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Antibody responses of rats after immunization with organic acid anhydrides as a model of predictive testing

by Xing-Dong Zhang, MD, Hans Welinder, PhD, Bo AG Jönsson, PhD, Staffan Skerfving, MD

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Objectives The sensitizing properties of organic acid anhydrides (OAA) were evaluated in a rat model.

Methods The development of specific immunoglobulin (Ig) E and Ig G in serum was investigated after immunization with 14 OAA and 3 OAA conjugates. Brown Norway rats were injected intradermally with 0.1 ml of 0.2 M OAA in liquid paraffin or 1.4 mg of rat serum albumin conjugate in saline. Serum samples were collected after 4 weeks. Antibodies were analyzed with enzyme-linked immunosorbent assay.

Results The serum titers of specific Ig E after immunization with the different free OAA varied from 150 to 6400. The rats immunized with 4-methylphthalic anhydride exhibited the highest titers. The specificity of Ig E was demonstrated by enzyme-linked immunosorbent assay inhibition tests. A good correlation was observed between the Ig E and Ig G titers. Immunization with OAA conjugates showed results parallel to the findings for the free compounds. Importantly, the Ig E titers for the OAA agreed well with findings from guinea pigs and with literature data from epidemiologic studies of exposed workers.

Conclusions The present animal model may be a valuable tool for predicting the sensitizing potential of OAA and possibly the sensitizing potential of low-molecular-weight compounds in general. Furthermore, the antibody specificity of the haptens and the variations in the magnitude of the antibody titers indicate a valuable approach for studies of quantitative structure-activity relationships.

Key terms animal model, immunoglobulin E, immunoglobulin G, occupational allergy, structure-activity relationship.

Knowledge about the chemical determinants of inhalant allergens that cause immune responses has both theoretical and practical significance. Thus interest in predictive testing of allergenicity (1, 2) and in quantitative structure-activity relationships (3—6) is growing.

There are several approaches for testing allergenicity in chemicals. Wass & Belin (7) suggested that the in-vitro reactivity of chemicals with proteins can be used to predict their sensitizing properties. Sarlo & Clark (8) introduced a stepwise procedure to detect chemical allergens, including both subcutaneous and inhalation sensitization of guinea pigs. The alternative use of a rat bioassay was described by Pauluhn (6). Topical sensitization of mice followed by the registration of alterations of the serum concentration of immunoglobulin (Ig) E and differential cytokine production is another interesting approach (5, 9, 10). The induction of specific antibodies may be a sensitive indicator of respiratory sensitization (1).

Knowledge of structure-activity relationships for respiratory sensitizers may be an important tool in predicting the immunogenicity of chemicals (3). Thus a need exists for animal experimental data on relevant chemicals to be combined with chemical, physical, and epidemiologic information.

Organic acid anhydrides (OAA) are reactive chemicals that induce specific Ig E antibodies in exposed workers (11—14). Ig E is involved in the pathogenesis behind rhinoconjunctivitis and asthma in association with exposure to OAA. Thus OAA are good model compounds for studies of the relationship between chemical characteristics and the induction of specific antibodies. Guinea pigs intradermally immunized with 13 OAA showed a wide variation of induced Ig G titers between the different OAA (15). However, while Ig G is the main anaphylactic antibody in guinea pigs, Ig E is important for the immediate allergic responses in rats, as well as in humans (16, 17).

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The aim of the present study was to evaluate antibody responses in rats after intradermal immunization with OAA as a model of predictive testing for allergenicity.

**Material and methods**

**Animals**

Male, inbred Brown Norway (BN) rats (Møllegaard Breeding Center Ltd, Denmark) were used. The strain origin was Zentralinstitut für Versuchstierzucht, Hannover, Germany. They weighed 100 to 150 g when obtained. The rats were acclimatized to the animal facilities for 1 week before the experiments. The microbiological monitoring of the animals and the animal facilities complied with the recommendations of the European Laboratory Animal Science Associations.

**Ethics**

The animal experiments were approved by the Animal Research Ethics Committee of the Lund University.

**Chemicals**

Succinic anhydride (SA), 4-methylhexahydrophthalic anhydride (4-MHHPA; cis-isomer with regard to the carboxyl groups and a mixture of cis- and trans-isomers with regard to the methyl group), and 1,2,3,6-tetrahydrophthalic anhydride (1,2,3,6-THPA) were obtained from Janssen Chimica (Geel, Belgium); meso-dimethylsuccinic anhydride (DMSA) was prepared from meso-dimethylsuccinic acid (Aldrich, Gillingham, United Kingdom) in our laboratory (identity and purity checked with gas chromatography with mass-spectrometric detection (GC-MS) and nuclear magnetic resonance); meso-diethylsuccinic anhydride (DESA) was obtained from Synthelec AB (Lund, Sweden); maleic anhydride (MA) and cis-hexahydrophthalic anhydride (cis-HHPA) came from Merck (Darmstadt, Germany); methylmaleic anhydride (MMA) and 3,4,5,6-tetrahydrophthalic anhydride (3,4,5,6-THPA) were from Sigma Chemical Co (St Louis, MO, USA); 4-methylphthalic anhydride (4-MPA) came from Merck (Darmstadt, Germany); 3,4,5,6-tetrahydrophthalic anhydride (3,4,5,6-THPA) and 4,4-methyltetrahydrophthalic anhydride (4,4-MTHPA) were prepared in our laboratory with identity and purity checked with GC-MS; phthalic anhydride (PA) was obtained from Mallinckrodt Inc (Paris, KY, USA); and trimellitic anhydride (TMA) came from Fluka Chemie AG (Buchs, Switzerland). The purity of the various OAA was >97%. However, DESA was found to isomerize during storage to the corresponding raceme form, which reduced the purity. Rat serum albumin (RSA, fraction V) was purchased from Sigma Chemical Company (St Louis, MO, USA), and liquid paraffin was obtained from Apoteksbolaget (Umeå, Sweden).

**Preparation of conjugates between anhydrides and rat serum albumin**

The conjugates were prepared by reacting the anhydrides with RSA. All the anhydrides were dissolved in water-free dioxane and dripped into a cooled (+4 to +8°C) and stirred solution containing 3 mg of RSA per milliliter of 0.1 M sodium hydrogen carbonate (NaHCO₃). The molar ratio between the added anhydride and RSA was 60:1. The mixtures were stirred for 18 hours. The anhydride-RSA conjugates were purified from low-molecular-weight compounds (<30 000 Da), and the 0.1 M NaHCO₃ buffer was substituted by 0.1 M ammonium hydrogen carbonate (NH₄HCO₃) via ultrafiltration processing.

**Figure 1.** Chemical structures of the organic acid anhydrides used in the experiments. (SA = succinic anhydride, DMSA = dimethylsuccinic anhydride, DESA = diethylsuccinic anhydride, MA = maleic anhydride, MMA = methylmaleic anhydride, cis-HHPA = cis-hexahydrophthalic anhydride, 4-MHHPA = 4-methylhexahydrophthalic anhydride, PA = phthalic anhydride, 4-MPA = 4-methylphthalic anhydride, TMA = trimellitic anhydride, 3,4,5,6-THPA = 3,4,5,6-tetrahydrophthalic anhydride, 1,2,3,6-THPA = 1,2,3,6-tetrahydrophthalic anhydride, 3,4-MTHPA = 3,4-methyltetrahydrophthalic anhydride, 4,4-MTHPA = 4,4-methyltetrahydrophthalic anhydride)
Determination of hapten density. The hapten density (HD) was determined for the conjugates by different methods. The "UV method" was a spectrophotometric one, according to Zeiss et al (11). In the "HCl method" the conjugates were hydrolyzed at 3 selected concentrations of hydrogen chloride (HCl) (0.1, 1, or 6 M), respectively. The samples were evaporated and then worked-up and analyzed by GC-MS according to Lindh & Jönsson (18). The hydrolysis conditions giving the highest yield were chosen for the determinations. The "TNBS method" was based on the titration of free amino groups by 2,4,6-trinitrobenzene sulfonic acid (TNBS), according to Snyder & Sobocinski (19).

Immunization

Free anhydride. Unconjugated, free anhydrides were dissolved in dioxane; then liquid paraffin was added to make a 0.2 M solution (the final concentration of dioxane was 3%). The rats were immunized via intradermal injection with 0.1 ml (in 2 portions of 0.05 ml) of the freshly prepared solutions. Each anhydride was injected into 7 animals. As a control, 7 rats were intradermally injected with liquid paraffin; the dose was 0.1 ml. Serum samples were collected 28 days after the immunization.

Conjugate. The procedures for the conjugates were the same for the free anhydrides, but with anhydrides conjugated to RSA. The dose was 1.4 mg of SA-RSA, or cis-HHPA-RSA, or MA-RSA, respectively, in 0.14 ml of 0.15 M sodium chloride (NaCl). Seven rats were intradermally injected with RSA as a control group, the dose was 1.4 mg.

Antibody determinations by enzyme-linked immunosorbent assay

Specific immunoglobulin E. The following steps were used for specific Ig E: (i) polystyrene microtiter plates (Nunc-Immuno Plate, Nunc, Kamstrup, Denmark) were coated by adding 100 μl/well with 0.015% (in phosphate-buffered saline) anhydride-RSA conjugate; (ii) uncoated protein-binding sites were blocked by adding 200 μl/well with 4% bovine serum albumin and the plates were stored after blocking solution at -18°C until use; (iii) sera taken from immunized BN rats were added to the plates at 100 μl/well in 2-fold dilutions, starting from 1:50, and incubated for 1 hour at room temperature; (iv) sheep anti-rat Ig E (ICN Immunobiochemicals Inc, Costa Mesa, CA, USA) of dilution 1:3000 was added at 100 μl/well and incubated for 1 hour at room temperature; (v) alkaline phosphatase-conjugated goat anti-rat Ig G (Fc Fragment; ICN Biomedicals) of dilution 1:5000 was added at 100 μl/well and incubated for 1 hour at room temperature. The plates were washed after each addition with a Titertek Microplate Washer (Flow Laboratories, Rickmansworth, United Kingdom); and (vi) substrate (p-nitrophenyl phosphate, Sigma Chemical Co) of 0.1% in diethanolamine buffer was added at 100 μl/well. After 2 hours at room temperature, the wells were read at 405 nm by a filter photometer (Titertek Multiskan, Ellob Oy, Helsinki, Finland). All the samples were analyzed in triplicate, with RSA-coated wells as controls for nonspecific binding. The result of each sample was expressed as the value of optical density (OD). The titer was the highest dilution at which the corresponding OD value was greater than the mean OD value of control rat sera plus 3 standard deviations. If the "mean OD + 3SD" was less than 0.05, the latter value was taken as the limit.

Cross-inhibition tests. Antisera of the rats immunized with 4-MHHPA were pooled and diluted at 1:50 in phosphate-buffered saline. Various anhydride-RSA or anhydrides conjugated to guinea pig serum albumin (GPSA) were added to the sera at concentrations of 0, 0.0064, 0.032, 0.16, 0.8 and 4 mg/ml, and after incubation at 4°C for 20 hours, specific Ig E against 4-MHHPA-RSA was analyzed.

Specific immunoglobulin G. The analysis for Ig G was performed according to the method for specific Ig E, but alkaline phosphatase-conjugated goat anti-rat Ig G (working dilution 1:4000; H + L, Zymed Laboratories, Inc, San Francisco, CA, USA) was added directly in the fourth step.

Statistics

For comparisons of the distributions between different groups, the Mann-Whitney U-test was used. The Spearman rank correlation (rs) method was applied to investigate the correlation between 2 variables which can be expressed in a rank order. Statistical significance refers to P<0.05 (2-tailed).

Results

The hapten density for all the conjugates (N=14) was analyzed by the TNBS method. It varied between 16 and 27 mol/mol for the different conjugates (table 1). For 6 of the conjugates, the molar absorbances were large enough to permit determinations also by the UV method. Four of these conjugates corresponded well with the results from the TNBS method, while MA-RSA and TMA-RSA showed a higher and lower hapten density, respectively. The hapten densities after the acidic hydrolysis were lower than those determined by the other 2 methods.

The titers of specific Ig E after the immunization of the rats with the free anhydrides varied from negative to 6400 (table 2). SA and 3,4,5,6-THPA did not give
positive results. Titers (median) below 1000 were obtained with DMSA, DESA, MA, MMA, 1,2,3,6-THPA, 3,4-MTHPA and 4,4-MTHPA, while titers above 1000 were obtained with cis-HHPA, 4-MHHPA, PA, 4-MPA, and TMA. The rats immunized with 4-MPA exhibited the highest titers, which were significantly different from the titers of all the other OAA. 4-MPA and DMSA also demonstrated significant differences in titers compared with their nonmethylated analogues PA and SA, respectively.

In the ELISA (enzyme-linked immunosorbent assay) inhibition tests with anti-4-MHHPA sera, the strongest inhibition was shown by 4-MHHPA-RSA at each concentration of <0.0064 mg/ml in the antisera, and the inhibition was >95% when the conjugate concentration in the antisera was 0.16 mg/ml. In addition, 4,4-MTHPA-RSA, 4-MHHPA-GPSA, and cis-HHPA-RSA showed inhibitions of >90% at the highest concentration (4 mg/ml) employed. For the same anhydride (4-MHHPA, cis-HHPA, 4-MPA), the inhibition of the RSA conjugate was larger than that of the corresponding GPSA conjugate (figure 2).

The titers of specific Ig G varied from 200 to 6400 (table 2). As for Ig E, 4-MPA exhibited the highest titers (P<0.05), and SA and 3,4,5,6-THPA had the lowest titers. There was a close correlation between the Ig E and Ig G titers for the various OAA (rs=0.92, P=0.0001).

The tested conjugates also induced the formation of positive specific Ig E and Ig G. The titers of Ig E varied from 50 to 800, and those for specific Ig G varied from 200 to 1600 (table 3). cis-HHPA-RSA induced higher titers than either SA-RSA or MA-RSA.

The lack of correlation (rs=0.3, P=0.5) between the Ig E titers and the reactivity of the anhydrides demonstrated by their hydrolysis rate constants according to Eberson & Landström (20) is shown in figure 3.

Table 1. Hapten densities of the conjugates between different organic acid anhydrides and rat serum albumin at the molar ratio of 60:1 as determined by a spectrophotometric method (UV method), the analysis of the corresponding acids after acidic [hydrogen chloride (HCl)] hydrolysis of the conjugates (HCl method), and indirect analysis by the determination of free amino groups by 2,4,6-trinitrobenzene sulfonic acid (TNBS method). (NA = not analyzed)

| Conjugate                        | UV method | HCl method | HCl method | TNBS method |
|----------------------------------|-----------|------------|------------|-------------|
| SA-RSA (succinic anhydride-rat serum albumin) | NA        | 11         | 6          | 23          |
| DMSA-RSA (dimethylsuccinic anhydride-rat serum albumin) | NA        | 7          | 6          | 22          |
| DESA-RSA (diethylsuccinic anhydride-rat serum albumin) | NA        | NA         | NA         | 23          |
| MA-RSA (maleic anhydride-rat serum albumin) | 48        | 11         | 0.1        | 23          |
| MMA-RSA (methylmaleic anhydride-rat serum albumin) | 28        | 7          | 0.1        | 22          |
| cis-HHPA-RSA (cis-hexahydrophthalic anhydride-rat serum albumin) | NA        | 10         | 0.1        | 20          |
| 4-MHHPA-RSA (4-methylhexahydrophthalic anhydride-rat serum albumin) | NA        | 13         | 0.1        | 22          |
| 3,4,5,6-THPA-RSA (3,4,5,6-tetrahydrophthalic anhydride-rat serum albumin) | NA        | 11         | 0.1        | 24          |
| 3,4,5,6-THPA-RSA (3,4,5,6-tetrahydrophthalic anhydride-rat serum albumin) | 21        | 10         | 0.1        | 24          |
| 3,4-MTHPA-RSA (3,4-methyltetrahydrophthalic anhydride-rat serum albumin) | NA        | NA         | NA         | 25          |
| 4,4-MTHPA-RSA (4,4-methyltetrahydrophthalic anhydride-rat serum albumin) | 26        | 19         | 6          | 27          |
| PA-RSA (phthalic anhydride-rat serum albumin) | 26        | NA         | NA         | 27          |
| 4-MPA-RSA (4-methylphthalic anhydride-rat serum albumin) | 13        | NA         | NA         | 22          |

Table 2. Titers of specific immunoglobulin (Ig) E and G by enzyme-linked immunosorbent assay of sera from rats (N=7) after intradermal immunization respectively with 0.1 ml of 0.2 M solutions of different organic acid anhydrides in liquid paraffin.

| Anhydride                  | IgE titer | IgG titer |
|----------------------------|-----------|-----------|
|                           | Median     | Range     | Median     | Range     |
| SA (succinic anhydride)    | <50       | <50       | <50       | <50       |
| DMSA (dimethylsuccinic anhydride) | 200     | 100-400   | 800       | 400-1600  |
| DESA (diethylsuccinic anhydride) | 800     | 400-800   | 1600      | 800-1600  |
| MA (maleic anhydride)      | 800       | 800-3200  | 1600      | 1600-3200 |
| MMA (methylmaleic anhydride) | 800     | 800-3200  | 1600      | 1600-3200 |
| cis-HHPA (cis-hexahydrophthalic anhydride) | 1600   | 1600-3200 | 3200      | 1600-3200 |
| 4-MHHPA (4-methylhexahydrophthalic anhydride) | 1600   | 1600-3200 | 3200      | 1600-3200 |
| 1,2,3,6-THPA (1,2,3,6-tetrahydrophthalic anhydride) | 800    | 200-1600  | 800       | 200-1600  |
| 3,4,5,6-THPA (3,4,5,6-tetrahydrophthalic anhydride) | <50   | <50-50    | <50       | <50-50    |
| 3,4-MTHPA (3,4-methyltetrahydrophthalic anhydride) | 800    | 400-800   | 1600      | 800-1600  |
| 4,4-MTHPA (4,4-methyltetrahydrophthalic anhydride) | 800    | 800-3200  | 1600      | 800-3200  |
| PA (phthalic anhydride)    | 3200      | 1600-3200 | 3200      | 1600-3200 |
| 4-MPA (4-methylphthalic anhydride) | 3200   | 3200-6400 | 6400      | 3200-6400 |
| TMA (trimellitic anhydride) | 1600      | 800-6400  | 1600      | 800-2000  |

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Discussion

Positive specific Ig E and Ig G titers were obtained after intradermal immunization of rats with various free OAA. However, the magnitude of the induced titers varied. SA and 3,4,5,6-THPA failed to give detectable antibody production, while the highest specific Ig E titers were shown by cis-HHPA, 4-MHHPA, TMA, and especially PA and 4-MPA. A close correlation was exhibited between the Ig E and Ig G titers. Immunization with the corresponding RSA conjugates showed positive titers for SA, MA, and cis-HHPA.

Equimolar amounts of the anhydrides were used for the immunizations. It is of course possible that the effective amounts differed because of the different levels of stability of the anhydrides before injection. However, in guinea pigs, similar titers were obtained after immunization with 0.3 M suspensions and 0.2 M solutions, respectively (Zhang et al, unpublished observations). Thus the exact amount of anhydride does not seem to be critical at the administered dose of immunization. A 10-fold decrease in concentration for very reactive OAA before immunization may have a substantial effect. However, very high titers were observed for the reactive MA in guinea pigs (15).

The antibody titers analyzed by ELISA may be dependent on the quality of the hapten conjugate used for the determinations. If the conjugates have a different hapten density, they may show different responses in the assays (21). However, there are indications that hapten densities in the range of 10–25 mol/mol are optimal and show a low variation in activity (21, 22). The conjugates in the present study had hapten densities of 16–27 mol/mol, as demonstrated by the TNBS method. These results were confirmed by the UV method. Lower values were obtained by the HCl method. However, the hapten densities analyzed by the HCl method also fell in the range of 10–25, except for DMSA-RSA and MMA-RSA, both of which had a hapten density of 7. On the other hand, hapten densities as low as 6 have been shown to exhibit only minor reductions in antibody titers (22). Thus we believe that the conjugates in our work were within their optimal hapten density range.

To identify and produce the optimal conjugates for a large number of haptens is expensive and time-consuming. Thus it has been suggested that the total Ig E induced...
by immunization may be used as an alternative indicator of the immunogenicity of a chemical (5). However, this possibility has to be tested further.

Ig E and Ig G are 2 different classes of anaphylactic antibodies in rats, both of which increase after active sensitization (23, 24). There was a close correlation between the Ig E and Ig G titers in this study. Both antibody classes showed similar patterns and levels of titers.

SA showed negative results, while a positive titer of Ig E was induced by MA. The present negative findings for SA, as compared with MA, can be explained by the flexibility of the succinic acid molecule and the fact that SA is an endogenous compound. In addition, MA may react by the double bond with thiol groups in proteins. However, the immunogenicity of MA is interesting, in light of the small size of the molecule. The antibody titers increased when SA was substituted with methyl groups (DMSA), and even more so when the substituents were ethyl groups (DESA). The titers increased still more when DESA was ring closed to the more rigid cis-HHPA. Further methylation to 4-MHHPA caused no additional increase in the titers. However, even higher titers were observed after immunization with the corresponding aromatic anhydrides PA and 4-MPA. When the methyl group in 4-MPA was replaced by a carboxyl group (TMA), the titers significantly decreased. Thus the chemical character of the substituent is important. The results of PA and TMA agree with the observed serum Ig E levels in mice after topical application of the free anhydrides (9). Substitution of MA by MMA and 3,4,5,6-THPA showed a different pattern of induced antibodies as compared with the effect caused by the corresponding structural changes of SA. A possible explanation may be the instability of the MMA and 3,4,5,6-THPA conjugates under in-vivo conditions (25). The isomerization of the double-bond position in 3,4,5,6-THPA to the 1,2,3,6-THPA isomer showed a significant positive effect on the Ig E titers. A corresponding effect is seen when the position of the methyl group in MTHPA is shifted from the 3- to the 4-position on the 6-carbon ring.

The specificity of the antibody readings was demonstrated by the low readings of the RSA controls on the ELISA microtiter plates, as well as the low readings for the control animals immunized with RSA. The specificity of the antibodies was further demonstrated by the ELISA inhibition tests of specific Ig E to 4-MHHPA. 4-MHHPA-RSA presented the highest inhibition. Other OAA showed different inhibitions, depending on their structural similarities with 4-MHHPA, as was demonstrated in an earlier work (26). Thus cis-HHPA-RSA and 4,4-MTHHPA-RSA showed inhibitions close to that of 4-MHHPA-RSA. Corresponding inhibitory potentials were obtained for the different RSA and GPSA conjugates. For the same anhydrides, the RSA-conjugates exhibited higher inhibitions than those of the corresponding GPSA conjugates.

![Figure 3. Correlation (rs=0.3, P=0.5) between induced Ig E titers and the hydrolysis rate constants of succinic anhydride (SA), dimethylsuccinic anhydride (DMSA), diethylsuccinic anhydride (DESA), cis-hexahydrophthalic anhydride (cis-HHPA), maleic anhydride (MA), methyl-maleic anhydride (MMA), and 3,4,5,6-tetrahydrophthalic anhydride (3,4,5,6-THPA) according to Eberson & Landström (20).](image)

However, the differences were limited and therefore demonstrated a high hapten specificity of the antibodies.

OAA induce antibody formation by conjugation in vivo; the conjugates are recognized as "nonself" proteins by the immune system. Thus antibody formation after immunization may be influenced by the chemical reactivity of the anhydrides (7). However, no correlation was seen between the hydrolysis rate constants and the antibody titers in the present work, as was observed in a previous study of guinea pigs (15). Thus there is no simple correlation between chemical reactivity and sensitizing potential. The chemical reactivity of a low-molecular-weight allergen is only one of several parameters which may affect sensitization.

Whereas the results of immunization with the free anhydrides may reflect several other characteristics of the anhydrides, including solubility and reactivity, immunization with the corresponding serum-albumin conjugates mainly reflects the "nonself" recognition of the conjugate. Thus immunization with free anhydrides and corresponding protein conjugates, respectively, may give different information on the sensitizing potential of the anhydrides. Immunization with cis-HHPA-RSA, MA-RSA, and SA-RSA showed positive antibody formation. If a hapten density of about 25 μmol/mol is assumed, the amount of free anhydride bound to the protein carrier used for immunization is about 1/30 of the doses of free anhydrides used for immunization. Thus immunization with the conjugate appears to be more efficient than with free anhydride. This difference may simply reflect the amount of free anhydride which reacts with proteins after injection. Interestingly, free SA did not induce detectable antibody levels, but SA-RSA did. A low sensitizing potential of SA has also been demonstrated by the lack of response to...
bronchial challenge in SA-sensitized guinea pigs (Zhang 
et al, unpublished data). Rats immunized with SA or MA 
bound to the RSA carrier induced similar titers but dif-
fered markedly after immunization with the unconjugat-
ed ones. This finding may indicate an effect of both chem-
icophysical characteristics and the epitopic structures on 
the sensitizing potential.

A correlation (rs=0.63, P<0.05) can be seen between 
the rat IgE titers in the present study and the IgG1 titers 
after the immunization of guinea pigs with the same an-
hydrides in a previous study (15) (table 4). Passive cuta-
neous anaphylaxis tests of specific guinea pig Ig E sug-
gested an enhanced effect on IgE by methyl group sub-
stitution. However, in the present study, methyl group sub-
stitution in PA and SA resulted in increased titers, while 
in cis-HHPA no effect was found and in MA there was a 
decreasing effect. Thus there may be an animal species 
difference.

A close correlation was observed between specific Ig 
G1 titers and lung resistance in guinea pigs sensitized with 
cis-HHPA (27). The relationship between specific Ig E 
and the airway response in rats was not evaluated by us in 
this study. However, a relationship between an increased 
dose of antigen, an increased Ig E titer, and enhanced air-
way resistance has been demonstrated earlier (28).

There are indications that chemical allergens that cause 
respiratory tract sensitization in humans also induce sen-
titization in experimental animals after intradermal or top-
ical immunization (5). However, there is no conclusive 
evidence that animal models can predict the sensitizing po-
tential of low-molecular-weight compounds. Furthermore, 
the antibody specificity to the hapten demonstrated by 
the inhibition tests and the variation in antibody titers af-
after immunization make the OAA an interesting model for 
studies on quantitative structure-activity relationships.

| Antigen                        | Rat IgE titer (median) | Guinea pig IgG, titer (median) |
|-------------------------------|------------------------|--------------------------------|
| SA (succinic anhydride)        | 50                     | 300                            |
| MA (maleic anhydride)         | 800                    | 51 200                         |
| MMA (methylmaleic anhydride)  | 200                    | 6400                           |
| cis-HHPA (cis-hexahydrophtallic anhydride) | 1600       | 12 800                         |
| 4-MPA (4-methylphthalic anhydride) | 1600          | 12 800                         |
| 1,2,3,6-THPA (1,2,3,6-tetrahydrophthalic anhydride) | 800    | 6400                           |
| 3,4,5,6-THPA (3,4,5,6-tetrahydrophthalic anhydride) | 800    | 800                            |
| 4,4-MTHPA (4,4-methyltetrahydrophthalic anhydride) | 300     | 182 400                        |
| 4-MHHPA (4-methylhexahydrophtallic anhydride) | 800     | 25 800                         |
| PA (phthalic anhydride)        | 3200                   | 51 200                         |
| 4-NPA (4-N-phenylphthalic anhydride) | 3200     | 51 200                         |
| TMA (trimellitic anhydride)    | 1600                   | 12 800                         |

humans to SA, DMSA, DESA, MA, MMA, 1,2,3,6-
THPA, 3,4,5,6-THPA, and 4-MPA. However, this lack 
of evidence may be due to a lack of investigations on these 
chemicals or due to the fact that they have little use in 
industry.

In conclusion, the magnitude of the specific Ig E titers 
in rats after intradermal immunization showed interesting 
relationships with the chemical structures of the various 
OAAs. Importantly, the results agreed well with those from 
a corresponding study of Ig G1 in guinea pigs, and with 
findings from exposed workers. Thus the present model 
may be a valuable tool for predicting the sensitizing po-
tential of low-molecular-weight compounds. Furthermore, 
the antibody specificity to the hapten demonstrated by 
the inhibition tests and the variation in antibody titers af-
fer immunization make the OAA an interesting model for 
studies on quantitative structure-activity relationships.

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