealed levels of HHPA ranging from 0.1 to 1.3 ppm (Table I). Samplers placed near mixing towers and mold pouring operations detected a mean HHPA concentration of 0.6 ppm (range 0.2 to 1.3 ppm). Samplers placed near

TABLE I. Total particulate and HHPA concentrations from area environmental samples

Sample location	Airborne concentration				
	Total particulates*,†	No. of HHPA samples	HHPA‡,†(ppm)		
1. Mixing towers and mold pouring	3.7 mg/m³	23	0.6 ± 0.26		$p < .01$§
2. Mold stripping and finishing area	2.3 ± 2.7 mg/m³	18	0.3 ± 0.20	NS	
3. Other locations in HHPA area	ND	5	0.3 ± 0.15		
4. Locations adjacent to HHPA area	ND	4	0.3 ± 0.23		

ND = not done; NS = statistically nonsignificant.
*Recommended standard (American Conference of Governmental Industrial Hygienists) for total particulate concentration is 10 mg/m³.
†Mean ± SD.
‡No recommended standard.
§Statistically significant difference (Student's t test, $p < 0.01$) between locations no. 1 and locations 2, 3, and 4.

mold stripping and finishing operations (including grinding) measured a mean of 0.3 ppm HHPA (range 0.1 to 0.5 ppm). The difference in area HHPA levels was statistically significant (Student's t test, $p < 0.01$). Samples in neighboring areas had mean HHPA concentrations of 0.3 ppm (range 0.2 to 0.4 ppm). All four area and three personal samples for total dust (mean 1.9, range 0.1 to 5.5 mg/m³) were below the threshold limit value recommended by the American Council of Governmental Industrial Hygienists and the Occupational Safety and Health Administration permissible exposure level (10 mg/m³ 8-hour time weighted average).[13] All but one of nine samples for trichloroethane (mean 54, range 4 to 520 ppm) were below the National Institute of Occupational Safety and Health ceiling value (350 ppm for 15 min) for this substance. Smoke tubes were used to demonstrate visually that air currents from the HHPA work area flowed to neighboring work areas. Based on these results the 27 workers were divided into three exposure groups. Loaders, mixers, and molders who worked near the mixing towers had the highest daily exposures to HHPA. Finishers, assemblers, and strippers had lower exposures to HHPA and were placed in the low exposure group. The self-selected employees working in low exposure areas adjacent to the HHPA work area were arbitrarily assigned to a different category.

Clinical evaluation

Twenty-one (64%) of 33 workers employed in the HHPA work area agreed to participate in the study. Six other self-selected workers from adjacent areas of the plant also requested evaluation because of increased frequency of respiratory symptoms that they associated with fumes originating from the plastic resin work area. Seven of the 27 workers reported symptoms of asthma (Table II). Four workers in this group gave clear histories of occupational asthma with exacerbation of symptoms at work, improvement away from the workplace, and no previous history of asthma symptoms before their present employment. Two of these workers with occupational asthma also noted nocturnal cough, shortness of breath, or wheezing; none had personal or family histories of atopy. Three of these workers had jobs adjacent to the HHPA work area. The fourth worker performed any necessary job in the area and was therefore included in the high exposure group. Work relatedness of asthma could not be determined in three workers, two of whom had symptoms of asthma before their current employment and the third who reported symptoms of cough and chest tightness related to use of a silicone spray. All seven asthmatic workers in the group also had developed occupationally related rhinitis and conjunctivitis.

Fifteen of the remaining 20 workers reported nasal and/or ocular symptoms while they were at work (Table III). Ten of these workers had occupationally aggravated rhinitis; 13 workers noted ocular symptoms. Two of these workers regarded sneezing and nasal pruritus as prominent symptoms.

Pulmonary function testing on the 27 workers demonstrated no airway obstruction on pre-shift testing (FVC and FEV_1 > 80% predicted, FEV_1/FVC > 0.7). All participants failed to demonstrate a signifi-

TABLE II. Summary of immunologic results in workers reporting asthma symptoms on questionnaire

					HHPA-HSA specific antibody		
					IgE		IgG
Worker no.	Job title	Atopic status	Asthma symptoms	Total IgE (IU/ml)	RAST % binding	SPBRIA HHPA-HSA binding per milliliter	ELISA OD 405 nm
1	Adj. area	NA	Occupational	ND	18.0*	ND	1.246†
2	Adj. area	NA	Occupational	67	16.6*	32.6‡	0.186
3	Adj. area	NA	Occupational/ nocturnal Sx	90	23.4*	9.6‡	1.562†
4	All jobs	NA	Occupational/ nocturnal Sx	84	8.7*	1.0‡	0.239
5	Assembler	AT	Prior hx of asthma	51	3.6	NS	1.834†
6	Finisher	AT	Prior hx of asthma	14	2.9	NS	−0.047
7	Stripper	NA	Related only to silicon spray	234	4.5	NS	0.099

Adj. = adjacent; NA = nonatopic; AT = atopic; ND = not done; NS = nonsignificant; hx = history; Sx = symptoms.
*Significant values defined as 2 × mean percent binding (2.5%) in nonexposed laboratory personnel.
†Significant values defined on 3 × highest level (0.227) in nonexposed laboratory personnel.
‡Significant values defined as 1 ng of HHPA-HSA bound per milliliter or >2 SD higher than the mean binding in nonexposed laboratory personnel.

cant post-shift decrement of FEV_1 when results were compared to pre-shift test results. There was no shift in pre-shift baseline spirometry performed 1 mo later. Similar results were obtained demonstrating no significant alteration in post-shift pulmonary function, and there was no significant change in FEV_1 on the second workday morning as compared to the previous day's results. No significant changes were noted on lung auscultation performed at the time of pulmonary function testing.

Immunologic evaluation

Immunologic tests were evaluated in relation to symptom and exposure categories. Table III demonstrates the results of immunologic profiles in different exposure groups. Specific IgE to HHPA-HSA was detected by RAST in 12 (44%) of 27 workers. Seven of these 12 employees were from the HHPA work area, and the other five were from adjacent work areas. The seven RAST–positive workers from the HHPA work area had worked in that area for an average of 4.2 yr, whereas the 14 RAST–negative workers from the HHPA work area had worked in this area an average of 1.6 yr. This difference was statistically significant (Student's t test, $p = 0.03$). Five of 11 workers in the high exposure group had significant RAST levels compared to two of 10 employees in the low exposure group (Fisher's exact test, $p = 0.3615$). Five of the six self-selected workers from an adjacent area of the plant (mean work time in area was 6 yr) also had significant RAST binding. All four of the workers with occupational asthma demonstrated high titers of HHPA-HSA–specific IgE by RAST, ranging from 8.7% to 23.4%; highest levels were present in the three workers adjacent to the HHPA work area. Complete blood counts in the 27 workers revealed elevated percentages of eosinophils in only three workers, all of whom were from this group reporting symptoms of occupational asthma. None of the three workers believed to have nonoccupational asthma demonstrated a significant RAST result, and all had eosinophil percentages of 2% or less. Workers with occupational asthma had statistically significant higher mean RAST binding than workers with nonoccupational asthma or asymptomatic workers (Table IV). Similar comparisons with workers who reported occupational nasal or ocular symptoms without asthma could not be made because a hypothesis of normality on the grouped data was rejected ($p < 0.05$). Of the 15 workers reporting only nasal and/or ocular symptoms at work, six had significant HHPA-HSA–specific IgE titers (Table IV). Two of five asymptomatic work-

TABLE III. Summary of immunologic results in workers classified by exposure

Exposure levels	Worker no.	Job title	Atopic status	Occupational symptoms		Total IgE (IU/ml)
				Nasal	Ocular	
High levels	4*	All jobs	NA	+	+	84
	8	Loader	AT	+	+	29
	9	Loader	AT	+	+	14
	10	Loader	NA	—	—	330
	11	Loader	NA	+	+	300
	12	Loader	?	—	+	123
	13	Molder	NA	+	—	465
	14	Mixer/molder	NA	+	—	14
	15	Mixer/molder	NA	+	+	192
	16	Molder	AT	—	—	750
	17	Molder	NA	—	+	ND
Low levels	5†	Assembler	AT	+	+	51
	6†	Finisher	AT	+	+	14
	7†	Stripper	NA	+	+	234
	18	Finisher	NA	—	—	27
	19	Finisher	?	—	+	14
	20	Finisher	?	—	—	<9
	21	Assembler	?	—	+	29
	22	Assembler/ stripper	?	—	—	23
	23	Assembler/ stripper	?	—	+	29
	24	Assembler/ stripper	NA	+	+	96
Unknown (adjacent areas)	1*	Adj. Area	NA	+	+	ND
	2*	Adj. Area	NA	+	+	67
	3*	Adj. Area	NA	+	+	90
	25#	Adj. Area	?	+	+	33
	26	Adj. Area	NA	+	+	96
	27	Adj. Area	NA	+	+	<9

NA = nonatopic; AT = atopic; + = symptomatic; — = asymptomatic; ND = not done; NS = not significant.
*Occupational asthma.
†Nonoccupational asthma.
‡Significant values defined as 2 × mean percent binding (2.5%) in nonexposed laboratory personnel.
§Heat inactivated.
‖Not heat inactivated.
¶Significant values defined as 3 × highest level (0:227) in nonexposed laboratory personnel.
#This employee had worked in the HHPA area in the past.
**Significant values defined as 1 ng of HHPA-HSA bound per milliliter or 2 SD higher than the mean binding in nonexposed laboratory personnel.

ers also demonstrated positive RAST tests. Only two of 10 RAST–positive workers had atopic backgrounds; reliable allergy histories were not available in the other two RAST–positive employees.

SPBRIA detected all RAST–positive sera except for one subject who could not be tested by both techniques. A significant linear correlation was noted between RAST and SPBRIA tests (Fig. 2). Moreover, RAST and SPBRIA were concordant in 23 of 25 tests when both positive and negative results were compared. The levels of radiolabeled conjugate binding in positive workers ranged from 1.0 to 32.6 ng/ml of ^{125}I–HSA-HHPA bound. Specificity of the assay was determined in two separate high-titered sera by sig-

HHPA-HSA specific antibody		
IgE		
RAST % binding	SPBRIA ng of HHPA-HSA bound per milliliter	IgG ELISA OD 405 nm
8.7‡	1.0§·**	0.239
8.4‡	1.0§·**	0.892¶
3.0	4.7‖·**	0.947¶
4.4	NS**	2.756¶
3.8	NS	0.109
3.1	NS	0.343
21.4‡	13.6§·**	0.707¶
6.5‡	17.4§·**	0.031
4.5	21.9§·**	1.200¶
7.5	1.0§·**	1.000¶
4.8	ND	0.868¶
3.6	NS	1.834¶
2.9	NS	−0.047
4.5	NS	0.099
6.3‡	10.0§·**	2.113¶
3.4	NS	0.469
3.6	NS	0.084
5.3‡	8.1§·**	0.077
3.7	NS	0.197
3.1	NS	0.377
3.3	NS	0.060
18.0‡	ND	1.246¶
16.6‡	32.6§·**	0.186
23.4‡	9.6§·**	1.562¶
15.3‡	4.5§·**	0.551
5.1‡	1.1	0.192
4.4	NS	0.011

nificant inhibition of radioactive binding after coincubation with unlabeled HHPA-HSA. All sera that were both SPBRIA– and RAST–positive were heat-labile after heating at 56° C for 4 hr. Positive SPBRIA results were obtained in two RAST–negative workers. Worker 15 had very high levels of specific allergen (21.9 ng/ml) that was abrogated by heating at 56° C for 4 hr or by preincubating with specific unlabeled antigen. The SPBRIA value of 4.7 ng/ml observed in serum from worker 9 was thought to be nonspecific because it did not exhibit heat lability or inhibition by homologous antigens.

Total IgE levels were determined in 25 workers. Six of seven workers with total IgE concentrations more than 100 IU/ml were from the high exposure group. There was a higher mean total IgE level in the high exposure versus the low exposure group (Student's t test, $p < 0.05$). However, no correlations were noted when total IgE levels were compared to symptoms reported by the workers.

Specific IgG to HHPA-HSA was demonstrated by ELISA in 11 of 27 workers illustrated in Table III. Seven of these positive sera contained specific IgE to the same antigen when sera was measured by RAST or SPBRIA and included two of the workers with occupational asthma. Seven high exposure workers had positive specific IgG antibody versus two workers each in the low and adjacent exposure groups. Three of five asymptomatic subjects had significant ELISA titers for HHPA–specific IgG (Table IV).

Exposure level of HHPA as assessed by job description and environmental sampling correlated with specific antibody reactivity (specific IgE, IgG, or both) (Fisher's exact test, $p = 0.048$). However, there was no similar correlation between symptomatic status and specific humoral responses in these workers. Although all workers with occupational asthma had specific IgE to HHPA conjugates, no consistent pattern of antibody responses was found in those workers with occupational nasal or ocular symptoms.

The specificity of IgE antibody to the anhydrides was investigated by use of SPBRIA. Inhibition of specific binding of workers' sera to radiolabeled HHPA-HSA was measured after coincubation with unlabeled HHPA-HSA, PA-HSA, or their respective sodium salts, NaPA or NaHHPA. A typical inhibition curve by use of serum from worker 3 is illustrated in Fig. 3. Unlabeled HHPA-HSA and PA-HSA reagents resulted in 50% inhibition at concentrations of 1.5×10^{-7} mol/L and 4.5×10^{-6} mol/L, respectively. NaHHPA required more than a hundredfold higher concentration to inhibit a similar degree of binding when it was compared to HHPA-HSA. NaPA was totally ineffective even at high concentrations. Similar results were obtained with serum from worker 13. Cross binding of HHPA workers' sera to PA-HSA conjugate was also demonstrated in the direct RAST system. Seven (58%) of 12 HHPA-HSA RAST–positive sera demonstrated significant binding to PA-HSA discs (mean RAST binding 15.8%). Negative HHPA-HSA RAST sera were also negative to PA-HSA–coupled substrate.

DISCUSSION

This study revealed symptoms of occupational asthma, rhinitis, and conjunctivitis in workers exposed to HHPA used as an ingredient in an epoxy resin molding system. Serologic RIA tests demonstrated significant levels of specific IgE to HHPA pro-

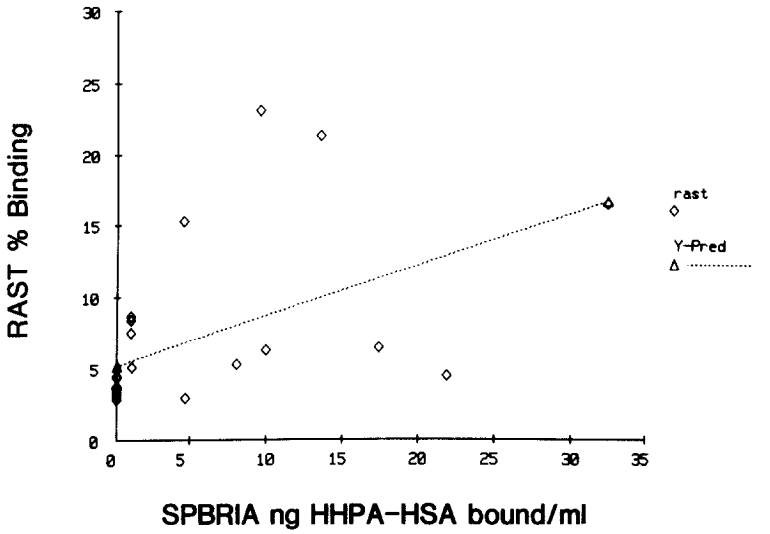


FIG. 2. Regression analysis of RAST (percent added radioactivity bound) vs. SPBRIA ng HHPA-HSA bound/ml. R = 0.512; F-ratio = 8.1638; significance level = 0.00891.

TABLE IV. Summary of immunologic results

	Significant elevations in antibody levels (no. positive/no. tested)				
		HHPA-HSA Specific Ab			
		IgE			
Clinical classification	Total IgE*	RAST† mean % binding	SPBRIA‡	IgG§	IgE, IgG, or both
Occupational asthma	0/3	4/4 (16.7)‖,¶	3/3	2/4	4/4
Nonoccupational asthma	1/3	0/3 (3.7)‖	0/3	1/3	1/3
Occupational rhinoconjunctivitis	4/14	6/15 (6.4)#	7/14	5/15	9/15
Asymptomatic workers	2/5	2/5† (5.1)¶	2/5	3/5	3/5
Nonexposed controls	—	0/4 (2.5)	0/6	0/4	0/6

*Total IgE level ≥100 IU/ml.
†RAST binding ≥5% total added radioactivity.
‡Radiolabeled HHPA-HSA bound ≥1 ng/ml.
§OD405 nm ≥ 0.277.
‖Student's t test $p = 0.024$.
¶Student's t test $p = 0.021$.
#Hypothesis of normality rejected.

tein conjugates and confirmed specific allergic sensitization in four workers with occupational symptoms of asthma/rhinitis. Historical findings did not permit absolute discrimination between allergic and irritant nasal or ocular responses to HHPA in those workers reporting occupational rhinitis, conjunctivitis, or both. However, the presence of significant HHPA-HSA titers of specific IgE by RIA in 10 of 17 workers, including two workers with sneezing, nasal pruritus, and coryza and the four subjects with occupational asthma, suggested that both upper and lower respiratory sensitization may occur. Although skin or organ challenge tests to HHPA conjugates would have helped to confirm these associations, they were not permitted in this study. These results clearly indicate that HHPA should be included in the list of phthalyl anhydrides that cause IgE-mediated respiratory disease.

Environmental sampling and clinical laboratory data indicated that exposure levels may influence the immunologic responses observed in individual workers. The high exposure group had significantly higher

mean total IgE levels and specific antibody responses when responses were compared to the low exposure group. Industrial sensitization also occurred in workers not directly involved in the HHPA work area; three of these workers developed occupational asthma. Subsequent environmental sampling and smoke tube analysis revealed that these workers were actually exposed to HHPA by air currents emanating from the adjacent HHPA work area. Their work duties also required intermittent and occasional access to the HHPA area. The finding of specific IgE in workers with low exposures demonstrates that HHPA is a potent industrial allergen and suggests that a low-dose exposure pattern over a period of time may be an important determinant of respiratory sensitization to HHPA.

Specific IgE to HHPA-HSA was demonstrated by both RAST and SPBRIA techniques. An IgE reagin was demonstrated to be involved in both systems by loss of significant binding after heat inactivation. The results of the two assays compared well and confirm that both are valid techniques for in vitro measurement of specific IgE to occupational allergens. The SPBRIA is advantageous in providing a quantitative level of specific IgE that may be useful for epidemiologic worker surveillance. Serum from one worker (Table III, worker 15) with a positive SPBRIA but negative RAST had definite reaginic activity because it was inactivated by heat and specifically inhibited by HHPA-HSA. This negative RAST result may have been due to interference by high affinity IgG since specific IgG was detected by ELISA in this case, although other workers with similar levels of specific IgG had significant RAST tests and clearly did not demonstrate any "blocking effect."[14, 15]

Serologic specificity of the SPBRIA test was verified by inhibition studies with the homologous conjugate at low molar concentrations. However, hapten specificity was not confirmed because the molar concentration of NaHHPA was at least two orders of magnitude higher than the HHPA conjugate before measurable inhibition of anhydride-conjugate binding was achieved. Although the salt of the PA phthalyl analog was almost totally ineffective in inhibiting binding, the protein conjugate of PA (PA-HSA) was an effective inhibitor in the SPBRIA assay system. Such results suggested that an epitopic configuration of NADs in the protein moiety of HHPA-HSA contributed major specificity to the IgE humoral response elicited by HHPA. Inasmuch as PA was not present in this plant, the inhibition and RAST (Fig. 3, Table IV) results also indicated that NADs formed by HHPA protein interaction cross-reacted with NADs to PA-HSA. Similar results were reported during investigation of trimellitic anhydride–exposed workers.[16] In this respect immune responses to PA and HHPA appear to be similar, at least in the two sera that were analyzed for cross inhibition relationships in this study.

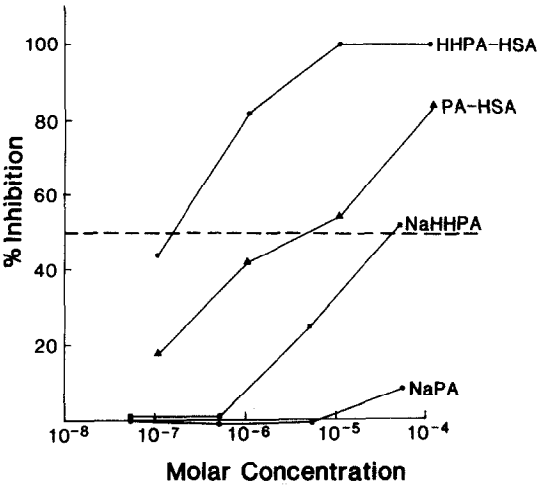


FIG. 3. Direct competitive inhibition of radiolabeled HHPA-HSA binding to sera from worker 3 in the SPBRIA by HHPA-HSA, PA-HSA, NaHHPA, and NaPA. Molar concentrations of haptens are plotted vs. percent inhibition. Fifty percent inhibition is demarcated by *dotted line.*

Although this combined clinical-immunologic cross-sectional survey of occupationally induced sensitization by HHPA was limited in scope, the data provided guidelines for future surveillance of HHPA–exposed workers. HHPA is a potent industrial sensitizer capable of inducing IgE-mediated respiratory disease, and future evaluation of exposure limits for HHPA in the workplace should reflect this capability. At the time this survey was conducted, the humoral immune responses of HHPA–exposed workers were complex as indexed by significant RAST, SPBRIA, and ELISA titers. Future prospective investigations are required to define more precisely the relationship between isotypic antibody reactions and clinical sensitivity.

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Anaphylaxis after contact with a jellyfish

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We report a case of an anaphylactic reaction to a jellyfish sting. The episode was manifested by hypotension and bronchospasm. The patient's basophils released histamine in response to nematocyst venom from the Chesapeake Bay sea nettle; this sensitivity could be passively transferred by a heat labile serum factor. This appears to be the first case report of such a reaction. (J ALLERGY CLIN IMMUNOL 75:672-5, 1985.)

The classic experiments of Portier and Richet,[1] who induced anaphylaxis in dogs with repeated injections of coelenterate protein, represent the beginnings of the scientific study of allergic disease. In spite of the fact that these investigations pointed out the potential for such reactions, there have been no laboratory-based clear demonstrations of human anaphylaxis after jellyfish stings. It has been generally assumed that the anecdotal case reports of severe reactions, and even death, after multiple jellyfish stings were largely toxic responses to the venomous nematocyst proteins.[2]

Abbreviations used
MTNV: Mixed tentacle nematocyst venom
D_2O: Deuterium oxide
Anti-IgE: goat anti-human IgE

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