

Regulatory and Compliance Activities to Protect Food-Allergic Consumers in Canada: Research in Support of Standard Setting and Consumer Protection

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An overview is presented of the activities of Health Canada and the Canadian Food Inspection Agency (CFIA) in the area of food allergens. Since the 1990s, changes were made in the *Food and Drug Regulations* in order to better protect allergic consumers by imposing labeling requirements to clearly identify sources of priority food allergens in prepackaged foods. Policies of application as well as risk management strategies are discussed with some statistics on allergen-related food recalls in Canada for the years 1997–2001. Health Canada's allergen method development program is a pioneering research initiative that was developed in the early 1990s in support of the changing Canadian regulatory environment. The objectives and some of the accomplishments of this program are presented. The development of the Canadian Compendium of Allergen Methodologies under a Web-based application to compile data on evaluated allergen detection methods will provide further support to compliance activities nationally, as well as to the international analytical community in both government and the food industry. Some emerging techniques for the confirmation of results generated by enzyme-linked immunosorbent assays are also discussed.

Food allergy is an on-going public health problem and continues to be a challenge to both the clinical community and the food industry. Health Canada, through its Food Program, and the Canadian Food Inspection Agency (CFIA) have played a proactive role in developing risk assessment and risk management procedures related to inadvertent consumption of allergenic ingredients by vulnerable consumers.

Since the creation of the CFIA in 1997, both Health Canada and the CFIA have shared the responsibility of ensuring the safety of foods offered for sale to Canadians. In brief, Health Canada's Food Directorate is in charge of risk assessment and standard setting, under the *Food and Drugs Act and Regulations*; the CFIA is in charge of risk management, practically manifested by compliance and enforcement of these same regulations. Health Canada is, in turn, responsible for assessing the enforcement activities related to food safety. The same scheme of shared responsibilities is applied to food allergens.

This paper presents the regulatory environment surrounding food allergens in Canada, in particular, some of the regulatory initiatives that were undertaken since the 1990s to protect allergic consumers, as well as various supporting efforts in research, method development, and method validation, that were implemented both by Health Canada's and CFIA's laboratories. New regulatory proposals leading to enhanced protection of allergic consumers will also be discussed. These measures are being supported by increasing technical capacity of method development and validation, aiming at the identification and confirmation of undeclared allergenic ingredients in foods.

Guest edited as a special report on "Methodological Aspects of Food Allergens" by Kristina Williams.

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Regulatory Environment Affecting Food Allergens in Canada

Health Canada's Food Directorate has the mandate to establish food labeling requirements where there are health and safety or nutritional quality concerns for the targeted food commodities. Notwithstanding several voluntary programs, such as the Allergy Aware Program (1), which was launched in 1991 in a number of Canadian restaurants, the need for more complete labeling of allergenic ingredients in foods sold in Canada was identified during consultations in 1993 with health agencies, consumers, industry, and government. These efforts were undertaken as part of a regulatory review of the *Food and Drugs Act and Regulations*. A Working Group consisting of representatives of Health Canada, the CFIA and practicing pediatric allergists working in consultation with the medical community set out to develop a scientifically based list of foods known to cause severe adverse reactions in hypersensitive individuals.

This Working Group summarized and published the outcome of its deliberations in a 1999 paper (2), which provided the scientific basis for the need to always label the substances identified on the list. Priority allergens listed were peanuts, tree nuts [almonds, Brazil nuts, cashews, hazelnuts (filberts), macadamia nuts, pecans, pine nuts, pistachios, walnuts], sesame seeds, milk, eggs, fish, crustaceans (crab, crayfish, lobster, shrimp), shellfish (clams, mussels, oysters, scallops), soy, wheat, and sulfites. The Working Group also made a recommendation that the plant species of all forms of hydrolyzed plant proteins, starches, and lecithin should be identified by their common names on food labels. Finally, food manufacturers, importers, distributors, and food service establishments were urged to develop an allergen prevention plan to manage allergy risks. Indeed, the Grocery Products Manufacturers of Canada had already introduced an Allergy Beware program in 1993 in conjunction with Health and Welfare Canada and the Allergy/Asthma Information Association.

The current *Food and Drug Regulations* state that all ingredients must be declared on the label of food products. Division 1 of these regulations requires almost all of prepackaged foods to include a list, all ingredients as well as their components. However, under Subsection B.01.009 (1), the components of 36 ingredients or groups of ingredients do not have to be declared on the label of a product. Among the items on this list are ingredients that can create problems for allergic individuals. For instance, margarine may contain milk products and meat may contain wheat fillers. These regulations are being revised by Health Canada to ensure that the 10 most common food allergens on the priority allergen list, as recognized by the Codex Alimentarius Commission in 1997, are consistently labeled for enhanced protection of allergic consumers. An overview of the suggested changes in regulations is presented in this paper.

In October 1999, an initial regulatory proposal entitled "Enhanced Labeling of Food Constituents Known to Cause Serious Adverse Reactions in Canadians" was brought

forward by Health Canada's Food Directorate. This proposal focused on dealing with labeling exemptions of Section B.01.009 of the Canadian *Food and Drug Regulations*, i.e., where components of foods such as margarine and flour, when used as ingredients of other foods, are not required to be shown on the label of such foods. This would no longer be applicable should any components of these so-called second generation ingredients be a major allergen or a gluten source. The initiative, however, was never intended to affect products currently exempted from labeling requirements under paragraphs B.01.008(2)(a), (d), and (e) of the *Food and Drug Regulations*. The foods included in these paragraphs are prepackaged products, packaged from bulk on retail premises, except prepackaged products that are a mixture of nuts; and prepackaged meat, meat byproducts, poultry, poultry meat or poultry byproducts that are barbecued, roasted, or broiled on the retail premises. The Food Directorate proposal was not intended to cover cross-contamination situations in which a major allergen might contaminate a food through inadvertent contact, airborne contamination, etc. Such situations, which continue to be problematic, are handled through other risk-management strategies, e.g., ensuring good manufacturing practices in food-processing establishments, and are often alleviated by "may contain" labeling, which, while not entirely satisfactory, is preferable to no labeling at all.

A joint Health Canada/CFIA policy team solicited comments from stakeholders likely to be affected by the regulatory amendments, particularly with regard to the inclusion of gluten on the list of substances associated with serious adverse reactions. The proposal was further developed by incorporating grains that are sources of gluten in the list of ingredients for which previous exemption was allowed (e.g., wheat, including spelt and kamut, or oats, barley, rye, or triticale and hybridized strains of these grains). It was also suggested to extend the enhanced labeling proposal to protein-containing products derived from priority allergens, unlike the European proposal, which includes allergens on the list and "products thereof". Some other new features were also introduced, such as a requirement that plant and animal sources be identified in the common name of hydrolyzed animal proteins and that the plant source of food starches be indicated on the labels of food containing them. The proposal focuses, therefore, on ensuring that consumers purchasing pre-packaged foods will be able to clearly ascertain whether or not the product contains a priority allergen ingredient when it is deliberately added to the product by the manufacturer.

New Regulatory Proposal Surrounding Food Allergens in Canada

Health Canada's Food Directorate Regulatory Proposal for enhanced labeling of allergenic ingredients addresses prepackaged food labeling exemptions that presently exist in the Regulations and focuses on prepackaged foods only. It does not create new labeling measures for bulk foods, foods packaged on retail premises, foods sold in vending machines, one-bite confections, food colors, and fruit and vegetable wax

coatings. In summary, these regulatory amendments would require that: (1) definitions for “gluten source” and “major allergen” be established in Section B.01.001 to ensure that enhanced labeling applies to a protein-containing product derived from a priority allergen; (2) the following major allergens or gluten sources be shown in the list of ingredients by their common name, followed by the name of the food specified in brackets, if it is not already identified in the common name: peanuts, tree nuts (including almonds, Brazil nuts, cashews, hazelnuts [filberts], macadamia nuts, pecans, pine nuts [pignolias], pistachios, and walnuts), sesame, milk, eggs, fish, crustacean, shellfish, soy, and wheat (including spelt and kamut, or oats, barley, rye, or triticale or any protein-containing part thereof, and hybridized strains of these grains); (3) added sulfites be declared in the list of ingredients when present at a level of 10 ppm or more in the final food product; (4) the plant and animal source be identified in the common name of all hydrolyzed proteins; (5) the specific source of the plant be identified in the common name of all forms of starch or modified starch; (6) the source of lecithin be identified in the common name of lecithin; (7) standardized vinegars and alcoholic beverages, including beer and wine, which are exempt from ingredient listing in general, be required to bear a statement that the product contains sulfites (or other potential allergens) if they are deliberately added.

The consultative process in Canada requires that a Schedule of Amendments be prepublished in the *Canada Gazette* Part I, that a formal public consultation period be allowed, and that a final (possibly) revised Schedule of Amendments be published in the *Canada Gazette* Part II, after which the amendments become effective. Prior to publication in the *Canada Gazette* Parts I and II, consideration is given to the Schedules of Amendments by a Special Committee of Council, essentially a Committee of Cabinet. By soliciting the input of various stakeholders prior to prepublication of the Schedule in the *Canada Gazette* Part I, this process ensures proper public and stakeholder input into the decision-making.

New Developments in the Regulatory Environment Surrounding Food Allergens Worldwide

Similar regulatory initiatives to those described above have been under way in the European Union, Australia, New Zealand, and the United States. There is an important similarity between the primary food allergens identified by both Health Canada and the U.S. Food and Drug Administration (FDA). The FDA has published a Food Allergen Compliance Policy Guide (3) for food manufacturers on the most common allergens: milk, fish, peanuts, tree nuts, eggs, wheat, soybean, and crustaceans (lobster, shrimp, crab). In March 2004, the U.S. Senate passed legislation (*Food Allergen Labeling and Consumer Protection Act of 2004*) to amend the *Federal Food, Drug, and Cosmetic Act* by removing labeling exemptions for allergens in spices, flavorings, colors, and food additives. A new joint Australia-New Zealand Food Standards Code requires the mandatory declaration for substances that can

cause severe adverse reactions, including cereals containing gluten, crustaceans, eggs, fish, milk, peanuts, tree nuts, soybeans, sesame, and sulfites (4). The European Commission recently issued a proposal (5) to amend the food labeling directive to ensure declaration on the label of any potential allergenic ingredient, irrespective of any other exemptions to ingredient labeling. The European allergen list is as follows: cereals containing gluten, crustaceans, eggs, fish, peanuts, milk and dairy products (including lactose), nuts and nut products, celery, mustard, sesame seeds, and sulfur dioxide and sulfites at concentrations of >10 mg/kg or 10 mg/L. All allergens on the previous list include “products thereof”.

At the international level, the Codex General Standard for the Labeling of Prepackaged Foods (6) stipulates that the following foods and ingredients known to cause hypersensitivity shall always be declared: cereals containing gluten, i.e., wheat, rye, barley, oats, spelt or their hybridized strains and products of these; crustaceans and products of these; eggs and egg products; fish and fish products; peanuts, soybeans, and products of these; milk and milk products (lactose included); tree nuts and nut products; and sulfite in concentrations of 10 mg/kg or more.

Policies of Application and Risk Assessment Related to Undeclared Food Allergens in Canada

The *Canadian Food Inspection Agency Act* states in Subsection 11 (2) that the agency is responsible for “...the enforcement of the *Food and Drugs Act* as it relates to food, as defined in Section 2 of that Act...,” in other words “for...any article manufactured, sold or represented for use as food or drink for human beings.” Health Canada has developed a *Policy on Food Allergens for Compliance Purposes* that the CFIA uses as a guideline to assess whether a food product poses a significant risk to the consumer and whether it should be recalled. In addition, further advice is provided by Health Canada through health-risk assessments, requested by the CFIA on a case-by-case basis.

Specifically, the Health Canada policy document on food allergens provides recommendations on compliance actions such as product recalls and public alerts, which can be taken under the following circumstances: (1) when peanut protein is detected in a food at levels ≥ 1 ppm and is not declared on the label, Health Canada would support a recall and a public alert; (2) when tree nuts, sesame seeds, soy, cow’s milk, eggs, fish, crustaceans and shellfish, wheat, and sulfites (>10 ppm) are added as ingredients/additives to a food without any declaration of their presence on the product label, Health Canada would support a recall; (3) when trace quantities of tree nuts, sesame seeds, soy, cow’s milk, eggs, fish, crustaceans and shellfish, and wheat are present in the food production process (and without a label warning), Health Canada would perform a health-risk assessment on a case-by-case basis.

Risk assessments related to food allergens are usually driven by mislabeling of prepackaged foods, discovered by

periodic ingredient verification or through incidents involving adverse responses or sickness in affected individuals following consumption of foods containing offending ingredients. Health Canada, at the request of the CFIA, undertakes risk assessments in such incidents to qualify and classify the health risk in any given situation. This is done by reference to the scientific and medical literature, to previous reports on adverse responses, and to information on prevalence. Also considered are whether the incidents are life-threatening, and an estimate of consumption of the offending substance that triggered the response. Despite the best efforts to obtain information about threshold levels triggering a response, there is currently very little reliable information in the literature, although there is considerable on-going research in this area.

Health Canada would determine a risk classification (7) for any given incident, as follows:

Health risk No. 1.—The situation described in the health-risk assessment poses a risk that may cause severe adverse health consequences or death and/or there is the potential for further spread with severe consequences.

Health risk No. 2.—The situation described in the health-risk assessment may cause temporary, not life-threatening, adverse health consequences or contribute to a long-term chronic illness. The probability of serious adverse consequences is low or remote.

Health risk No. 3.—The situation identified is an indication of a breakdown in good manufacturing practices and is not likely to result in an adverse health consequence. It is recommended to correct the problem identified.

Most incidents involving allergens fall into the Nos. 1 and 2 categories.

The above-described policy was developed in 1998 and was based on the best-available knowledge at the time regarding assessment of risk associated with exposure to substances known to be the cause of the most frequent and severe allergic or allergic-type reactions in Canada. The policy recognizes numerous unknowns with regard to quantitative risk assessment of food allergens and that research laboratories are continuing to improve analytical methods for the detection of food allergens. As these methods become available, in conjunction with the introduction of new regulatory amendments and increased knowledge on threshold levels and allergic reactions, it is anticipated that the policy will be further refined.

Risk Management Strategies and Recalls Related to Undeclared Food Allergens in Canada

CFIA's role in managing risks associated with food allergies is both to promote and determine compliance, on the part of industry, with all applicable regulations and guidelines. Another role is to encourage the development of plans to control allergens in all domestic and imported goods offered for sale in Canada. The CFIA has encouraged the development, by manufacturers, of allergen prevention plans, to manage the risks associated with those foods known to

cause severe adverse reactions. Part of this strategy includes a thorough evaluation of manufacturing and ingredient control procedures in addition to complete and accurate labeling. More specifically, in order to support the industry's efforts, the Agency publishes documents on its Website, such as the *Guide to Food Labelling and Advertising*, which refer directly to declaration of allergens. The agency has also prepared 2 resources to help importers obtain product information from their suppliers, which include accurate declaration of allergens: "Tool for Managing Allergen Risks in Domestic and Imported Food Products" and "Allergen Checklist for Food Suppliers and Manufacturers." These actions are undertaken in addition to regular educational sessions, direct meetings, consultations, and information on the Agency's Website. The CFIA also implements inspection programs and projects related to assessing allergen threats to food safety. Inspection programs include such activities as verifying the accuracy of labels on domestic and imported products, and inspections and audits of domestic manufacturers and importers to assess their compliance with the applicable legislation.

Compliance and enforcement activities are issues of fundamental concern in the food allergen area, because of the prevalence of repeat offenders in both the domestic manufacturing and food imports sectors. Figure 1 presents the number of allergen-related recalls in Canada for the 4 year period from 1997 to 2001, with an indication of the classes of these recalls, which correspond to the classes of health risk previously described.

It should be noted that 58% of these allergen recalls were Class 1 recalls. The increasing number of recalls for the year 2000–2001 coincides with a new counting scheme for allergen recalls, based on incidents as well as on increased testing capacity within the Agency's laboratories. A review of the Class 1 recalls of all types over the same 4 year period, including non-allergen related food incidents (e.g., microbiological, chemical, extraneous, and other) reveals that the majority of these Class I recalls were allergen-related: 75% in 1997–1998; 69% in 1998–1999; 58% in 1999–2000; 75% in 2000–2001. This high proportion of allergen-related Class 1 recalls can be

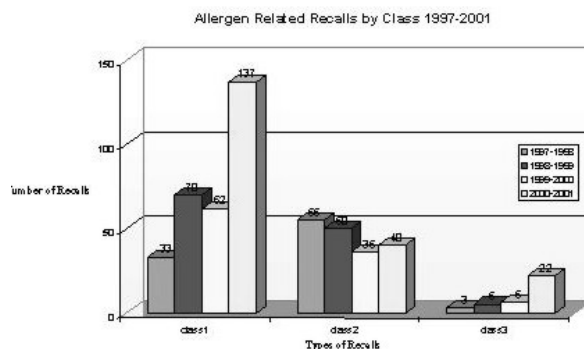


Figure 1. Allergen-related recalls by class (1997–2001).

explained by possible increased attention to particular allergens, increased consumer awareness and reporting, the effect of enforcement activities as more analytical techniques to detect allergens become available, and greater awareness on the part of the food industry of the risks posed by allergens.

Table 1 presents the list of allergens that were implicated in recalls throughout the same 4 year period. A higher percentage of recalls was triggered by peanuts/tree nuts, dairy, sulfites, and eggs compared to other allergens. One immediate explanation for this observation is directly linked to available analytical methodologies applied within CFIA laboratories to detect markers of allergens in investigated food products. As further methods become available and are validated, it is expected that the proportion of recalls for other priority allergens will increase. The laboratories' role constitutes a pillar of allergen control strategies. Analytical results for trace amounts of allergen markers in food samples represent an indispensable tool to support CFIA's programs as well as the decision-making process to manage the risks related to allergens in food.

The Allergen Method Development Program: Research in Support of Enforcement and Policy Development

To support on-going policy development strategies in relation to food allergens, Health Canada's Bureau of Chemical Safety launched an ambitious method development program in the early 1990s to address the lack of methodologies for detection of undeclared allergens in foods. Methods were developed to detect peanut (8, 9), soy (10), milk (Yeung et al., unpublished results), egg (11), hazelnut (12), Brazil nut, and crustacean tropomyosins (13). Most of these methodologies have been transferred to CFIA laboratories and constituted the backbone of the Canadian compliance program with regard to the presence of undeclared allergens in foods.

With the emergence of several commercially available test kits for the measurement of allergens, the research program has been re-aligned to address new challenges in allergen testing, identified in collaboration with the CFIA, through the newly created Allergen Methods Committee (AMC). Among the key objectives of the Health Canada/CFIA allergen method development program are the following: (1) to maintain the independence of federal testing capabilities for allergens by providing back-up techniques and by filling the gaps in allergen methodologies where no commercially available method exists; (2) to promote the dissemination of methods developed in-house through existing technology transfer opportunities and to increase collaboration with national and international partners in government, universities, and the diagnostic industry; (3) to collaborate in the evaluation of commercially available test kits and in the development of a compendium of methodologies for allergen detection/determination in foods; (4) to collaborate with other international agencies in attempts to ensure a common approach in the reporting of results for allergens in foods; (5) to develop and evaluate alternatives to enzyme-linked immunosorbent assay (ELISA)-based methodologies for confirmation of results, using either protein separation techniques coupled with mass spectrometry (MS) identification of key proteins/peptides, or DNA-based techniques; (6) to develop characterized fractions of protein markers for the priority allergens and to establish a repository of immunoreagents specific to these fractions. The AMC plays an important role in coordinating these efforts to deliver on the set objectives, in line with CFIA's needs.

The Committee membership consists of Health Canada and CFIA allergen researchers, specialists from the CFIA regulatory laboratories conducting allergen testing, and CFIA laboratory program management. The Committee provides a communications linkage between laboratories and facilitates a coordinated approach to method development and to delivery and advancement of research projects. In addition, the

Table 1. Allergens implicated in Class 1 recalls in Canada: 1997–2001

Allergens	1997–1998	1998–1999	1999–2000	2000–2001	Total recalls	Overall % ^a
Peanut and tree nuts	15	32	21	35	103	34
Dairy	1	2	7	70 ^b	80	26
Sulfites	1	11	16	17	45	15
Egg	5	7	12	6	30	10
Soya	4	5	2	5	16	5
Wheat	0	2	1	4	7	2
Seafood	1	0	0	0	1	<1
Sesame seed	1	0	0	0	1	<1
Multiple allergens	5	11	3	0	19	6
Total recalls by year	33	70	62	137	302	100

^a Percentages are estimates based on review of CFIA's data.

^b Increase could be due to improved method detection and targeted enforcement activity.

Committee promotes consistency in the implementation of methods and reporting of results in a manner that meets the needs of all the laboratories involved, as well as CFIA program planners and Health Canada's risk assessors.

The Committee has coordinated interlaboratory evaluations of both commercial and in-house allergen detection methods. Through this activity, a set of formalized guidelines and performance criteria for the evaluation of allergen methods has been drafted to assist in the development of the Canadian Compendium of Allergen Methodologies.

Canadian Compendium of Allergen Methodologies—Joint Health Canada and CFIA Approach to Evaluate/Validate Allergen Detection Methods

With the emergence of several allergen detection methodologies came a need to adopt a consistent approach to evaluate/validate these methods, if they are to be used in enforcement/compliance activities. The reporting of results for the presence of allergen markers in investigated food commodities is dictated by risk-assessment needs. According to Health Canada's existing procedures, these results are to be quantitative and expressed in concentration of proteins (e.g., μg protein/g commodity). It is therefore important to qualify the calibrators or standard solutions used in these protein-based methodologies in terms of their protein content. Despite the inconsistencies that may be encountered with protein determination methods, the AMC has agreed to estimate the protein content of standards or calibrators by using a standardized protein assay: the bicinchoninic acid assay (BCA) with bovine serum albumin (BSA) as a reference. Although subject to discussion, and despite the variation in nature and behavior of soluble allergen proteins in comparison to BSA, it was agreed to use BSA as common reference for reporting purposes. These reference parameters are reported along with the assay results, if used as part of an investigation.

Another challenge faced in the development of the Compendium of Methodologies is to achieve an evaluation process that meets the requirements of various methodology

users, without necessarily imposing a limit of detection that all methods must be capable of meeting. It remains to users to decide on the suitability of existing/evaluated methods, according to their own needs. Nonetheless, it is important to establish a uniform procedure for method evaluation where possible, by designating one or several reference materials that can be used in fortification experiments (naturally incurred or artificial fortification) for the food matrixes of interest. The AMC has defined a reference material as a "material that is representative of the allergenic food commodity, that is well characterized, and that can be produced/supplied with reproducible characteristics."

The AMC has set general criteria for the introduction of allergen analytical methods into the compendium. The general principles behind this set of criteria are: (1) The Compendium of Methodologies aims at reporting the performance of methods that were evaluated according to an agreed-upon experimental protocol. Inclusion in the compendium does not imply endorsement or approval of specific methods.

(2) The evaluation protocol will be designed and implemented in total collaboration with the method developer, whether the developer is a test kit manufacturer or a private, university, or government laboratory, and will be tailored according to the specificities of each allergen studied and the corresponding reference materials developed.

(3) Two levels of evaluation will be targeted: (a) an entry level, where a method is experimentally evaluated by a single AMC-affiliated laboratory and that of the method developer; and (b) a full evaluation level, where results are generated by at least 3 AMC-affiliated laboratories and that of the method developer.

(4) A designated reference material should be defined for the method to be evaluated and must be of some validity of use for more than the targeted test kit, thereby allowing an evaluation of the performance of several comparable test kits or methods.

(5) Levels of fortification and matrixes to be targeted will be defined according to the claims of the method author; typically, 3 levels of fortification will be evaluated, a blank, $2 \times$ limit of quantitation (LOQ) and $5 \times$ LOQ.

Table 2. Summary of results for hazelnut assay interlaboratory evaluation before introduction to the Compendium of Allergen Methodologies

Commodity	Spiking level, ppm	Lab No. 1		Lab No. 2		Lab No. 3		All 3 labs		
		Avg. % rec.	SD	Avg. % rec.	SD	Avg. % rec.	SD	Avg. % rec.	<i>n</i>	SD
Cookie	8	90.1	12.8	101	20	114.3	11	101.8	30	18
	20	101.3	19.3	84	7	87.2	10	90.8	30	15
Dark chocolate	8	138.7	34.3	126.3	27	124.8	15	129.9	30	27
	20	122	14.1	104.8	11	90.1	7	105.6	30	17
Milk chocolate	8	99.8	23.4	131.3	18	111.9	11	114.3	30	22
	20	105.1	20.1	112.8	13	97	11	104.9	30	17

(6) The number of replicates has been defined so that the evaluation process does not impact negatively on activities of the participating laboratories, whose primary mission is to deliver on compliance activities rather than to be involved in laborious evaluation of methodologies.

(7) The report of performance on evaluated methods will be posted on a Web-based version of the allergen Compendium of Methodologies, which will make available more data on existing methodologies and allow users from the analytical community to choose methods according to their needs.

(8) The generated data will be shared in agreement with all involved parties, including the method developer, and will enable a better understanding of data provided by a third party using one of these evaluated methods.

(9) Federal laboratories are free to use whatever testing methodologies they consider appropriate to fulfill the requirements of their allergen testing. Such information will not be posted on the Web-based application, as it is subject to change and may be regarded as an endorsement of a particular method.

This evaluation process was recently applied to a Health Canada-developed methodology for the detection of hazelnut proteins. Three AMC-affiliated laboratories (a Health Canada laboratory and 2 CFIA laboratories) analyzed blind samples that had been fortified with hazelnut at 3 different levels (0, 8, and 20 ppm). Results for this interlaboratory evaluation are shown in Table 2. All samples were correctly identified as positive or negative, and recoveries varied from 90.8 to 129.9%. A report of performance on this methodology is being prepared, before its introduction into the Compendium of Allergen Methodologies. In parallel, Health Canada's allergen laboratory has taken part in a similar interlaboratory evaluation for commercially available test kits for the detection of peanut markers in food commodities. The protocol of evaluation was led by the AOAC Research Institute (AOAC-RI), in collaboration with the FDA and targeted the detection of 5 µg/g total peanut. The results of this evaluation will also be summarized and the evaluated methodologies introduced into the Compendium of Allergen Methodologies, once the corresponding authorizations have been obtained from the AOAC-RI.

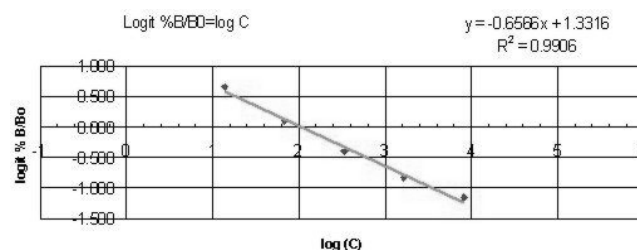


Figure 2. Example of calibration curve for seafood assay. The IC₅₀ is estimated to be 6 ng/mL shrimp protein extract.

Latest Accomplishments of the Canadian Allergen Research Program

A multiresidue determination system using ELISA-based techniques has been developed for screening purposes (14). This multi-nut assay has the ability to test for 5 different allergen markers in chocolate: 4 tree nuts (almond, Brazil nut, cashew nut, hazelnut) and peanut, in a single run with a detection limit <1 µg/g protein for each allergen. This assay is meant for use with samples identified as a result of complaints from consumers with allergic histories related to more than one commodity. A screening assay would help pinpoint the analytical methodology to be used for quantitative determination of any undeclared allergen.

An ELISA was also developed to target the detection of crustacean proteins, mainly invertebrate tropomyosin (13). The assay was developed using shrimp-specific antibodies but shows reactivity to other crustaceans (lobster, crab, and scampi). Figure 2 presents an example of a calibration curve for the seafood assay giving a 50% inhibition concentration (IC₅₀) of 6 ng/mL shrimp protein. This assay was optimized for application to processed foods such as frozen prepared meals, soups, and fish sticks.

Efforts are also being directed toward confirmatory methods using protein isolation coupled with MS identification of peptide markers of allergenic proteins. With MS, it is possible to analyze tryptic digests of extracts to look for specific protein signatures. This technique is meant to

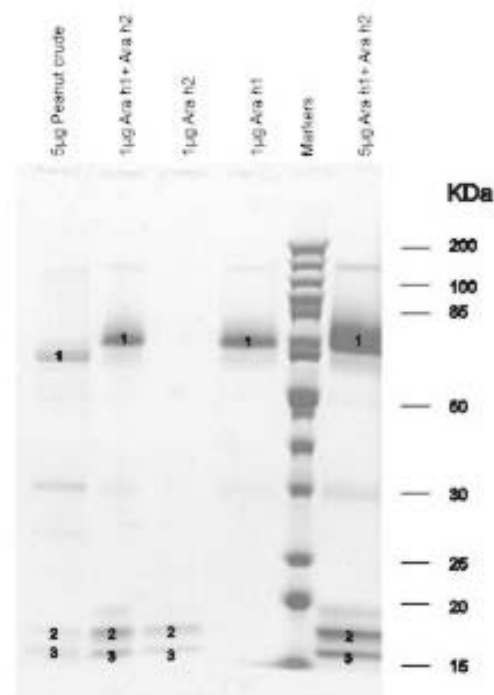


Figure 3. 1D gel separation of peanut protein extract and mixtures of Ara h1 rich and Ara h2 rich standards. Bands 1–3 were excised for tryptic digestion and LC/MS/MS analysis.

Table 3. Results of NCBI database search using the Micromass Proteinlynx Global Server v1.1^a

	Band 1	Band 2	Band 3
5 µg Peanut extract	Ara h3/4 (1640, 8) Ara h1 (1178, 9)	Ara h2 (67, 2)	Allergen II (12, 1)
1 µg Ara h1 + Ara h2 mix	Ara h1 (6732, 19)	Ara h2 (581, 4)	Allergen II (379, 2)
1 µg Ara h2	ND ^b	Ara h2 (498, 4)	Allergen II (339, 2)
1 µg Ara h1	Ara h1 (5732, 19)	ND	ND
5 µg Ara h1 + Ara h2 mix	Ara h1 (18687, 26)	Ara h2 (1641, 6)	Allergen II (3177, 4)

^a Values in parentheses indicate score and number of peptides matched.

^b ND = Not determined.

corroborate results obtained by ELISA-based methodologies. Preliminary studies were performed on peanut protein extracts. A phosphate-buffered protein extract from defatted roasted peanuts (8) was separated 1D by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Identified protein bands were cut and excised into small pieces before an in-gel tryptic digestion was performed with the robotic system MassPrep station. The generated peptides were extracted and analyzed by capillary liquid chromatography (LC)/tandem MS (LC/MS/MS) on a Waters Capillary LC/QTOF2 system. The system was configured for use with either a short C18 precolumn to trap and desalt protein digests and rapidly elute them, or both a pre-column and an analytical column (PepMap C18). All MS data were acquired with the Micromass Masslynx v3.5 under the Survey mode. The data generated were analyzed either by the Proteinlynx Global Server v1.1, with databases including SWISSPROT, TrEMBL, NR (compact version of NCBI), or by submitting the peak list data to Matrix Sciences' Mascot search engine for MS/MS search of the National Center for Biotechnology Information (NCBI) database.

The major peanut allergen proteins were successfully identified by in-gel digestion of the selected bands from the 1D gel electrophoresis separation of the peanut protein extract (Figure 3, Table 3). Two peanut allergen protein standards obtained from colleagues at the U.S. Department of Agriculture were also characterized with the same methodology (Figure 3). The high score in the search gives good confidence that an excellent match was obtained. In some cases, where the numbers of peptide matches were up to 26, the peptide coverage was as high as 45% of the protein. The high-resolution and high-mass accuracy of the instrument requires only a few peptide hits (sometimes as low as 1) to obtain an unequivocal identification of the protein involved. Investigation of the applicability of such a technique to extracts obtained from food products, which gave a positive response for peanut protein with the ELISA, is under way. The development of the appropriate sample preparation steps appears to be critical prior to protein separation and detection with LC/MS/MS.

Conclusions

The Canadian regulatory environment surrounding the declaration of allergens in food is in constant evolution. A strong interdepartmental collaboration was developed between Health Canada and CFIA laboratories. This collaboration resulted in the establishment of a relevant method development program for allergen determination in food, to be used in compliance/enforcement activities. It is a firm belief that such cooperation, along with increased international collaboration in understanding threshold levels of reaction to allergens, harmonization of risk management tools (analytical methods, reference materials, reporting units, etc.), and further consultation with the food industry and allergic consumers, will result in the prevention of unexpected exposure of susceptible individuals to allergenic food commodities and the overall improvement of consumer protection.

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