

Cockroach allergenic activity: Analysis of commercial cockroach and dust extracts

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Previous investigations demonstrated that cockroach whole bodies and feces are important sources of allergens in the induction/exacerbation of bronchial asthma. The current study investigated different cockroach source materials, commercial extracts, and house dust extracts for cockroach allergenic activity. In general, extracts from four different sources of either American or German cockroaches contained similar amounts of allergenic activity by RAST inhibition. Three commercial American cockroach extracts compared by RAST inhibition had similar allergenic activity on an equal protein basis. Skin test results correlated house dust reactivity to both commercial and inhouse cockroach wholebody extracts and to fecal extracts. Six different samples of house dust obtained from vacuum cleaners in the New Orleans area and three commercially obtained house dust extracts contained varying quantities of cockroach allergenic activity by RAST inhibition. These studies demonstrate that commercial cockroach extracts vary in allergenic activity and that all house dust extracts tested contain cockroach allergens. (J ALLERGY CLIN IMMUNOL 1991;88:895-901.)

Key words: Cockroach, allergens, commercial extracts, dust, skin test, RAST

A number of studies have previously established that cockroach allergens are important sensitizing agents. This finding is particularly true in populations with asthma living in large economically deprived urban areas.¹⁻⁹ During infestation, cockroaches can produce a variety of materials that may contain significant allergenic activity. Bernton and Brown¹⁰ reported skin test results that suggested the presence of German cockroach allergens in feces. These studies were supported by the observations of Choovivathanavanich in Thailand,¹¹ as well as our own studies, that demonstrated reactivity to cockroach fecal extracts by skin test, RAST, and immunoprinting.^{9, 12, 13}

Since cockroach allergens are believed to be inhaled, cockroach components, such as body parts and feces, must disintegrate and become incorporated into

Abbreviations used

AWBE:	American cockroach whole-body extract
GWBE:	German cockroach whole-body extract
AFE:	American cockroach fecal extract
GFE:	German cockroach fecal extract
PNU:	Protein nitrogen unit

respirable house dust. Choovivathanavanich et al.¹⁴ first noted in 1970 a relationship between skin test reactivity to house dust and to cockroach extract. Additional studies by Hulett and Dockhorn¹⁵ concluded that cockroach allergen may be as important as the mite in the etiology of house dust allergy. Sanders et al.¹⁶ found cockroach allergen in commercial house dust extracts with an ELISA-inhibition assay.

The presence of cockroach proteins in respirable house dust was confirmed by investigations of Swanson et al.¹⁷ who used high-volume air samplers and demonstrated cockroach antigens in dust samples obtained from homes in Harlem, N.Y., but not Rochester, Minn. More recent studies by Kang et al.¹⁸ did not detect cockroach antigens in commercial house dust extracts by double immunodiffusion or immunoelectrophoresis; however, they could demonstrate cockroach antigens in autologous house dust extracts obtained from the homes of cockroach-sensitive sub-

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jects with asthma. The current study was undertaken to investigate commercial, as well as local, house dust preparations for the presence of cockroach allergens and to compare commercial cockroach source materials and cockroach extracts for allergenic activity.

MATERIAL AND METHODS

Study subjects

Sixty-nine atopic subjects with asthma, observed at our allergy clinics at Tulane University School of Medicine, Charity Hospital of New Orleans, and the Louisiana State University Medical Center at Shreveport, were chosen for study. Informed consent was obtained, and all subjects were skin prick tested with AWBE, AFE, GWBE, and GFE. These extracts were prepared from Carolina Biological Supply (Burlington, N.C.) source material, as described below, at 1 mg/ml in 50% glycerol. Subjects were also skin prick tested with commercial cockroach (prepared from American, German, and Oriental cockroaches), and house dust extracts obtained from Hollister-Stier Laboratories, Spokane, Wash. Skin test reactions with mean wheals ≥ 3 mm were considered positive in subjects who reacted to histamine but were negative to diluent. Blood was obtained from an additional nine subjects with asthma, who were previously revealed to be skin test and RAST positive to cockroach extracts, for sera used in RAST and RAST-inhibition studies.

Cockroach extracts

American or German cockroaches of both sexes (obtained from the Carolina Biological Supply Co.) were received in the shipping crate and kept at room temperature for 5 to 7 days. Pieces of apple were added to each crate to provide food and moisture for the insects. Cockroaches were killed by freezing at -70°C , and whole bodies and fecal pellets were identified macroscopically and removed separately with forceps. American and German cockroach source materials were obtained from Biopol Laboratory, Inc. (Spokane, Wash.), Allergon, Inc. (Carthage, Mo.), and Tommie D. Edwards (Raleigh, N.C.). These firms supply starting material for commercially licensed allergenic extracts.

Whole-body and fecal extracts were prepared by homogenization in saline with a Waring blender (Waring Products Division, New Hartford, Conn.) (three 1-minute pulses). The homogenates were mixed overnight at 4°C and centrifuged (18,000 g), and the supernatants were concentrated (YM2-MWCO, 1000 daltons, Amicon Corp., Danvers, Mass.) The concentrates were centrifuged (105,000 g), supernatants were aliquoted, dry weights and protein concentrations (Coomassie blue protein assay, Pierce Chemical Co., Rockford, Ill.) were determined, and aliquots were frozen and stored at -20°C . Protein concentrations expressed as a percentage of dry weight were 47.4% GWBE, 7.7% GFE, 57.6% AWBE, and 12.9% AFE.

Commercial extracts of American cockroaches from Greer Laboratories (Lenoir, N.C.) and Center Laboratories

(Port Washington, N.Y.), or a mixture of three cockroach species (Hollister-Stier Laboratories), were also obtained for additional analysis. Extract concentrations were expressed as PNUs per milliliter (as per label) or milligram per milliliter (dry weight determination).

House dust extracts

Three different commercial house dust extracts (Greer, Center, and Hollister-Stier Laboratories) were analyzed for cockroach allergen. In addition, six different samples of house dust were obtained from the vacuum cleaners of homes in the greater New Orleans area. These dust samples were extracted by homogenization in saline with a Waring blender as described above. After centrifugation, the supernatant was concentrated by Amicon ultrafiltration and recentrifuged, and supernatants were aliquoted, dry weights were determined, and aliquots were frozen and stored at -20°C .

RAST inhibition

For RAST inhibition, 50 μl of inhibitor was incubated overnight with 50 μl of sera pool (composed of equal volumes of nine sera), diluted 1:10 with phosphate-buffered saline in the presence of cockroach allergen-coated disks (coated with extract at a ratio of 1 μg per disk) on a rotator at 24°C . Disks were then washed, and ^{125}I -labeled anti-IgE was added and incubated overnight. Disks were washed again, and the activity bound was determined in a gamma counter. Results were expressed as the percent inhibition of radiolabeled anti-IgE bound as compared to a control reaction with phosphate-buffered saline inhibitor.

Statistical analysis

Associations between skin test reactivity to different reagents were determined by chi-square analysis. RAST-inhibition curves were compared by linear-regression analysis.

RESULTS

Comparison of cockroach source materials

Four different sources for American and German cockroach allergens were extracted and analyzed for activity by RAST inhibition. For this assay, RAST disks were coated with our standard extract prepared from cockroaches from Carolina Biological Supply. All four German cockroach sources yielded extracts with similar allergenic activity (Fig. 1, A). Of the four sources assayed for American cockroach allergenic activity, the extract from Carolina Biological Supply was the most active (Fig. 1, B).

Comparison of commercial cockroach extracts

The American cockroach extract prepared from Carolina Biological Supply was used as an internal

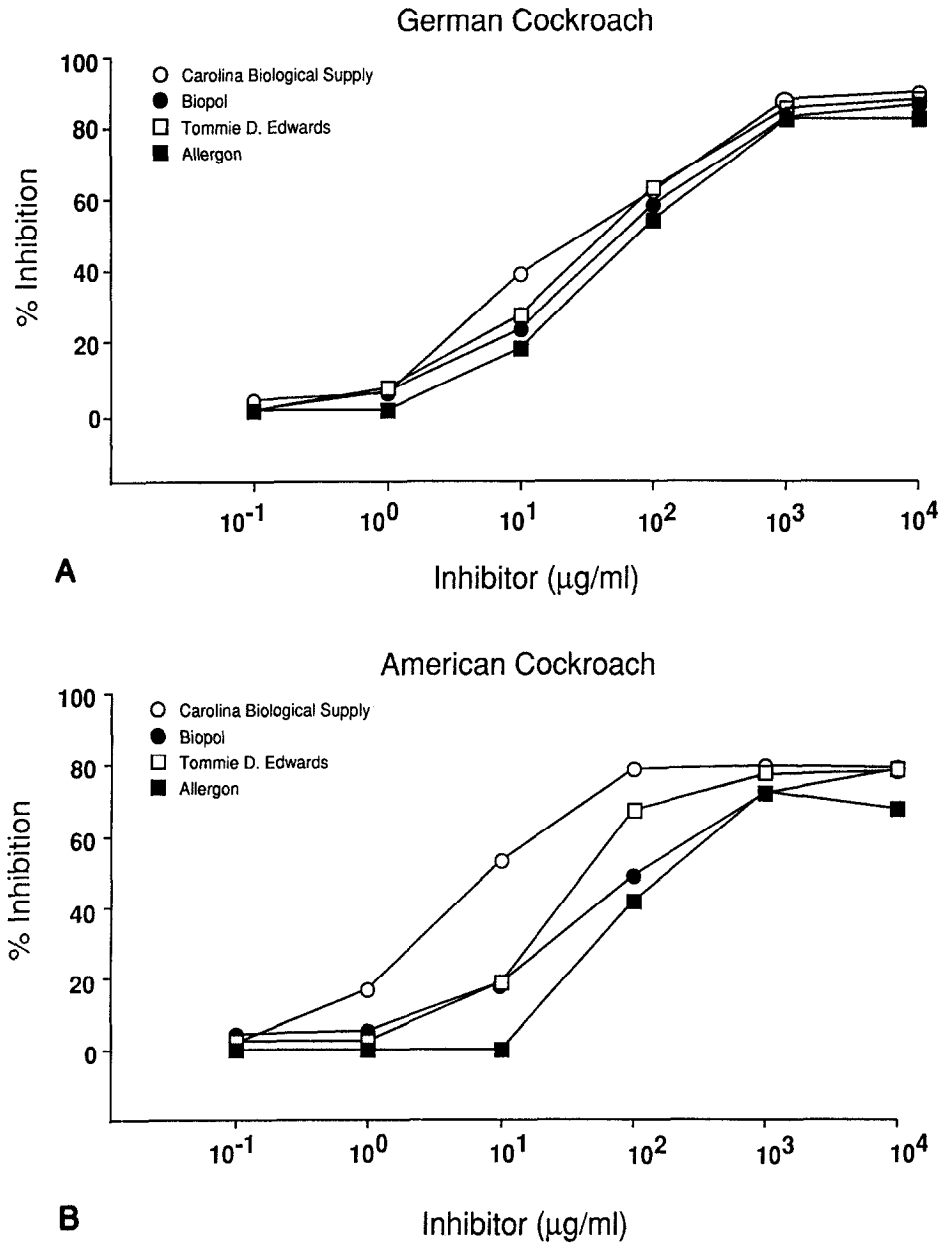


FIG. 1. Analysis of four different source materials for cockroach allergen. Extracts prepared from four different cockroach-allergen source materials were analyzed by RAST inhibition for activity of **A**, German, or **B**, American cockroach allergens. RAST disks were coated with GWBE or AWBE cockroach allergen, respectively.

standard. Two commercial extracts of American cockroaches (Greer and Center Laboratories) and a mixture of three cockroach species (Hollister-Stier Laboratories) were compared by inhibition of American cockroach RAST (Fig. 2). On an equal protein basis (protein nitrogen units), all three extracts contained similar allergenic activity (Fig. 2, A). However, on a weight/volume (microgram per milliliter) basis, our

AWBE standard had greater activity as compared to the other extracts (Fig. 2, B).

Skin test reactivity

Significant skin test reactivity was observed to cockroach, as well as to house dust extracts (Tables I, II, and III). A comparison of skin test reactivity demonstrated a significant correlation for commercial

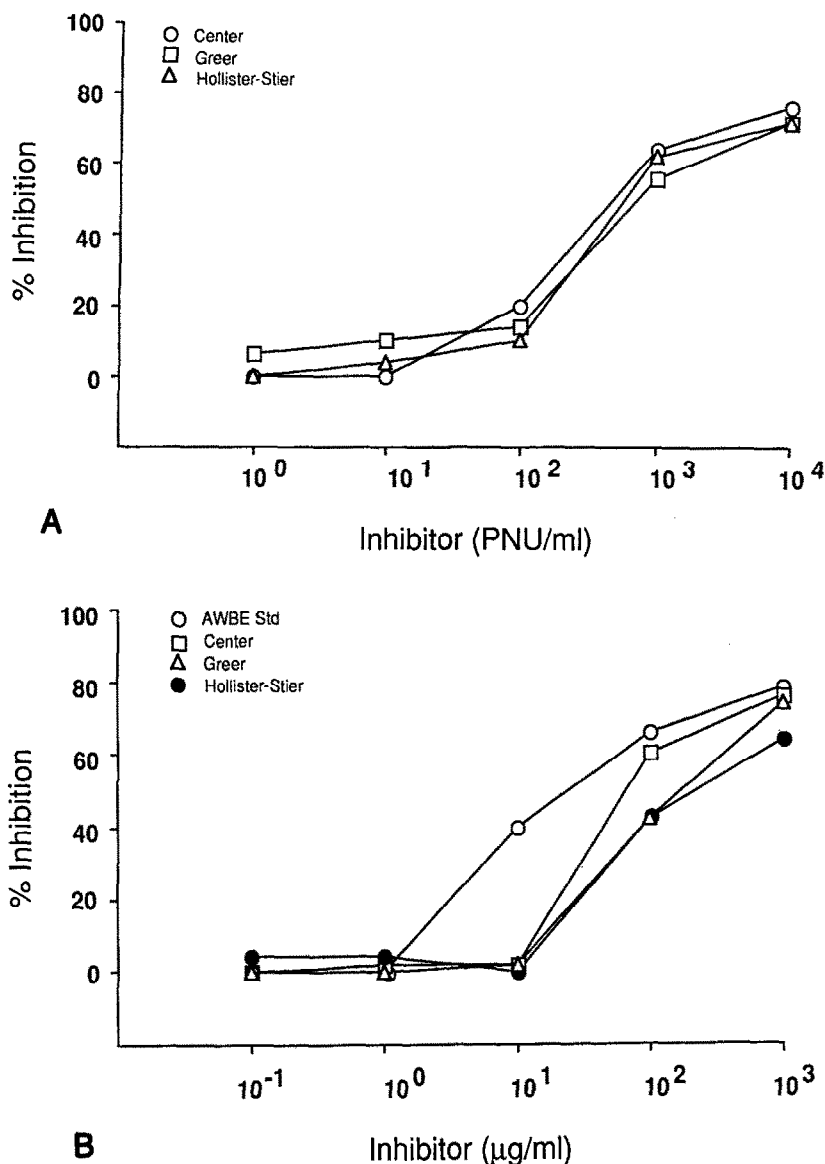


FIG. 2. Comparison of commercial cockroach-allergen extracts by RAST inhibition. Two commercial extracts of American cockroach allergen (Center and Greer Laboratories) and a third cockroach extract (a mixture of three cockroach species) were analyzed for allergenic activity by RAST inhibition. RAST disks were coated with our standard AWBE. Results were expressed as **A**, PNUs or **B**, micrograms per milliliter. A convenient method of converting PNUs per milliliter to estimate milligrams of protein per milliliter is by multiplying (PNUs per milliliter) (1×10^{-6} mg/PNU) (6.25).

cockroach mix to commercial house dust extract ($p = 1.5 \times 10^{-5}$; Table I), GWBE, and/or AWBE to commercial house dust extract ($p = 3.82 \times 10^{-5}$; Table II), and fecal extracts to commercial house dust extract ($p = 1.86 \times 10^{-4}$; Table III).

Cockroach allergenic activity in house dust extracts

Three different commercial house dust extracts (Greer, Center, and Hollister-Stier Laboratories) each had German cockroach allergenic activity by RAST inhibition (Fig. 3). In addition, samples of house dust

obtained from six homes in the New Orleans area were also assayed for German cockroach allergenic activity (Fig. 4). All samples contained significant, yet varying, amounts of allergenic activity. Negative controls, such as peanut or castor extracts (data not presented), demonstrated no significant inhibition at these concentrations.

DISCUSSION

Although characterization and standardization are advanced for most allergenic extracts, house dust preparations are anachronistic because of their crude,

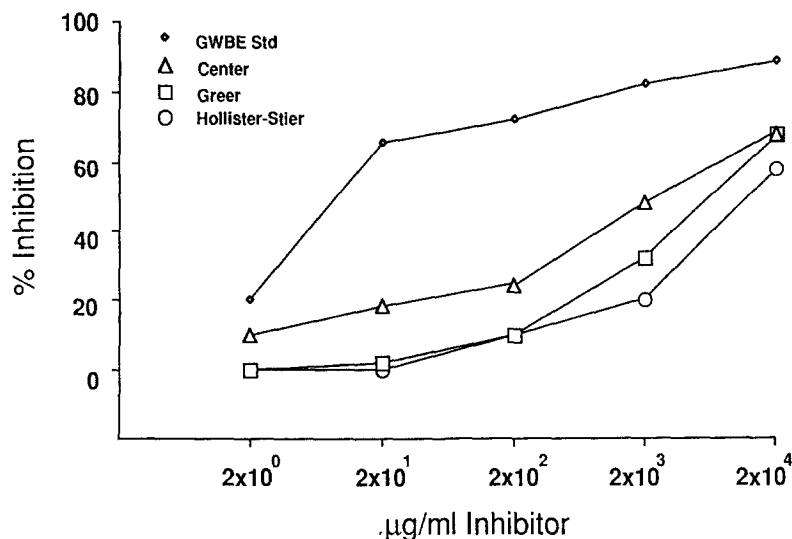


FIG. 3. Analysis of commercial house dust-allergen extracts for cockroach allergenic activity. Aqueous extracts of commercial house dust, obtained from Center, Greer, or Hollister-Stier Laboratories, were analyzed for German cockroach allergenic activity by RAST inhibition.

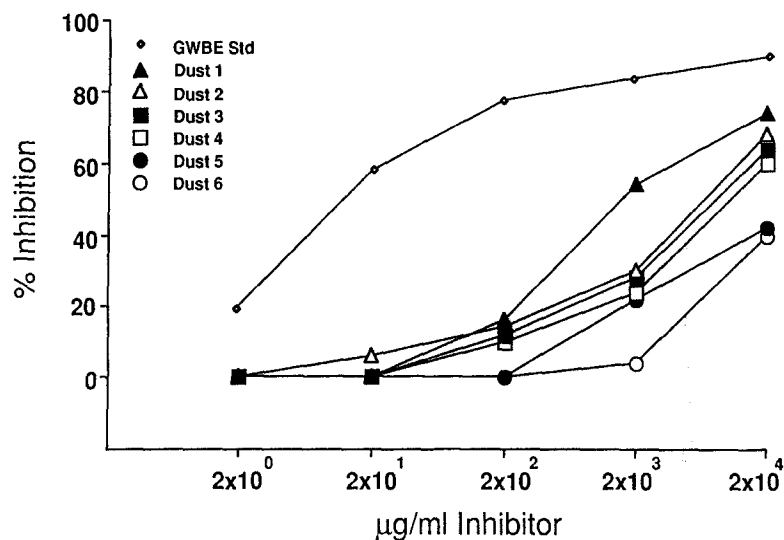


FIG. 4. Cockroach allergenic activity in local house dust extracts. Dust samples obtained from houses in the New Orleans area were extracted and analyzed for German cockroach allergenic activity by RAST inhibition.

variable, and undefined composition. The advisory panel of the Food and Drug Administration has even recommended that house dust extracts be withdrawn from the market.^{19, 20} Since standardized extracts of particular allergens will eventually superannuate crude dust extracts, the major allergenic components of house dust must be identified, and their relative importance must be determined.

Mite allergens are considered the most important components of dust extracts, but all dust allergens are not mite related.²⁰⁻²⁷ Our results indicate that cockroach allergens are also important relevant components of the allergenic activity in house dust extracts.

The association of skin test reactivity to dust and to several cockroach sources were highly significant in our population of cockroach-sensitive subjects with asthma. This finding suggests that cockroach allergens are part of dust allergenic activity and, furthermore, that among cockroach-sensitized subjects, these allergens are clinically important.

Another important aspect of these skin test results is the association of dust reactivity with all cockroach extracts. This included commercial whole-body extracts and laboratory extracts of whole body and of fecal material. Thus, commercial extracts are valid testing material. More importantly, the association of

TABLE I. Comparison of skin test reactivity of commercial cockroach and house dust extracts

	Cockroach mix		
	+	-	Total
House dust			
+	49	12	61
-	0	8	8
Total	49	20	69

$p = 1.5 \times 10^{-5}$

TABLE II. Comparison of skin test reactivity to GWBEs and/or AWBEs with house dust extract

	GWBEs and/or AWBEs		
	+	-	Total
House dust			
+	47	14	61
-	0	8	8
Total	47	22	69

$p = 3.82 \times 10^{-5}$.

dust and fecal extracts suggests that fecal material is the means by which cockroach allergen is incorporated into house dust. However, skin test correlations cannot distinguish between shared allergens and parallel sensitivities to cockroach and dust. RAST-inhibition studies can provide such direct evidence of shared epitopes, however, and do indicate that cockroach allergens contribute to the allergenic activity of dust extracts. A significant portion of the allergenic activity that is recognized in dust by pooled sera of cockroach-sensitive subjects was due to cockroach allergens.

RAST inhibition also demonstrated that cockroach source materials from different suppliers contain comparable amounts of allergenic activity, although American cockroach source materials were more variable in RAST-inhibitory activity than German cockroach sources. Since source materials for each species were obtained from the same set of suppliers, differences in preparation should be comparable for both species. Thus, the variability among American cockroach sources may be intrinsic, intraspecies variability, although this assumption requires validation.

Commercial American cockroach extracts demonstrated similar, but not identical, inhibition activity against a RAST with a laboratory extract of Ameri-

TABLE III. Comparison of skin test reactivity to cockroach fecal and house dust extracts

	GFE and AFE		
	+	-	Total
House dust			
+	43	18	61
-	0	8	8
Total	43	26	69

$p = 1.86 \times 10^{-4}$.

can cockroach, when extract was expressed on a weight/volume basis. These materials were essentially identical when they were expressed as PNUs. This variability probably reflects slight differences in relative protein content. Such variability would not be adequately controlled for on a weight/volume basis, as it would be when these materials were diluted on a PNU basis.

Thus, some variability exists among AWBE sources, and yet both AWBE and GWBE are similar among different suppliers. Furthermore, AWBE commercial extracts contain similar amounts of allergenic activity. Although standardization of extracts should be encouraged, the commercial extracts surveyed appear to be sufficiently potent and uniform to detect cockroach sensitivity among suspected allergic subjects with asthma. Most importantly, the presence of cockroach as a substantial portion of dust allergenicity is confirmed, and we have demonstrated this activity in dust extracts as well as local dust samples. This finding implies that cockroach allergens are widely distributed in house dust and in derived preparations.

Further analysis of cockroach allergens in homes is required with techniques such as the recently developed, double-monoclonal, antibody assay.²⁸ This procedure is more sensitive and specific than RAST inhibition and has been used to demonstrate cockroach allergens in house dust far in excess of mite and cat allergens.²⁹ Such procedures may provide reliable quantitative estimates of dust allergenic activity that is cockroach derived. This method in turn will establish a basis for determining threshold levels of cockroach allergens required for an allergic reaction.

Significant numbers of dust-sensitive subjects with asthma are sensitized to cockroach allergens, and cockroach allergen is present and important in house dust extracts. This finding suggests that a significant component of dust sensitivity is, in fact, cockroach sensitivity. Thus, a definitive diagnosis of dust-sen-

sitive subjects should include testing for cockroach sensitivity. Cross-reactivity between cockroach allergens and other arthropods that may be intrinsic to house dust also may play a role in the overall biologic activity. This hypothesis requires further investigation. Testing for cockroach and other dust components will also relieve the practicing allergist from a reliance on dust extracts that may be superannuated by standardized extracts.

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