American Industrial Hygiene Association Journal

ISSN: 0002-8894 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/aiha20

IMMUNOCHEMICAL QUANTIFICATION AND PARTICLE SIZE DISTRIBUTION OF AIRBORNE PAPAIN IN A MEAT PORTIONING FACILITY

M.C. Swanson , J.M. Boiano , S.K. Galson , L.W. Grauvogel & C.E. Reed

To cite this article: M.C. Swanson , J.M. Boiano , S.K. Galson , L.W. Grauvogel & C.E. Reed (1992) IMMUNOCHEMICAL QUANTIFICATION AND PARTICLE SIZE DISTRIBUTION OF AIRBORNE PAPAIN IN A MEAT PORTIONING FACILITY, American Industrial Hygiene Association Journal, 53:1, 1-5, DOI: 10.1080/15298669291359230

To link to this article: https://doi.org/10.1080/15298669291359230

Published online: 04 Jun 2010.

AIHA JOURNAL Advances in Industrial & Environmental Hygiene

AM. IND. HYG. ASSOC. J. 53(1):1–5 (1992)

IMMUNOCHEMICAL QUANTIFICATION AND PARTICLE SIZE DISTRIBUTION OF AIRBORNE PAPAIN IN A MEAT PORTIONING FACILITY*

M.C. Swanson[a†‡]
J.M. Boiano[b]
S.K. Galson[b]
L.W. Grauvogel[c]
C.E. Reed[a†]

[a]Mayo Clinic/Mayo Foundation, 200 1st St. SW, Rochester, MN 55905; [b]National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226; [c]Cole Associates, Inc., 2211 E. Jefferson Blvd., South Bend, IN 46615

The use of enzymes in industry continues to expand. With this increased use comes a concerted need to better understand potential respiratory health hazards to exposed workers and to quantify exposure levels that cause impaired health. To this end, projects were undertaken by the National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluations Program and Cole Associates whereby this information was collected. Data concerning medical evaluation and aspects of industrial hygiene are the subjects of two separate reports from these respective groups. This method/results report includes a description of (1) a sensitive immunoradiometric assay for the quantification of airborne papain and its particle size distribution, (2) measurement of papain from both general area and personal breathing zone air samples obtained from a meat processing plant that used this immunochemical analysis, (3) a sampling strategy, and (4) an improved air sample processing technique. Airborne papain was measured at levels ranging from low nanogram to microgram per cubic meter concentrations. Approximately half of the papain activity was associated with particles having an aerodynamic diameter of less than 9.4 μm. These data point to a need for containment and controls in the manufacture and use of such compounds. This approach can be considered by the hygienist as an effective tool to be used in conjunction with epidemiologic studies to help set standards that are practical, safe, and maintained.

For the standard principles of industrial hygiene to be applied effectively to the control of airborne occupational allergens, it is necessary to have information about the concentration of the allergen in the air. The increasing commercial use and production of enzymes derived from bacteria, fungi, and, in the case of papain, botanical sources has continued to expose individuals and cause asthma or allergic sensitization. Despite early recognition of hypersensitivity and asthma caused by digestive ferments[1] and allergy to papain specifically,[2] the use of this enzyme continues to expand in a wide variety of consumer products such as foods, drugs, and cosmetics and industries such as breweries and tanneries.

The potential for serious respiratory hazard during the production of detergents containing bacterial enzymes was first reported by Flindt et al.[3] and Pepys et al.[4] in 1969. Novey et al.[5] reported a dual asthma response to papain with IgE and precipitating antibodies. With continued exposure even on a chronic low-level basis, these precipitating antibodies acting in immune complexes may compound the problem of asthma with pulmonary interstitial disease.[5] Recognition and elucidation of the problem of enzyme sensitization resulted in remedial actions to improve plant ventilation measures and dust control early in the 1970s. For example, to control dust in the detergent industry, the invention of an enzyme granule that embeds the enzyme in a matrix of organic salt spheres about 600 μm in diameter was purported to reduce exposure to

*This study was supported by grants from National Institute of Allergy and Infectious Diseases, AI21255, and the Mayo Foundation.

†Corresponding authors.

‡Quan-Tec-Air, Inc., 126 11th Ave. SE, Rochester, MN 55904.

airborne, respirable enzyme dusts and thus reduce the incidence of respiratory sensitization. In 1984, Liss et al.[6] conducted a study to test the development of clinical sensitivity to enzymes in plant workers where no enzymes had previously been used in any form; the enzyme was produced by using current granule technology. Even under these conditions, area, personal, and particle size air samples showed half the airborne enzyme present in respirable particles (<5 μm), significant concentrations in area samples, and total dust levels often exceeding the 1 mg/m³ safety guidelines on personal samples. Their enzyme assay was based on the sample's ability to digest N,N-dimethyl casein.[7] This enzyme assay, developed in 1971, has three main constraints: (1) it is subject to enzyme deactivation by various environmental actions, (2) it requires the use of high-volume air samples to collect sufficient air sample quantities for detection, and (3) it does not allow quantification of personal exposure by using conventional personal sampling pumps.

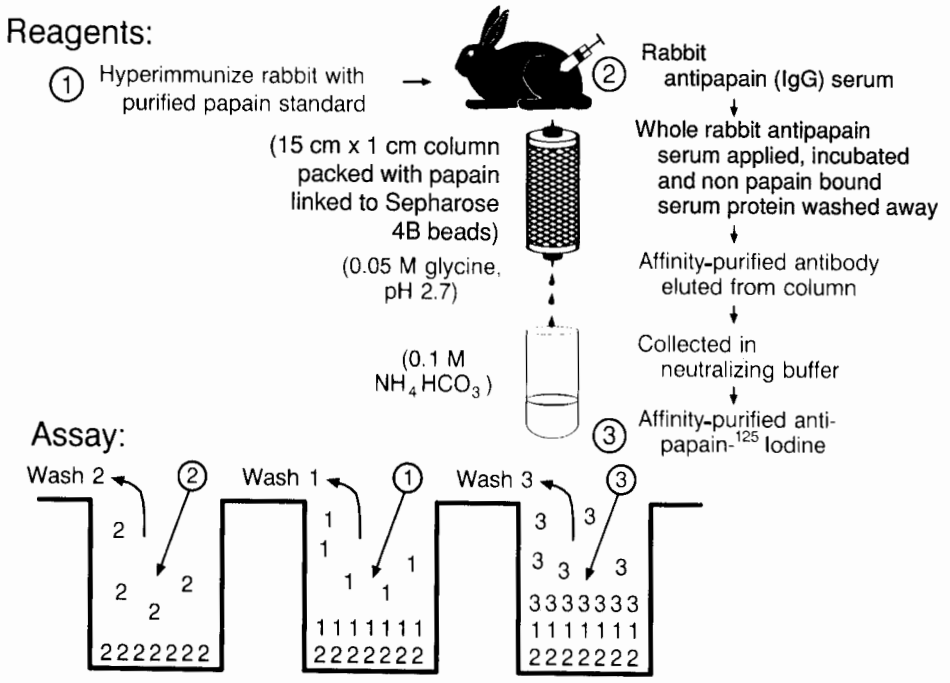

FIGURE 1. Diagram of antigens, antibodies, and assay for papain. (1) papain, (2) rabbit antipapain, (3) affinity-purified antipapain.

In the last 10 yr, sensitive immunoassays have been developed to measure airborne proteolytic enzymes. Wells et al.[8] described a modified radioallergosorbent test for papain. The sensitivity of this method (5 ng/m³) indicated that personal breathing zone air samples might be utilized effectively for quantifying exposure during an 8-hr work shift. Subsequently, Agarwal et al.[9] developed another immunochemical technique by using a two-site immunoradiometric assay for quantifying Esperase® in the air of a dry bleach factory. This was the first time personal breathing zone air samples had been successfully quantified by using immunochemical techniques. The sensitivity of the assay allowed as little as 2 ng/m³ of airborne Esperase to be detected with a sampler flow rate of 2 L/min over an 8-hr period.

This paper reports the development of an improved air sample processing technique and an ultrasensitive, two-site immunoradiometric assay for measurement of papain in personal breathing zone samples by utilizing conventional industrial hygiene air-sampling equipment. Assay sensitivity extended easily into the picogram per cubic meter range with full-shift samples collected at 2–3 L/min. General area and personal breathing zone air samples were collected in a meat processing plant in the upper midwest.[10] When seasoning mixtures containing papain were used to tenderize the meat, these air samples demonstrated similar values, indicating widespread airborne distribution of the antigen in the workplace.

The authors found about half the airborne immunochemical activity papain in particles of less than 9 μm (mass median aerodynamic diameter).

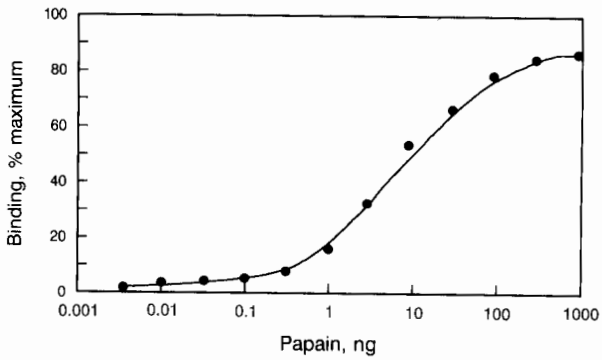

FIGURE 2. Typical calibration curve with the papain standard

EXPERIMENTAL MATERIALS AND METHODS

Air Sampling

A total of 20 personal breathing zone and 20 general area air samples were collected on polytetrafluoroethylene (PTFE) membrane filters (Quan-Tec-Air, Inc., Rochester, Minn.) rated 99% efficient at 0.3 μm. The filters were loaded into 37-mm plastic cassettes attached via flexible tubing to battery-operated pumps, which were operated at flow rates ranging from 2 to 3 L/min. Sampling trains were calibrated before and after each use. Proper airflow rate and sample integrity were checked periodically during the work shift. Field blanks were prepared and included with the samples. The filters were stored at –20°C until they were processed for analysis.

Particle sizing was accomplished with a five-stage Andersen cascade impaction head (Andersen Instruments, Atlanta, Ga.) mounted on a Quan-Tec-Air Air-Sentinel® with a flow rate of 300 L/min. PTFE filters were used for particle impaction on Stages 1–4 and as a final filter on Stage 5. The particle size cut points for each stage were 9.4, 4.5, 2.7, 1.6, and less than 1.6 μm, respectively. This sample was taken on the processing floor where a liquid solution of seasoning and papain is applied to the meat.

Antigens/Antibodies

Papain and rabbit antipapain antibodies were purchased commercially (Calbiochem, Behring Diagnostics (Hoechst), La Jolla, Calif.). The papain used as the standard was described as having 100 000 papain units/g. The powder was weighed and dissolved to a concentration of 10 mg/mL in phosphate buffer (pH 7.5) containing 0.1% iodoacetate and 50% glycerol. A 1% solution of pure papain at 278 nm and a 1-cm light path should have an absorbance of 25.[11] This preparation had an absorbance of only 1.5 or about 600 μg/mL pure papain equivalents. All concentrations were expressed in terms of dry weight of the papain powder. Affinity-purified antipapain antibodies were obtained by covalently linking papain to cyanogen bromide–activated Sepharose 4B beads (Pharmacia, Piscataway, N.J.). Excess papain was washed away and the papain–Sepharose 4B beads were packed into a 15-cm × 1-cm column. One-half mL of rabbit antipapain serum was passed through the column, and the unbound portion was washed away. The bound antipapain antibody was eluted from the column by using 0.05 M glycine-HCl (pH 2.7) and collected in an equal volume of 0.1 M NH_4HCO_3 neutralizing buffer (Figure 1). Approximately 60 mL of eluate was concentrated (20×) by using an YM50 ultrafilter (Amicon, Lexington, Mass.) at 35 psi.

Air Filter Processing

Filters used for collection of airborne papain were PTFE-laminated to a Quan-Tec-Air polyester spun support backing. After exposure, the PTFE membrane was delaminated from the polyester support backing, then placed into a test tube with 0.5 mL of 0.1 M phosphate buffer containing 0.2% bovine serum albumin and 0.1% iodoacetate (pH 7.5) in 50% glycerol, and vortexed for 30 sec. The membrane was allowed to soak in this solution overnight at 4°C. The sample was then vortexed and centrifuged. The supernate fluid was promptly used in the immunoassay for papain, as the enzymatic activity of papain is retarded, but not stopped, with the use of glycerol and 0.1% iodoacetate. Further, freeze-thaw cycles without glycerine will damage the immunoreactivity of the enzyme.

Immunoassay

This two-site immunoradiometric assay required 100 μL per well of crude polyclonal rabbit antipapain (1:500) absorbed overnight to the surface of Immulon-2 microtiter wells (Dynatech, Alexandria, Va.) by using 20 mM carbonate-bicarbonate buffer, titrated to pH 9.2. The wells were washed three times and 100 μL of samples were delivered (papain standards or air sample extracts) and kept overnight at room temperature in a humid box. The wells were washed again and 100 μL (30 ng) of affinity-purified antipapain antibody radiolabeled with ^{125}iodine[12] were added to the wells. They were incubated as before, washed, and counted. Bound counts proportionally reflect papain content, as shown by a typical calibration curve of the papain standard (Figure 2). The samples were counted on a RIASTAR multidetector Packard gamma counter (Packard Instrument Co., Meriden, Conn.). The standard curve was plotted by using a smoothed spline function.

RESULTS

Papain was detected in all personal breathing zone air samples batched together in a single assay (Table I). Two samples were collected on consecutive days from a quality technician, who

TABLE I. Full-Shift Personal Breathing Zone Concentrations

	Papain Concentration (ng/m³)			
Job Description	*N*	*Range*	*Mean*	*SD*
Quality technician	2	450–600	525	106
Packer–Line 1	6	240–1700	988	618
Packer–Line 2	6	290–1100	472	309
Packer–Line 3	6	220–1300	695	398

TABLE II. General Area Papain Concentrations

Location Description	*Papain Concentration (ng/m³)*
Compounding room	1400,1700
Corridor, outside compounding room	<1, <1
Spray Line 1	2100, 1000
Spray Line 2	1200, 1100
Spray Line 3	1100, 1200
Spray Line 4	Lost, 80
Freeze tunnel	20, 20
Scale platform	220, 190
Preprocessing	2, <1
Office	<1, <1

TABLE III. Particle Size Distribution Associated with Immunochemical Papain Activity

Stage	*Cut Point (μ)*	*Papain (pg/m³)*
1	9.4	1003
2	4.5	402
3	2.7	168
4	1.6	226
5	<1.6	110

blended dry powdered seasonings containing papain into a tank containing water. These samples demonstrated airborne exposure levels of 450 and 600 ng/m^3. Eighteen workers who manually bagged meat products treated with the spice/papain solution had exposures ranging from 220 to 1700 ng/m^3.

General area air-monitoring results demonstrated airborne papain in the compounding room and at all sampled locations on the production floor. Two separate samples were collected in each location. Papain concentrations on the main production floor diminished with increasing distance from the conveyor lines where the meat was treated and ranged from 2100 ng/m^3 on top of a sprayer cover to 20 ng/m^3 next to a freeze tunnel located about 50 ft away (Table II). There are no standards for papain; however, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends a ceiling limit of 60 ng/m^3 for subtilisins (bacterial proteolytic enzymes primarily used in the detergent industry and assumed to have comparable biological potency to papain).

About half the airborne papain was associated with particles having an aerodynamic diameter less than 9.4 μm (Table III). Scanning electron photomicrographs illustrated the sizing efficiency of the impactor (Figure 3).

DISCUSSION

Although ACGIH recommends a ceiling limit value of 0.06 μg (60 ng) per cubic meter of air for subtilisins (proteolytic enzymes of *Bacillus subtilis*), this may not be universally applicable to all enzymes. Many well-characterized allergens (e.g., mites, ragweed, *Alternaria*) are proteases. These allergens have been associated with symptoms in the nanogram per cubic meter of air range, suggesting that 60 ng/m^3 of papain will provoke respiratory symptoms in sensitive individuals.[13] Indeed, the majority of the air samples reported here were well above this value. It should be pointed out that the papain standard was not a pure preparation. Because it was defined by weight and its true papain content was approximately one-seventeenth of its gravimetric weight, the values reported are overestimates for papain content.

The relatively insensitive characteristics of prior analytical techniques and the high-volume air samples required to provide sufficient sample quantities for detection call for improved

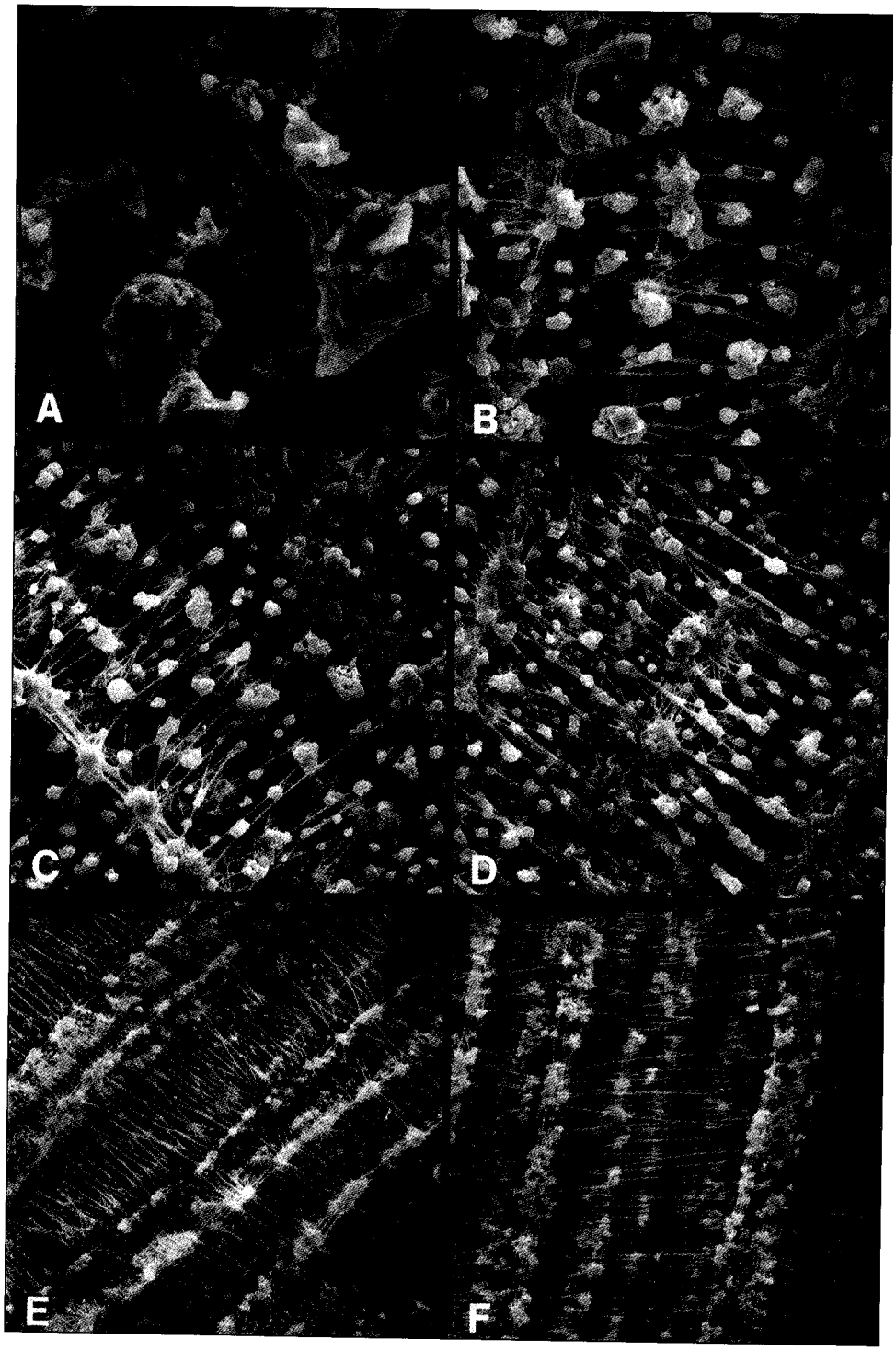

FIGURE 3. Scanning electron photomicrographs of sizing stages for airborne particles. A: >9.4 μm; B: <9.4, >4.5 μm; C: <4.5, >2.7 μm; D: <2.7, >1.6 μm; E: <1.6 μm; F: unexposed. A–D: 1000×. E and F: 500×.

collection and assay techniques, which will provide better data from which to make recommendations. Of course, an air sample obtained over an extended period of time cannot reflect momentary changes in airborne concentration. Although area samples may adequately estimate average concentrations within a work site, it is generally well accepted that personal breathing zone samples can indicate higher levels and hence greater exposure potential.

Certainly, personal breathing zone samples better reflect individual exposure; however, samples taken over an entire shift will likely underestimate short-term peak exposure levels that may occur briefly during a particular task. For this purpose, task-related personal sampling is desirable and most importantly, it is feasible. For example, given a limit of detection of approximately 0.3 ng per sample, one could collect a short-term air sample (i.e., 15 min) by using a personal sampling pump at 3 L/min and still achieve detection of 7 ng/m^3, which is roughly an order of magnitude below the ACGIH threshold limit value for subtilisins.

Variability among replicate assays may be as much as 100% as a result of compounding errors at any or all of the handling steps. Typically, if replicate assays are performed by using the same reagents within a reasonable time period, the variability should not exceed 50%. This is to say, the absolute values may vary to this degree; however, the values relative to one another will not change.

In conclusion, the analytical techniques and flexible sampling strategies are available for evaluation of papain exposure levels in industries that use papain or enzymes in general. The effectiveness of changes intended to control worker exposure should be monitored by measuring the concentration of papain in the air. Success of changes in engineering controls or operating conditions is measured by the reduction in airborne papain found both in general area samples and in task-related personal samples during activities involving potential for acute exposure at a point source. The final and most important criteria for success are observed reductions in workers' symptoms and in the incidence of sensitization. Although standards cannot be recommended from these data, it is hoped that this overall approach will be used to help link exposures with symptoms, thereby allowing relevant standards to be set and maintained.

ACKNOWLEDGMENT

The authors appreciate the critical reviews given to this article by Dr. William R. Solomon, University of Michigan Medical Center; Mr. Jere Ingram, CIH, Clorox Company Technical Center; and Dr. Balki Balakrishman, Berkeley Antibody Company. The authors also wish to thank Ms. Toni Buss for her secretarial assistance.

REFERENCES

1. **Beecher, W.L.:** Hyperesthetic Rhinitis and Asthma due to Digestive Ferments. *Ill. Med. J. 59*:343 (1931).
2. **Osgood, H.:** Atopic Sensitivity to Caroid (Papain). *J. Allergy 16*:254 (1945).
3. **Flindt, M.L.H.:** Pulmonary Disease due to Inhalation of Derivatives of *Bacillus Subtilis* Containing Proteolytic Enzymes. *Lancet 1*:1177 (1969).
4. **Pepys, J., F.E. Hargreave, J.L. Longbottom, and J. Faux:** Allergic Reactions of the Lungs to Enzymes of *Bacillus Subtilis*. *Lancet 1*:1181 (1969).
5. **Novey, H.S., L.E. Marchioli, W.N. Sokel, and I.D. Wells:** Papain-Induced Asthma—Physiological and Immunological Features. *J. Allergy Clin. Immunol. 63*:98–103 (1979).
6. **Liss, G.M., J.R. Kominsky, J.S. Gallagher, J. Melius, S.M. Brooks, and I.L. Bernstein:** Failure of Enzyme Encapsulation to Prevent Sensitization of Workers in the Dry Bleach Industry. *J. Allergy Clin. Immunol. 73*:348–355 (1984).
7. **Dunn, E. and R. Brotherton:** The Use of N,N-Dimethyl Casein in the Determination of Proteolytic Enzymes in Washing Products and Airborne Dust Samples. *Analyst 96*:156–163 (1971).
8. **Wells, I.D., R.E. Allan, H.S. Novey, and B.D. Calver:** Detection of Airborne Industrial Papain by a Radioimmunoassay. *Am. Ind. Hyg. Assoc. J. 42(4)*:321–322 (1981).
9. **Agarwal, M.K., J.W. Ingram, S. Dunnette, and G.J. Gleich:** Immunochemical Quantitation of an Airborne Proteolytic Enzyme, Esperase®, in a Consumer Products Factory. *Am. Ind. Hyg. Assoc. J. 47(2)*:138–143 (1986).
10. **Galson, S.K. and J.M. Boiano:** *Health Hazard and Technical Assistance Report: E.S.I. Meats, Inc.* NIOSH Report No. HETA 87:112-1922. Cincinnati, Ohio: National Institute for Occupational Safety and Health, August 1988.
11. **Windholz, M., ed.:** *The Merck Index.* 10th ed. Rahway, N.J.: Merck & Co., 1983. p. 1007.
12. **Greenwood, F.C. and W.M. Hunter:** The Preparation of ^{131}I Labeled Human Growth Hormone of High Specific Radioactivity. *Biochem. J. 89*:114–123 (1963).
13. **Agarwal, M.D., M.C. Swanson, C.E. Reed, and J.W. Yunginger:** Immunochemical Quantitation of Airborne Short Ragweed, *Alternaria*, Antigen E and Alt-1 Allergens: A Two Year Prospective Study. *J. Allergy Clin. Immunol. 72*:40–45 (1983).