

**2026** **Cockroach (CR) reactivity patterns differ between African American (AA) and Caucasian (C) individuals.** MC, Soto-Aguilar, RD, deShazo, T, Kordash, B, Morse, M, Amend, G, Plunkett, L, Baldwin, L, Freshwater. University of South Alabama, Mobile, AL; Bayer Corporation, Spokane, WA; BioSTAT Consultants, Inc, Kansas City, KS.

CR allergy is a major cause of asthma and affects up to 60% of asthmatics living in intercity dwellings, many of whom are more allergic to CR than dust mites. Using purified American (AC) and German CR (GC) extracts, sixteen highly CR allergic (n = 6 AA, n = 10 C) adult subjects were studied. The subjects were tested by the ID<sub>50</sub>EAL method using strict criteria for skin reactivity and statistical evaluation based on the dose response curve estimated from summation of erythema (ΣE) versus skin test dilution. One subject did not meet the statistical criteria for a valid AC assay, and one subject did not meet the statistical criteria for a valid GC assay. The mean entry puncture test responses were ΣE = 96 mm (AC) and 99 mm (GC) for the AA population and ΣE = 71 mm (AC) and 65 mm (GC) for the C population.

There was a marked difference between the skin test responses of AA and C subjects. While the mean D<sub>50</sub> of AA subjects was 21.65 (range 16.12-26.51) for AC and 23.08 (range 16.02-26.59) for GC, C subjects indicated a mean D<sub>50</sub> of 13.36 (range 9.85-16.10) for AC and 13.24 (range 10.55-15.84) for GC. German CR RAST results were also bimodal with a mean of 13,446 counts (range 4,885-23,145) (Class 5) for the AA subjects and a mean of 2,079 (range 367-5,365) (Class 2) for the C subjects. SDS-PAGE immunoblots revealed that the AA subjects reacted more strongly and more consistently to proteins in the 30 KD to 40 KD region.

These results reveal a higher sensitivity to CR allergens by AA individuals. Possible reasons include genetic predisposition, increased environmental exposure, or other unrecognized factors. These data may help explain the higher morbidity and mortality seen with AA asthmatic individuals.

**2027** **High level expression of German cockroach calycin allergen Bla g 4 in the yeast *Pichia pastoris*.** LD Vailes, LK Arruda, MD Chapman. University of Virginia, Charlottesville VA.

The major *Blattella germanica* (German cockroach) allergen Bla g 4 is a member of the calycin protein family. This family contains several other major allergens including beta-lactoglobulin from cow's milk, rat and mouse urinary proteins, dog allergens (Can f 1 and Can f 2), bovine and equine allergens (Arruda *et al.* JBC 1995; 270: 31196). Recombinant Bla g 4 (rBla g 4) produced in *E. coli* using the pGEX-4T1 expression system, shows excellent reactivity on skin testing of cockroach allergic patients. However the yields are low (typically 0.25mg Bla g 4/L of culture). In order to obtain high levels of rBla g 4, the *Pichia pastoris* expression system was used. PCR-amplified Bla g 4 DNA was subcloned into the EcoRI and NotI sites of the pPIC9 vector, which contains the *S. cerevisiae* α-factor signal sequence, to target protein expression to the secretory pathway. pPIC9 containing Bla g 4 DNA was linearized with Bgl II, to generate the His<sup>+</sup>Mut<sup>S</sup> (Methanol utilization slow) GS115 phenotype following transformation. In addition, *Sal* I linearized pPIC9 was used to generate either His<sup>+</sup>Mut<sup>+</sup> (Methanol utilization plus) or His<sup>+</sup>Mut<sup>S</sup> GS115 or KM71 phenotype transformants, respectively. Twenty-four colonies were identified by screening for Mut<sup>+</sup> and Mut<sup>S</sup> in minimal medium with or without Methanol, and 7/10 selected colonies had the correct Bla g 4 insert, as analyzed by PCR and sequencing. Four of these transformants secreted Bla g 4 in the supernatant, following culture in YPD media strictly at 30° C. A 23kd band was detectable on Coomassie blue-stained SDS-PAGE gel after 24 hours of culture, comprising most of the total protein in the supernatant. Time course experiments revealed that Bla g 4 production peaks at 72-96 hours for all four transformants. The yield was as ~40mg protein/L culture. The results suggest that yeast rBla g 4 will make possible further studies on the structure of calycin allergens and on the

immunological responses to this cockroach allergen in patients with asthma.

**2028** **A Clinical and Immunologic Study to Assess Risk of TMA-Induced Respiratory Disease as Related to TMA Exposure Level.** LC Grammer, MA Shaughnessy, B Kenamore, Northwestern University Medical School, Chicago, IL.

**Background:** The objective of this study was to determine whether there are TMA exposure levels that are very unlikely to cause immunologically mediated respiratory disease. If so, employees at those exposure levels would not need to participate in surveillance studies.

**Methods:** A 3-year clinical and immunologic survey study of 298 employees who had never had an immunologically mediated respiratory disease due to TMA was conducted at a facility that manufactures TMA. Each employee was assigned an exposure classification from 1 (highest) to 5 (lowest) based on industrial hygiene data and job description.

**Results:** Of the 27 individuals in exposure class 1, three (11%) developed IgG mediated disease and one (4%) developed IgE mediated disease; of the 60 class 2 employees, two (3%) developed IgG and two (3%) developed IgE mediated disease; of the 86 class 3 employees, three (3%) developed IgG and two (2%) developed IgE mediated disease. Of the 101 class 4 employees and the 24 class 5 employees, none developed an immunologically mediated respiratory disease due to TMA during the 3-year period of follow-up.

**Conclusions:** Employees in classes 4 and 5 whose TMA exposure levels averaged less than 0.0005 mg/m<sup>3</sup> did not develop disease over the 3-year period of surveillance. Since individuals in class 4 and 5 are at low risk of developing immunologically mediated respiratory disease due to TMA, it appears that they do not warrant routine inclusion in surveillance studies.

**2029** **Occupational Asthma and Rhinitis among Egg-Processing Workers.** LE Pinkerton, M Massoudi, D Bernstein, R Biagini, RD Hull, A Ruder, M Brown, M Boeniger, E Ward, CDC, NIOSH, Cincinnati, OH

We conducted a cross-sectional study evaluating the respiratory and immunologic effects of egg proteins among 42 egg-processing workers and 33 unexposed workers. Egg-processing workers are exposed to aerosolized egg proteins and potentially exposed to chlorine. Respiratory and nasal symptoms were assessed using a standardized questionnaire. Occupational asthma (OA) was defined as work-related respiratory symptoms with a 20% decrement in peak expiratory flow rate. Egg-related OA was defined as OA with a positive skin prick test to at least one egg protein. Rhinitis was defined as reporting 2 of the following 3 symptoms: itchy, runny nose with or without sneezing; nasal stuffiness; and itchy, watery eyes. The prevalence of atopy (positive skin prick test to two or more common aeroallergens) was 24% among both egg-exposed and unexposed workers. Fifteen (36%) of the egg-exposed and none of the unexposed workers had a positive skin prick test to at least one egg protein (p<0.001). Among the 15 workers sensitized to eggs, 13 (87%) had a positive skin prick test to ovalbumin, 8 (53%) to ovomucoid, 7 (47%) to lysozyme and 9 (60%) to conalbumin. Nine (60%) of 15 sensitized workers were atopic compared to only 1 (4%) of 27 unsensitized egg-exposed workers (p<0.001). Among workers sensitized to eggs, 5 (33%) had egg-related OA; 2 of these also had rhinitis. Three (20%) had rhinitis without egg-related OA. Seven (17%) of the egg-exposed workers had OA. Two (7%) of 27 unsensitized egg-exposed workers had OA compared to 5 (33%) of 15 sensitized workers (p=0.08). The overall prevalence of egg-related OA among this group of egg-exposed workers was 12% compared to 5% to 10% in previous studies. Over one-half of sensitized workers had egg-related OA and/or rhinitis. Although atopic workers appear to be at higher risk of sensitization to egg proteins the prevalence of sensitization among nonatopic workers was still substantial (6 of 32).