Mustard: A Priority Food Allergen in Canada

A Systematic Review
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A Systematic Review
Table of Contents

Executive Summary .................................................. 1

Issue ................................................................. 2

Objective ......................................................... 2

Methods ............................................................ 2
  Systematic Data Collection ........................................ 2
  Organization and Tabulation of Data .......................... 2

Results .............................................................. 3
  A. Characterization of Mustard ................................. 3
  B. Pivotal Clinical Studies (Table 1, Appendix 2) .......... 5
  C. Non-Pivotal Clinical Studies (Table 2) ..................... 7
  D. Other Relevant Clinical Studies (Table 3) ............... 8
  E. Case reports (Table 4) ..................................... 8

Discussion ......................................................... 9

Conclusions ...................................................... 13

Recommendations ............................................... 14

References ......................................................... 15

Appendix 1 ........................................................ 31

Appendix 2 ........................................................ 33

Appendix 3 ........................................................ 36
Executive Summary

Health Canada scientists have conducted a systematic literature review of the available information on the allergenicity of mustard in order to determine the scientific validity of including mustard on the list of priority food allergens in Canada. Information that was considered relevant to the evaluation was assessed using Canadian criteria established to amend the list of priority allergenic foods (http://www.hc-sc.gc.ca/fn-an/pubs/label-etiquet/crit/index-eng.php).

A total of 42 publications from the scientific literature were considered relevant to the assessment of mustard as a food allergen. The following evidence was available to substantiate the addition of mustard to the list of food allergens in Canada:

• There are Canadian case reports supporting the occurrence of mustard food allergies in children and adults in Canada;
• A credible cause-effect relationship is supported by positive double-blind, placebo-controlled, food challenge (DBPCFC) studies designed to assess the allergenicity of mustard;
• Reports describe severe systemic reactions, including anaphylaxis following exposure to very small amounts of mustard within foodstuff;
• Mustard is affirmed on the most recent list of 14 allergens to be declared on labels (updated in 2007) by the Commission of the European Communities (CEC, 2007) and mustard is recognized as an allergen by the International Union of Immunological Societies (IUIS, 2009);
• All three types of mustard seed are available in Canada and mustard is used in cooking and in processed and pre-packed foods;
• Results from characterization studies of allergenic proteins indicate that proteins in mustard are resistant to degradation by heat and digestive enzymes which makes these proteins more likely to withstand food processing;
• The thermostable allergenic proteins in mustard have the potential to be hidden within certain ingredients, preparations and mixtures in processed and pre-packaged foods;
• Individuals known to be sensitive to allergenic proteins from one type of mustard seed are likely sensitive to other types;
• Additional factors which make mustard allergy relevant to the Canadian scenario include the potential cross reactivity between mustard and rape seed and the facts that, Canada is a major producer of both of these crops and sensitization to mustard can be acquired through dermal and respiratory exposure.

This scientific evidence fulfilled the Canadian criteria for addition of a new food to the list of priority allergens in Canada. In satisfying all of the criteria, mustard was recommended for addition to the Canadian list of food allergens and that the Food Allergen Labelling Regulations, which require enhanced labelling for priority allergens in pre-packaged foods, be applied accordingly in order to ensure that mustard allergic consumers in Canada are duly protected.
Issue

On July 26, 2008, Health Canada published its proposed amendments to the *Food and Drug Regulations* (1220 – Enhanced Labelling for Food Allergens and Gluten Sources and Added Sulphites) in *Canada Gazette*, Part I (CGI). Just over 140 comments were received from the general public, patient groups, health professionals, a consumer organization, and governmental agencies during the public consultation regarding the proposed amendments.

Of the responses, 22% requested that mustard and/or mustard seeds be added to the list of priority allergens in the regulatory amendments based on observations or personal experiences of adverse reactions associated with the consumption of mustard and/or food products containing mustard and/or mustard seeds.

In response to the feedback received during the public consultation, Health Canada initiated a systematic review of the available literature in order to determine the scientific validity of the inclusion of mustard on the list of defined priority food allergens in Canada. Foods that are listed in the definition of the “food allergen” as proposed in the amendments to the *Food and Drug Regulations* would require that the source of the food allergen to be declared on the label of pre-packaged food products.

Objective

In order to determine the scientific validity of including mustard on the Canadian list of priority food allergens, the information obtained from a systematic review of available literature regarding the potential allergenicity of mustard must fulfill the Canadian criteria for amending the list of priority allergenic foods.

Methods

Methods for the management and evaluation of available scientific information have been previously outlined in order to ensure a consistent and transparent approach for the assessment of the potential allergenicity of a food or food ingredient (http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/allergen/index-eng.php).

Systematic Data Collection

An electronic database search of publications in English, French or Spanish was conducted utilizing the following databases: Ovid Medline (R) In-Process & Other Non-Indexed Citations and Ovid Medline (R) 1950 to Present; Ovid Medline (R) In-Process & Other Non-Indexed Citations, November 18, 2008; Ovid Embase, 1980 to 2008 Week 45; FSTA Direct, last updated 10 Nov 2008. Details of the search terms used are provided in Appendix 1.

Organization and Tabulation of Data

Studies fulfilling the selection criteria (see methods) were reviewed and assessed based on the strength of evidence. Publications that could not be categorized by these criteria were referenced in the results under the heading (A) Characterization of Mustard. Evidence from publications that fulfilled the strength-of-evidence parameters were tabulated under the following categories:
(B) Pivotal Clinical Studies (Table 1: evidence from randomized and non-randomized controlled trials)

1. Double-blind, placebo-controlled food challenge
   a. Randomized
   b. Non-randomized

2. Single-blind, placebo-controlled food challenge
   a. Non-randomized

(C) Non-Pivotal Clinical Studies (Table 2: evidence from quasi-experimental studies)

(D) Other Relevant Studies (Table 3: evidence from non-experimental descriptive studies (comparative/correlation))

(E) Case reports (Table 4: evidence from non-experimental descriptive studies)

1. Canadian Reports
2. International Reports

Results

A total of 358 publications were identified through the database search using the terms denoted in Appendix 1. However, based on the inclusion and exclusion criteria, only 42 publications were selected and considered relevant to the objectives of this review. Of these 42 publications, 27 were grouped by the strength-of-evidence to support regulatory recommendations in Tables 1 to 4. The remaining 15 publications were not included in the risk analysis; however, the publications provided information with regard to the characterization of mustard and were considered relevant to the evaluation.

A. Characterization of Mustard

Mustard is an herbaceous flowering plant (Angiospermae) belonging to the family Brassicaceae (formerly known as, Cruciferae), which includes but is not limited to cabbage, cauliflower, brussels sprouts, turnips, radishes, broccoli and fodder (rape) crops (Rancé, 2003).

Mustard seeds are sold as whole, ground into powder or processed further into prepared mustard. Prepared mustard is commonly used as a condiment and mustard seeds and powder are increasingly being used in cooking and in processed and pre-packaged foods as a seasoning or flavouring agent, emulsifier, and water binding agent for texture control (AAFC, 2009a).

The major types of mustard seeds used in cooking and food processing are: white (Sinapis alba or yellow mustard), brown (Brassica juncea or oriental mustard) and black (Brassica nigra or black mustard). Commercially sold mustard powder is usually a mixture of ground black and white mustard seeds and prepared mustard sauce is composed of mustard seeds, salt, vinegar, wheat flour, and other spices and additives. White mustard seeds are much larger and a lot less pungent than the brown variety and are the main ingredient in North-American-style mustards. White and brown mustard seeds are blended to make English-style mustards and brown mustard seeds are the main ingredient in European and Chinese-style mustards (AAFC, 2009a).
All three types of mustard seed are available in North America. In fact, Canada is a world leader in the international mustard seed market accounting for about 35% of world production and 50% of global exports (AAFC, 2009b).

Based on information provided by Agriculture Agri-Food Canada, yellow mustard seeds contain: 20–30% protein, 24–35% oil, 6–12% lipids and 12–18% carbohydrates (AAFC, 2009a). Mustard also contains irritants that may cause non-immune reactions mimicking allergic reactions, for example, capsaicin, the irritant ingredient of capsicum, and isothiocyanates. Capsaicin is capable of releasing substance P, which may induce non-IgE-mediated mast cell de-granulation (Niinimäki et al., 1995). It is therefore important to base the diagnosis of a mustard allergy on evidence of IgE-mediated response. Early studies investigated whether the adverse reactions associated with the consumption of mustard were attributable to an isothiocyanate sensitivity. Mustard extracts treated with myrosinase, which degrades isothiocyanates, did not reduce the cutaneous allergenic potency of the extract. Similar treatment with proteolytic enzymes found that the cutaneous allergenicity was reduced; this demonstrated that the allergic responses to mustard are elicited by protein (Leanizbarrutia et al., 1987).

The major allergenic proteins in mustard have been identified and characterized. The major allergen of mustard is a 2S albumin, which is a seed storage protein composed of one heavy chain and one light chain (39 and 88 amino acids), linked by two disulfide bridges (Menéndez-Aris et al., 1988). This seed storage protein has also been isolated from rapeseed, leguminous plants (peas and soya), walnuts, sesame seeds and Brazil nuts and is resistant to thermal degradation (Bartolome et al., 1997; Bush and Helfe, 1996, Menéndez-Aris et al., 1988; Teuber et al., 1998). The major 2S albumin of yellow mustard is Sin a 1 and it is a thermostable protein that is resistant to digestion by trypsin and degradation by other proteolytic enzymes (Dominguez et al., 1990; González de la Peñe et al., 1996; Menéndez-Arias et al., 1990; Monsalve et al., 1993; Palomares et al., 2005). Sin a 1 is able to interact with membrane lipids (Oñaderra et al., 1994). This interaction is postulated to facilitate the uptake of Sin a 1 at the intestinal barrier, thus increasing the resistance of Sin a 1 to protease digestion. Sin a 1 binding to B-cell membranes would allow cross-linking of cell surface proteins, promoting B-cell activation and a subsequent immune response (Oñaderra et al., 1994).

Characterization of the major allergen found in oriental mustard (Brassica juncea), Bra j 1, revealed that Bra j 1 and Sin a 1 have a homologous epitope (González de la Peñe et al., 1991; Monsalve et al., 1993; Palomares et al., 2007). These findings imply that individuals known to be sensitive to one species of mustard are likely to show sensitivity to other species. Furthermore, a marked in vitro cross-reactivity between the principal allergen of rape seed (Bn III) and Sin a1 have been described in the literature (Asero et al., 2002; Bartolome et al., 1997). However, cross-sensitivity between other Brassicaceae species appears to be rare (Rancé, 2003). The proposed reason for this observation is that the proteins in cruciferae leafy vegetables are more susceptible to digestion and thermal degradation than the allergic proteins in mustard (Astwood et al., 1996; Caballero et al., 2002).
B. Pivotal Clinical Studies (Table 1, Appendix 2)

Three clinical trials are considered pivotal to the strength-of-evidence to support regulatory recommendations and are tabulated in Table 1 with detailed summaries provided in Appendix 2. Two double-blind, placebo-controlled food challenge (DBPCFC) studies (Figueroa et al., 2005; Morisset et al., 2003) and one single-blind, placebo controlled food challenge (SBPCFC) study (Rancé et al., 2000 & 2001) were identified in the literature.

Of the DBPCFC studies, one study design was randomized and conducted mostly with adult subjects (Figueroa et al., 2005) and the other study design was non-randomized conducted mostly with children (Morisset et al., 2003). In both studies, the number of subjects recruited and who actually participated in the challenges was limited. Out of 38 subjects who were recruited in the Figueroa et al. (2005) trial, only 24 subjects participated in the oral challenge. Subsequently, out of the 30 subjects who were recruited in the Morisset et al. (2003) trial, 24 of them participated in the oral challenge. The most sensitive subjects who had a history of anaphylaxis were excluded from the challenges.

Both DBPCFC studies masked the strong taste of mustard in other food products. Figureoa et al. (2005) challenged subjects with increasing doses of masked yellow mustard sauce (80, 240, 800, 2400 and 6480 mg) containing S. alba mustard seeds (14% w/v) at 15-minute intervals until a clinical reaction was observed or a cumulative dose of 10 g of the mustard sauce was administered. Morisset et al. (2003) challenged subjects with increasing doses of masked mustard seasoning (10, 30, 100, 300, and 900 mg) containing 33.6% of B. juncea mustard seeds at 20 minute intervals until a cumulative dose of 1340 mg of mustard seasoning was administered. Both groups of investigators specified that mustard products used in the trials were free of metabisulfite which ensured that any reactions observed after the challenge were attributable to a mustard allergy.

In the Figueroa et al., (2005) trial, 14 out of the 24 challenged subjects (58%) were considered to show a positive reaction specific to mustard. Of the positive reactions, the most frequent symptom observed was oral allergy syndrome (OAS) in 10 subjects (71%). This reaction was considered mild and was characterized by pruritus and mild angioedema of the lips, tongue, palate and throat, and was followed by a rapid resolution of symptoms. One subject showed angioedema and bronchial asthma (1/14; 7%) after mustard sauce ingestion and another subject reacted with systemic anaphylaxis (1/14; 7%). In these two cases, the eliciting dose was 156.8 mg of mustard sauce and the reaction was considered moderate and severe, respectively, according to the criteria of Brown (2004). The lowest dose eliciting a reaction was 44.8 mg of mustard sauce. The mean cumulative reactive dose of mustard sauce (±SD) was 891.4 ± 855.2 mg, equivalent to 124.8 ± 119.7 mg of mustard seeds (S. alba).

The results of this study also report a significant association between mustard hypersensitivity and mugwort pollen sensitization (97% of patients). Furthermore, approximately 40% of the subjects were allergic to at least one other food product within the Brassicaceae family. This suggests cross-reactions between mustard and taxonomically related foods. Exercise induced anaphylaxis was also associated with 2% of the cases.
In the Morisset et al. (2003) trial, 7 out of the 30 challenged subjects (23%) were considered to show a positive reaction specific to mustard. Symptoms of positive reactions included: eczema, urticaria, rhinitis, conjunctivitis, abdominal pain, diarrhea, pruritus, sneezing, erythema, and wheezing with a predominance of skin manifestations (5/7; 72%), followed by respiratory/gastro-intestinal (4/7; 57%) symptoms. Skin manifestations alone are considered mild reactions, whereas gastrointestinal symptoms and respiratory symptoms are considered moderately severe (Brown, 2004). There were no reports of anaphylaxis or symptoms indicative of hypoxia or hypotension, which are considered severe reactions. The cumulative dose tested was 1340 mg of mustard seasoning. The lowest eliciting doses were noted at 440 mg and 40 mg mustard seasoning. The dose of 40 mg of mustard seasoning resulted in the subject experiencing rhinitis and urticaria. This dose of mustard seasoning (40 mg) was calculated to be equivalent to 13.5 mg of mustard seeds (*B. juncea*), which is roughly equivalent to 0.8 mg of mustard proteins (*B. juncea* mustard seeds contain 6% mustard protein). This subject was described as being sensitized to mustard pollen and to rape pollen. It was reported that the subject lives in an area of a mustard seasoning factory which emits an unpleasant smell. In this case, skin reactivity was observed with the two species of mustard, (*B. nigra* and *B. juncea*) indicating cross sensitization.

In addition to the study design and the age of the subjects, another difference between these two DBPCFC studies is worth noting. The Morisset et al. (2003) study found that a positive skin prick tests (SPT) and the presence of specific IgE as determined by radioallergosorbent tests (RAST) were not predictive of a positive outcome. In contrast, Figueroa et al. (2005) demonstrated a significant relationship between SPT mean wheal diameter (performed with a commercial mustard extract) and challenge outcome, obtaining a threshold value of 8 mm, with a specificity of 90% and a sensitivity of 50%.

The SBPCFC study by Rancé et al. (2000 & 2001) investigated 36 children (22 male and 14 female) aged 10 months to 15 years (average age 5.5 years) who had positive mustard SPT and compared these subjects to 22 control subjects without a history of food allergies. Specifications for the mustard seeds used for the SPT were provided ([mustard seed powder, including Sinapis alba and Brassica juncea, 1:10 w/v, protein concentration 5 mg/ml]; however, it was not clear whether the same source of mustard was used for the oral challenge.

Of the 36 challenged subjects, 15 had positive reactions (42%) and 21 were considered not allergic to mustard. Of the subjects with positive reactions to mustard, 8 (53%) of the subjects had initially exhibited reactions to mustard under the age of 3 years. Based on this observation, the authors suggest that there may be sensitization to mustard though lactation or in utero.

After the challenge, symptoms included: urticaria (14 cases), rhinoconjunctivitis (3 cases), angioedema (1 case), oral allergy syndrome (1 case), and eczema (1 case). These reactions are considered mild (Brown, 2004). However, the 54 initial clinical features of the subjects included asthma (5 cases, 9%) and, in one case, laryngeal edema with oral allergy syndrome and rhinoconjunctivitis (2%). These symptoms pose a higher risk for hypoxia and are therefore considered more severe than the predominantly dermatological manifestations observed after the SBPCFC. The SBPCFC cumulative reactive dose varied from 1 to 936 mg of mustard powder. The mean cumulative reactive dose was 153 mg of mustard powder. No reactions to placebo were observed. It is also worth noting that (24/36) 67% of the subjects were also allergic to other foods, including peanuts, eggs and milk.
C. Non-Pivotal Clinical Studies (Table 2)

Six studies that were conducted using an open allergenicity assessment, which included mustard as one of the foodstuffs tested, were identified in the literature. These assessments utilized labial (LFC) or oral food challenges (OFC) and/or a combination of skin prick test (SPT), RadioAllergoSorbent Test (RAST) and determinations of serum immunoglobulin E (IgE) specific to mustard in order to verify an allergic response and quantify the prevalence of reactions to certain foodstuff. These studies are tabulated in Table 2.

Niinimäki et al. (1989) conducted SPT and RAST on 50 subjects with a reported history of reactions to spices and pollen. Mustard was included in a battery of spices tested in a 5% (w/v) test solution. Of the subjects tested, 58% had positive reactions for mustard to either one or both SPT or RAST. Furthermore, there were positive SPT results for 3 children, ages 1 to 1.5 years old, who were breastfed for 11 months and had never orally ingested mustard. These results suggest the possibility of the transfer of mustard allergens through human milk and supports the view expressed by Rancé et al. (2000 & 2001) that there is possible sensitization to mustard though lactation or in utero.

Rancé et al., (1994) conducted a similar study designed to assess the prevalence of allergic reactions to various spices among children with a history of food and pollen allergies. In this study, 83 children were evaluated with SPT and IgE against a variety of specific spices including mustard. Of the 83 subjects, 23 (28%) had SPT and IgE positive results for mustard. Out of the 23 subjects, 11 reacted to mustard; they showed symptoms associated with Oral Allergy Syndrome, urticaria and conjunctivitis. These symptoms are considered mild (Brown, 2004). The mustard allergy was further confirmed by LFC and OFC in 7 of the 23 subjects.

Rancé and Dutau (1997) examined over 25 food allergens among 142 children with a history of food allergies. Subjects submitted to LFC for various foodstuffs and when the results of the LFC were negative, SBPCFC were conducted. Twenty-three subjects (16%) had a positive response to mustard, 16 by LFC and 7 by SBPCFC. Mustard was the 3rd most common food allergen in this study; egg (75%) and peanut (60%) were the most common. Rancé and Dutau (1998) also reported a similar prevalence of mustard allergy (12%) among 45 children allergic to 3 or more different foods. In 2002, Rancé and Dutau reported a mustard allergy prevalence of 7% among children previously identified as having bronchial asthma by pulmonary function (Rancé and Dutau, 2002). However, the high prevalence of mustard allergy among children in France, as described by Rancé et al., appears to be more frequent in the southwest region rather than in the other regions of France (Moneret-Vautrin, 2006). Niinimäki et al., (1995) reported a slightly higher prevalence of positive responses: 22 of 49 (44%) of subjects, with an average age of 16.5 years, and who had a history of allergies to spices and birch pollen, had elevated IgE specific to mustard.
D. Other Relevant Studies (Table 3)

With regard to the literature, three non-experimental descriptive studies were identified as being relevant to the assessment of the allergenicity of mustard. These studies are tabulated in Table 3.

A retrospective analysis conducted by André et al. (1994) examined which foods were most frequently associated with anaphylactic reaction over a 9-year period. Mustard was associated with 3% of severe reactions. The authors noted an increasing trend in the frequency of sensitization to mustard over time. This observation is in agreement with the opinion expressed in an article by Rancé (2000).

In a prospective prevalence study, 544 children with a history of food allergies (confirmed by a food challenge) were investigated (Rancé et al., 1998; Rancé et al., 1999a & 1999b). Of the 544 children, 49 (9%) tested positive for an allergy to mustard via SPT and/or specific IgE. Mustard was the 4th most common allergen identified with this study. In addition, one child within the group reacting positively to mustard was reported as having an anaphylactic reaction. However, the anaphylaxis-eliciting dose is not specified in the report. The cumulative dose used for all allergens tested was from 0.1 to 10 g of lyophilized food.

A cohort study conducted by Caballeros et al. (2002) in 29 subjects who tested positive for an allergy to mustard by a SPT and mustard-specific IgE, reported anaphylactic reaction in 14 of 29 subjects (48%) with an overall systemic reactions in 19 of 29 subjects (65%). Symptoms ranged from loss of consciousness, dyspnea, angioedema, generalized urticaria, gastrointestinal symptoms, oral allergy syndrome, conjunctivitis, and rhinitis. The most frequent symptoms were angioedema (55%) and urticaria (34%) (Caballeros et al., 2002). These symptoms are, for the most part, graded as mild (Brown, 2004). However, they can be severe in cases of generalized urticaria and rapidly evolving angioedema involving the face and neck (including the glottis). Symptoms such as these can present a high risk of airway obstruction and hypoxia.

E. Case reports (Table 4)

A total of 15 case reports of allergic responses to mustard were identified in the literature. Two case reports documented cases in Canada and the remaining 13 reports documented international cases including: Spain (5 reports), Italy (2 reports), Sweden (2 reports), France (2 reports), Germany (1 report) and Turkey (1 report). These case reports are tabulated chronologically by publication date in Table 4 (1) (Canadian reports) and Table 4 (2) (International reports). These case reports provide descriptions of the severity of reactions to mustard as well as identifying the sources of mustard exposure and cross-contamination.

1. Canadian Reports

Yip and Zimmerman (1999) report five cases of a mustard allergy in children (4 boys and 1 girl). Of the five children, three are 3 years of age or less and the other two are 5-7 years old. All of the children had a reported history of multiple food allergies and 3 of the 5 children were atopic.

Reactions to mustard included: angioedema, airway obstruction, urticaria, wheezing, vomiting immediately after exposure and swelling of the lips. Two cases had at least one episode of airway obstruction or anaphylaxis requiring emergency hospital attention. These symptoms are considered moderate to severe (Brown, 2004). In at least one case, the allergen was hidden within the glazing of a prepared ham. The sensitivity to mustard was supported by positive SPT in all cases. However, based on the clinical history of the subjects, none of the cases were orally challenged with mustard due to the high risk of a severe reaction.
The other Canadian report concerned a single case. A 50 year-old woman had a history of anaphylactic type reactions after exposure to mustard. This clinical history was supported by a positive SPT for mustard. Further details about this case were not available (Connors et al., 2006).

These Canadian reports provide the only North American data with regard to allergic reactions to mustard. None of the Canadian reports included testing of IgE specific to mustard or oral challenge.

2. International Reports

For most international cases, the information provided was limited because none of the case reports included oral challenges. However, the case reports provided valuable information regarding the severity of reactions, which were induced by the ingestion of small amounts of mustard. Reactions ranged from acute anaphylaxis to generalized skin manifestation, with the majority of cases reporting severe to moderate acute reaction to mustard. Of the 13 international case reports, describing 22 individual cases of allergic reactions to mustard, 15 individuals reported anaphylactic-type reactions that required emergency medical intervention. Reactions were reported after ingestion of small amounts of mustard: some as a result of cross-contamination in fast food, and in one case, a reaction was elicited by the smell of mustard (Malet et al., 1993). This suggested that the dose may only need to be minute to elicit a severe reaction to mustard in food. All cases tested positive for mustard allergy via SPT and/or IgE.

Discussion

An overall assessment of the available literature suggests that a strong scientifically based database exists to assess the potential allergenicity of mustard. However, the following limitations of the systematic literature review were taken into consideration when determining the scientific validity of including mustard on the Canadian list of priority food allergens.

There were a small number of DBPCFC and OFC studies identified in the literature. However, there is agreement among researchers in the field that the difficulty of masking the strong taste of mustard limited the attempts to perform DBPCFC studies. Furthermore, the clinical history of the majority of subjects included in allergenicity studies indicates a high risk of severe systemic reactions to the ingestion of mustard and therefore the use of oral challenges to confirm that mustard was the food ingredient responsible for eliciting the allergic reaction was considered an unethical health risk.

A large number of publications identified in the initial database search were screened out of our assessment because the studies were only relevant to either skin, respiratory and/or occupational exposures. These data were not considered to be pertinent to the issue of food allergenicity; however, it is recognised that this information is important for those in the clinical field to assess the possibility of occupational or environmental disorders, particularly in areas where mustard is cultivated and/or processed.
There were wide inconsistencies in the reporting of the data, including the amount and level of detail of information provided, and the description and interpretation of clinical symptoms. Data relevant to the assessment of the allergenicity of mustard were often contained within publications in which the main objective of the study did not include reporting specifically on the allergenicity of mustard. This fact made the identification of relevant information more challenging and may have led to the exclusion of available information on mustard. Furthermore, many of the studies published by the same groups of investigators may have reported data from earlier publications within the more recent data publications and/or published the same data in different languages. Our review included publications in English, French and Spanish. Therefore, when feasible, duplications of data were identified and eliminated from the interpretation of the database.

Despite the identified limitations of the systematic literature review, the available database provides valid science-based information for regulatory decision-making that fulfills the Canadian criteria for the introduction of food to the priority list of food allergens.

The first criterion of the Canadian adopted Joint Expert Committee on Food Additives (JECFA) recommendations stipulates the existence of a credible cause-effect relationship, based upon positive DBPCFC studies or unequivocal reports of reactions with typical features of severe allergic or intolerance reactions. The existing database includes two positive DBPCFC studies; one with a randomized study design conducted with adult subjects (Figueroa et al., 2005) and one with a non-randomized study design conducted with children (Morisset et al., 2003). A positive non-randomized SBPCFC conducted with children (Rancé et al., 2000 & 2001) was also considered pivotal in the evaluation of the mustard cause-effect relationship because the category of evidence from these studies is considered strong as per the strength-of-evidence criteria established by the ACAAI (2006). In addition to these studies, supporting studies were categorized by the strength of the evidence provided by the study designs and evaluated accordingly (Tables 1-4 & Appendix 2).

The prevalence of positive mustard allergies among the challenged subjects in the pivotal clinical studies were as follows: Morisset et al. (2003) reported a confirmation of 23% of the children who had a previous reported history of mustard reactions exhibited mustard specific IgE reactions after an oral challenge with mustard seasoning. This rate is low compared to the results of Rancé et al. (2000 & 2001) who reported 42% of previously sensitized children had an IgE-confirmed reaction to an oral challenge with mustard seed extract and the 58% reported by Figueroa et al. (2005) in atopic adults challenged with mustard sauce.

In the current database, the vast majority of the study populations are atopic or have a family history of atopy. In the Figueroa et al. (2005) trial, 92% of the subjects had a history of atopy and in the Rancé et al. (2000 & 2001) trial, 81% of the children had a family history of atopy. The term atopy describes the genetic predisposition to become IgE-sensitized to allergens commonly occurring in the environment and to which everyone is exposed but the majority do not produce a prolonged IgE-antibody response. Thus, atopy is a clinical definition of an IgE-antibody high-responder (Johansson et al., 2001 & 2004). The use of atopic study populations in clinical trials is not considered a misrepresentation of the risk of the allergenic potential of mustard because this sensitive segment of the general population represents the majority of those individuals who are susceptible to food allergies. The capacity of mustard to elicit an IgE-mediated response is valid whether the individual is atopic or not.
Subjects with a clinical history of anaphylaxis were excluded from participating in the oral food challenges. In the Figueroa et al. (2005) trial, 11% of the subjects were excluded based on their clinical history of severe systemic reactions. Nonetheless, anaphylaxis was reported in the challenge studies. One out of 14 subjects without a history of anaphylaxis had an anaphylactic reaction after being challenged with mustard sauce and 2% experienced anaphylaxis in association with exercise (EIA) (Figueroa et al., 2005). Other clinical presentations included: Oral allergy syndrome (OAS); urticaria, and angioedema (AE), which are considered mild reactions (Brown, 2004). However, when these reactions are complicated by laryngeal edema; bronchial asthma (BA) or respiratory symptoms, particularly in individuals with a previous history of asthma, these reactions are considered moderate to severe.

There is evidence within the database to support the conclusion that the amount of mustard required to elicit a reaction may be very small; however there is insufficient information to estimate a dose-threshold (Taylor et al., 2004). Estimations of the eliciting dose in the pivotal studies range from 124.8 ± 119.7 mg of mustard seeds in the Figueroa et al. (2005) trial to the lowest dose reported by Morisset et al. (2003) of 40 mg of mustard seasoning (equivalent to 13.5 mg mustard seeds and 0.8 mg of protein) in one child. Most of the case reports indicate that the food eliciting the allergic reactions was mustard sauce or mustard hidden in other sauces, such as chicken dip, mayonnaise and salad dressing. Only one case report (Kanny et al., 1995) estimated the concentration of mustard in the dip responsible for causing the reaction as 0.15 mg of mustard in 100 mg of dip. Other case reports only indicated that the amount of mustard associated with the allergic response was small or present in trace amounts which included reports of cross-contamination of fast food, a hidden source of mustard in the glaze of a ham and, in one case, the smell of mustard (Malet et al., 1993).

The second criterion of the Canadian adopted JECFA recommendations calls for reports of severe systemic reactions following the exposure to foodstuff. Risk of severe reactions and anaphylaxis appear to be higher in adults than in children based on the results of the DBPCFC (Morisset et al., 2003; Figueroa et al., 2005). Anaphylactic reactions are reported in 2% of children (Rancé and Dutau, 1997; Rancé et al., 1998; Rancé et al., 1999a & 1999b) and in up to 48% of adults with a confirmed mustard allergy (Caballeros et al., 2002). In a Canadian case report, 2 out of 5 children described had severe reactions to the ingestion of mustard, which required emergency medical intervention. These cases were not confirmed by an oral challenge with mustard due to the high risk of another severe reaction. Of the 13 international case reports, describing 22 individual cases of allergic reactions to mustard, 15 individuals reported anaphylactic-type reactions that required emergency medical intervention. Other severe reactions described in case reports included: laryngeal edema; generalized urticaria and bronchial asthma.

The third and last criterion of the Canadian adopted JECFA recommendations requires the assessment of all available Canadian prevalence data in children and adults, supported by appropriate clinical studies or alternatively available data from other countries. Prevalence data are not available for Canada or for many other regions of the world.
world. However, mustard allergy could be estimated as the 3rd/4th most common food allergy among children in France (Rancé et al., 1998; Rancé et al., 1999a & 1999b; Rancé and Dutau, 1997; Rancé and Dutau, 1998; Rancé and Dutau, 2002) and is probably the most common allergy among spices (Rancé et al., 1994; Niinimäki et al., 1989; Niinimäki et al., 1995). These European data are supported by the current database as previously discussed under criterion 1 and 2. Furthermore, mustard is affirmed on the most recent list of 14 allergens to be declared on labels (updated in 2007) by the European Commission (EC, 2007) and mustard is recognized as an allergen by the International Union of Immunological Societies (IUIS, 2009).

Other notable observations from the current database include the occurrence of mustard allergy symptoms beginning in subjects under the age of 3 years. In one trial, this age group represented up to 53% of the subjects (Rancé et al., 2000; Rancé et al., 2001) and in another study (Niinimäki et al., 1989), positive SPT results were reported for 3 children, ages 1 to 1.5 years old, who were breastfed for 11 months and had never orally ingested mustard. Several hypotheses are discussed in the literature as to a possible explanation for the early onset of mustard allergies. Suggestions include: sensitization in utero, during lactation, or the presence of mustard in baby foods. This issue will require further investigation. Furthermore, there is also supporting evidence of cross-sensitization with pollen and other aero-allergens. Figueroa et al., (2005) reported 83% of adult cases had primary respiratory sensitization (mustard dust exposure or cross-reactivity to aero-allergens). It is apparent that sensitization and reactions to mustard can be elicited via cross-sensitization with other aero-allergens or through contact or inhalation of mustard dust in areas where mustard is cultivated. This is important for Canadian consumers because Canada is a major producer of mustard seeds.

In addition to the Canadian adopted JECFA recommendations (CAC, 1999) for the introduction of food to the Codex priority list of food allergens by individual countries, the proposed amendments to the Canadian Food and Drug Regulations define food allergens with an emphasis on the protein portion of the food being responsible for eliciting allergic reactions. The major allergenic proteins in mustard have been identified and characterized. Sin a 1 is the seed storage protein in yellow mustard associated with allergic reactions. It is resistant to degradation by heat and digestive enzymes and interacts with membrane lipids (González de la Peñe et al., 1996; Monsalve et al., 1993, Oñaderra et al., 1994). These characteristics suggest that the allergenic proteins in mustard can remain intact throughout food processing and digestion which would elicit an allergic reaction in a susceptible individual. Furthermore, the seed storage proteins found in mustard have also been isolated from Brazil nuts, walnuts and sesame seeds, which are foods currently defined as priority food allergens in Canada (Bush and Helfe, 1996, Menéndez-Aris et al., 1988; Teuber et al., 1998). Similar structural features of Sin a 1 with proteins characterized in other types of mustard indicate that individuals who are sensitive to one species of mustard are likely to show sensitivity to other species.
Prepared mustard is commonly used as a condiment and mustard seeds and powder are becoming increasingly used in cooking and in processed and pre-packaged foods as a seasoning or flavouring agent, emulsifier, and water binding agent for texture control (AAFC, 2009a). Current Canadian Food and Drug Regulations exempt components of certain ingredients, preparations and mixtures from declaration in the list of ingredients on food packages, which may result in, as in the case of mustard, pre-packaged products with undeclared sources of mustard. Since food allergic consumers must rely on information provided on food labels in order to avoid foods that contain the ingredients to which they are likely to react, Health Canada has proposed regulatory amendments to identify these potential hidden sources of food allergens. In accordance with the proposed regulatory amendments, Health Canada would require the declaration of the mustard on the label of pre-packaged food products, either in the list of ingredients or in a statement beginning with the words “Allergy and Intolerance Information – Contains:” when mustard protein is present in the pre-packaged food product.

Conclusions

An assessment of the current mustard allergenicity database provides: international data supporting a credible cause-effect relationship; reports of severe systemic reactions including anaphylaxis following exposure to very small amounts of mustard within foodstuff; evidence that mustard allergy is common in some regions of Europe and has been affirmed on the European Commission's list of priority allergens; Canadian case reports supporting the occurrence of mustard food allergies in children and adults in Canada; evidence that all three types of mustard seed are available in Canada and mustard is used in cooking and in processed and pre-packed foods; results of characterization studies indicating that the allergenic proteins in mustard are resistant to degradation by heat and digestive enzymes and thus likely to withstand food processing; information that mustard is used in food processes that can result in “hidden” sources of food allergen; and, evidence that individuals known to be sensitive to allergenic proteins from one type of mustard are likely sensitive to other types. Additional factors which make mustard allergy relevant to the Canadian scenario include the potential cross reactivity between mustard and rape seed and the facts that Canada is a major producer of both these crops and sensitization to mustard can be acquired through dermal and respiratory exposure.

This scientific evidence fulfils the Canadian criteria required to add new allergens to the list of priority allergens.
Recommendations

Based on the conclusions of this report, it is recommended that mustard be added to the Canadian list of priority food allergens and that the proposed amendments to the *Food and Drug Regulations* relating to the labelling of food allergens be applied accordingly.
References


Menéndez-Arias, L., Dominguez, J., Moneo, I., and Rodriguez, R. (1990). Epitope mapping of the major allergen from yellow mustard seeds, Sin a I. Molecular Immunology. 27(2) 143-150


<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>Study Design Details</th>
<th>Subjects</th>
<th>Clinical History</th>
<th>Symptoms &amp; Signs (before challenge)</th>
<th>Symptoms &amp; Signs 1 Severity of Rxn 2 after Challenge</th>
<th>Diagnostic Tests 3</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Prevalence</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figueroa et al., 2005</td>
<td>Prospective Questionnaire &amp; CH Increasing Dose: 80, 240, 800, 2400, 6480 mg w/ 15 min interval until symptoms appeared or cumulative max dose of 10g Mus Followed by an open arm up to 25g Mus 24/38 (83%) CH w/ Mus 14/38 (37%) excluded because SEV of symptoms or did not agree to enter the food CH</td>
<td>38 SUB</td>
<td>Hx of A in 11% of SUB (exclude from CH) Age: 5/38 ≤ 14y Average: 21.9±8.6y Sex: 20F:18M Hx of atopy in 92% of SUB Hx of primary Res Sen in 83% of SUB</td>
<td>A: 11% EIA: 3% OAS: 47% U/AE 42%</td>
<td>Positive Rxn 14/24 (58%) Type of Rxn: OAS 10/14 (71%) SEV: mild AE+BA 1/14 (7%) SEV: mod A 1/14 (7%) in a SUB w/ out previous Hx of A SEV: sev</td>
<td>SPT to a panel of Aero-allergens and food extracts IgE to mugwort pollen, mustard, cabagge, broccoli etc.</td>
<td>Mean cumulative dose (until reaction appeared or max dose reached): 891.4 ±855.2 mg of Mus sauce equivalent to 124.8 ±119.7 mg of Mus Eliciting dose in most severe cases: 156.8mg of Mus sauce lowest dose eliciting a reaction was 44.8mg of Mus sauce</td>
<td>Mus sauce Meta-bisulfite free*</td>
<td>58% of SUB positive for Mus allergy</td>
<td>Cross-Rxn w/ mugwort pollen: 97% SUB Other food Sen 42% SUB Sen to Brassicaceae 100% SUB Assoc. EIA 2% SUB</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hc: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/ w/: w/; (y) years old.  
1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: Systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).  
2 Severity of reaction: Refer to Methods (Brown, 2004).  
* To avoid reactions due to sulphite intolerance.
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<th>Symptoms &amp; Signs(^1) (before challenge)</th>
<th>Symptoms &amp; Signs(^1) &amp; Severity of Rxn(^2) after Challenge</th>
<th>Diagnostic Tests(^3)</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Prevalence</th>
<th>Comment</th>
</tr>
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<tbody>
<tr>
<td>Morisset et al., 2003 France</td>
<td>Doses 10, 30, 100, 300, 900 every 20 min with a cumulative dose of 1340 mg Mus seasoning amount selected based on routine consumption</td>
<td>30 SUB Ch:28/30 A: 2/30 Age: 3-20 y Sex: 11F:19M</td>
<td>Hx of Rxn to ingestion of Mus Screened for Mus allergy by PST and IgE.</td>
<td>U,AE,AD BA, abdominal pain, diarrhea.</td>
<td>Positive Rxn 7/30 (23%) Type of Rxn: SK e.g., pruritus, erythema 5/7 (72%) SEV: mild GI/Res e.g.: abdominal pain, diarrhea, sneezing, wheezing 4/7 (57%) SEV: mod</td>
<td>SPT to Mus seed, Mus flour and metabisulfite-free Mus Mustard specific IgE</td>
<td>Lowest dose inducing symptoms: 1 Ch 40 mg Mus (0.8 mg of protein). Subject Sen by mustard pollen and rape pollen Another Ch 440 mg Mus</td>
<td>Mus seasoning (B. juncea seed) containing 34% Mus seed and 6% Mus protein Metabisulfite-free*</td>
<td>23% SUB positive for Mus allergy</td>
<td>SEV of certain Rxns argues for an informative labelling, Mus often masked allergen in many manufacture sauces</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hc: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: Systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).

2 Severity of reaction: Refer to Methods (Brown, 2004).

3 Diagnostic Tests: EUSA: enzyme-linked immunosorbent as say; IgE: serum immunoglobulin E; RAST: RadioAllergoSorbent Test; SPT: skin prick test.

* To avoid reactions due to sulphite intolerance.
### Table 1: Pivotal Clinical Studies (detailed summary in Appendix 2)

#### 2. Single-Blind, Placebo-Controlled Food Challenge (SBPCFC)

a. Non-randomized

<table>
<thead>
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<tr>
<td>Rancé et al., 2000 &amp; 2001 France</td>
<td>SUB selected for by positive Mus SPT Compared SUB to 22 controls w/out Hx of food allergy Increasing doses: 1.5, 10, 20, 50, 100, 250 and 500 mg Mus</td>
<td>36 Ch Age: 10 m–15 y Ave. 5.5y Sex: 22M:14F</td>
<td>15/30 ch w/ previous hx of food allergy Family Hx of atopy 81% 8/15 SUB (53.3%) exhibited Rxn to Mus under the age of 3 years</td>
<td>Of the 54 initial clinical features: AD 52% U/AE 37% BA 9% laryngeal edema + OAS + C 2%</td>
<td>Mustard allergy confirmed in 15/36 (42%) Most common reaction: U 14/15 (93%) SEV: mild</td>
<td>SPT IgE</td>
<td>1 to 936 mg Mus powder Mean cumulative dose: 153 mg Mus powder</td>
<td>Mus powder</td>
<td>42% SUB positive for Mus allergy Symptoms started ≤3 y of age in 53% of the subjects</td>
<td>67 % SUB were also allergic to other foods-peanuts, eggs &amp; milk Possible Sen in utero or lactation Mustard in baby food</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hc: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EIA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: Systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).

2 Severity of reaction: Refer to Methods (Brown, 2004).


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</tr>
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<tbody>
<tr>
<td>Niinimäki et al., 1989 Finland</td>
<td>Open study assessing allergy to spices including Mus using SPT and RAST</td>
<td>50 SUB</td>
<td>Hx allergy to spices &amp; birch pollen 64%</td>
<td>Gastric pain</td>
<td>Positive Rxn to SPT 29/50 (58%)</td>
<td>SPT</td>
<td>0.648 g Mus dissolved in glycerol &amp; saline to make 5% (w/v) test solution</td>
<td>Commercial powdered Mus</td>
<td>58% SUB positive for Mus by either one or both SPT/RAST</td>
<td>40/50 (80%) SUB positive SPT &amp; RAST to birch pollen</td>
</tr>
<tr>
<td>Rancé et al., 1994 France</td>
<td>Open study assessing allergy to spices including Mus using SPT and IgE</td>
<td>83 SUB (Ch) tested for allergy to spices</td>
<td>Hx of pollen and food allergy</td>
<td>Chronic U or recurrent AE/E</td>
<td>Positive SPT for Mus in 23/83 SUB (28%)</td>
<td>SPT IgE</td>
<td>Not reported</td>
<td>Commercial extract</td>
<td>39/83 SUB (46%) positive (SPT &amp; IgE) allergy to spices</td>
<td>23/39 SUB (59%) positive allergy to mustard 7/23 SUB (30%) confirmed by LFC or OFC Pollen allergy existed in 56% of Ch allergic to spices</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Ren: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).

2 Severity of reaction: Refer to Methods (Brown, 2004).

### Table 2: Non-Pivotal Clinical Studies

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<thead>
<tr>
<th>Author, Year, Country</th>
<th>Study Design Details</th>
<th>Subjects</th>
<th>Clinical History</th>
<th>Symptoms &amp; Signs&lt;sup&gt;1&lt;/sup&gt; (before challenge)</th>
<th>Symptoms &amp; Signs&lt;sup&gt;1&lt;/sup&gt; Severity of Rxn&lt;sup&gt;2&lt;/sup&gt; after Challenge</th>
<th>Diagnostic Tests&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Prevalence</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Niinimäki et al., 1995 Finland</td>
<td>Open study assessing allergy to spices including Mus using SPT, RAST: IgE at 2m and 2.9y intervals</td>
<td>49 SUB</td>
<td>Age: 1 to 51y; Ave: 16.5y Sex: 23M: 26F</td>
<td>Hx allergy to spices &amp; birch pollen Hx of atopy</td>
<td>Atopic dermatitis w/ Res symptoms 57% Chronic E</td>
<td>Positive Rxn to Mus specific IgE 22/31 (71%) SUB w/ positive SPT</td>
<td>SPT RAST: Total IgE Mus specific IgE</td>
<td>4 mg powdered spice and 50 μL of saline on the skin</td>
<td>Native Mus (Sinapis alba &amp; Brassica nigra)</td>
<td>31/49 (63%) SUB positive SPT 22/31 (71%) SUB positive for Mus allergy 22/49 (45%) SUB positive for Mus allergy</td>
</tr>
<tr>
<td>Rancé &amp; Dutau, 1997 France</td>
<td>Open allergy study assessing over 25 allergens including Mus using SPT, IgE LFC and SBPCFC (when LFC negative)</td>
<td>142 SUB (Ch)</td>
<td>Age: 7m to 15y; Ave: 4.5 y Sex: 95M:47F</td>
<td>Hx of food allergy</td>
<td>Multiple presenting symptoms in 66% Ch AD 61% Rash 32% AE 25% BA 24% A 4%</td>
<td>Positive for Mus allergy 23/142 (16%) 16/23 in LCF 7/23 in SBPCFC</td>
<td>SPT IgE</td>
<td>1 mg to 5 g for all allergens tested</td>
<td>extracted from local food</td>
<td>23/202 SUB (11%) positive for Mus allergy</td>
</tr>
</tbody>
</table>

**Abbreviations:** Adult: Ad; Challenge: Ch; Children: CH; Challenge: CH; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1. Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).
2. Severity of reaction: Refer to Methods (Brown, 2004).
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<tbody>
<tr>
<td>Rancé &amp; Dutau, 1998 France</td>
<td>Open allergy study assessing foodstuffs including Mus using SPT, IgE and open LFC</td>
<td>45 Ch</td>
<td></td>
<td>Family Hx of atopy: 78% SUB</td>
<td>U 30% AE 26% E 20% BA 10% A 2%</td>
<td>Positive allergy to mustard 12% SUB</td>
<td>SPT IgE</td>
<td>Mean dose by OFC 900mg (1mg to 10gr)</td>
<td>Details not provided</td>
<td>Positive allergy to Mus 12% SUB</td>
</tr>
<tr>
<td>Rancé and Dutau, 2002 France</td>
<td>Open allergy study assessing foodstuffs including Mus documented by DBPCFC in BA patients identified by pulmonary function</td>
<td>163 Ch</td>
<td></td>
<td>Family Hx atopy 91% Hx of ≥ 1 food allergies BA for ave. 5.5 y</td>
<td>Asthma induced by food allergens potentially severe. A 6%</td>
<td>SPT Ig E</td>
<td>Details not provided</td>
<td>Various food extracts</td>
<td>Positive allergy to Mus 7% SUB</td>
<td>Prevalence of asthma induced by food allergen: 10%</td>
</tr>
</tbody>
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Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hc: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rec: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EA: exercise induced anaphylaxis; GE: generalized eczema; Gl: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: Systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).

2 Severity of reaction: Refer to Methods (Brown, 2004).

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<th>Prevalence</th>
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<tr>
<td>André et al., 1994, France</td>
<td>Retrospective Analysis 9 y period investigating foodstuff most frequently associated with A Rxn</td>
<td>580 SUB 480 Ad 100 Ch</td>
<td>Hx of adverse Rxn to food</td>
<td>60/580 SUB Hx severe Rxn to food A 52/60 AE 6/60 Broncho-spasm 2/60</td>
<td>Not applicable to study design</td>
<td>SPT IgE</td>
<td>Details not provided</td>
<td>Mustard</td>
<td>3% of SUB positive for ser Rxn to Mus</td>
<td>An increase in the frequency of sen (1%) to Mus was noted</td>
</tr>
<tr>
<td>Rancé et al., 1998 &amp; 1999 a, b France</td>
<td>Prospective prevalence study of food allergy validated by SPT/IgE/LFC</td>
<td>Ch 544 Age 0-15 Sex 343M: 201F</td>
<td>Hx food allergy confirmed by food challenge Family Hx atopia 71%</td>
<td>AD 275/544 (51%) U/AE 165/544 (30%) BA 47/544 (9%) A 27/544 (5%)</td>
<td>49/544 (9%) positive Rxn to Mus AD 21/49 (43%) U/AE 21/49 (43%) BA 2/49 (4%) A 1/49 (sev) (2%) GI 1/49 (2%) OAS 1/49 (2%)</td>
<td>SPT IgE</td>
<td>0.1–10g for all allergens tested Dose specific to mustard: not reported</td>
<td>Test substances extracted from local food</td>
<td>49/544 (9%) SUB positive for Mus allergy</td>
<td>Mus 4(^{th}) most common allergy identified within a population with multiple food allergy</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; Ch: challenge; Ch: children; DBPCFC: double-blind placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

1 Symptoms and signs: A: anaphylaxis; AD: atopic dermatitis; AE: angioedema; BA: bronchial asthma; C: conjunctivitis; CV: cardiovascular; E: eczema; EIA: exercise induced anaphylaxis; GE: generalized eczema; GI: gastrointestinal; OAS: oral allergy syndrome; R: rhinitis; SK: skin; SR: Systemic reaction; U: urticaria (Refer to appendix 3 for a glossary of terms).

2 Severity of reaction: Refer to Methods (Brown, 2004).

Table 3: Other Relevant Studies

<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>Study Design Details</th>
<th>Subjects</th>
<th>Clinical History</th>
<th>Symptoms &amp; Signs(^1) (before challenge)</th>
<th>Symptoms &amp; Signs(^1) Severity of Rxn(^2)</th>
<th>Diagnostic Tests(^3)</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Prevalence</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caballeros et al., 2002 Spain</td>
<td>Cohort</td>
<td>29 SUB Hx Mus allergy Age: 15-58y mean 27.3±10y Sex: 10M:19F</td>
<td>Family Hx of atopy 50% SUB allergy to other vegetables 17% allergy to non-vegetables 52% symptoms of pollinosis</td>
<td>Mus allergy confirmed by SPT and IgE</td>
<td>19/29 (65%) SUB systemic Rxn 14/19 (74%) A AE 16/29 Dyspnea 11/29 U 10/29 OAS 8/29 Most common symptom U &amp; AE SEV: mild-sev</td>
<td>SPT total IgE Mus specific IgE</td>
<td>Details not provided Trace amounts reported to elicit Rxn</td>
<td>Mus extract of B. nigra at 1:10 w/v</td>
<td>Not applicable study population 100% positive allergic Rxn to Mus</td>
<td>Mustard seed allergens are resistant to digestion and high temperature No challenge Study due to risk of severe Rxn</td>
</tr>
</tbody>
</table>

Abbreviations: Ad: adult; CH: challenge; Ch: children; DBPCFC: double-blind placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

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2 Severity of reaction: Refer to Methods (Brown, 2004).

### Table 4: Case Reports

#### 1. Canadian Reports

<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>Cases</th>
<th>Clinical History</th>
<th>Symptoms &amp; Signs&lt;sup&gt;1&lt;/sup&gt; Severity of Rxn&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Diagnostic Tests&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yip and Zimmerman, 1999 Canada</td>
<td>5 children</td>
<td>Case 1: Atopic boy w/ Hx of multiple food allergies since 18 month (sesame seed, fish)</td>
<td>Case 1: 3 episodes of severe Rxn requiring emergency medical treatment AE and at least one episode of airway obstruction</td>
<td>SPT positive for several Mus preparations</td>
<td>Details not provided</td>
<td>Mustard sauce Salad dressing glazed ham</td>
<td>2/5 Cases Severe 2/5 moderate 1/5 mild. One case due to hidden source of mustard in the glazing of a prepared ham &amp; one case in fast food</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 2: Atopic boy w/ Hx of sen to kiwi, peanut</td>
<td>Case 2: 3 episodes of U immediate after exposure to mustard. No mention of emergency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 3: boy w/ Hx of BA and multiple food allergies (eggs, sesame, peanuts)</td>
<td>Case 3: at least one episode of Ax reaction to fast food (mustard and sesame)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 4: Atopic girl w/ E since infancy; BA; Allergy to milk, egg and peanut</td>
<td>Case 4: U and wheezing following ingestion of mustard. No indication of emergency visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 5: boy w/ Hx BA and multiple food allergies</td>
<td>Case 5: vomiting, swelling of the lips No indication of emergency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connors et al., 2006 Canada</td>
<td>1 female</td>
<td>No detailed Hx provided Hx of A type Rxn to Mus</td>
<td>Symptoms of A after ingestion of Mus</td>
<td>SPT</td>
<td>Details not provided</td>
<td>Fast food Mustard sauce</td>
<td>Very limited info provided</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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2 Severity of reaction: Refer to Methods (Brown, 2004).

### Table 4: Case Reports

#### 2. International Reports

<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>Cases</th>
<th>Clinical History</th>
<th>Symptoms &amp; Signs(^1) Severity of Rxn(^2)</th>
<th>Diagnostic Tests(^3)</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paconesi et al., 1980 Italy</td>
<td>1 male</td>
<td>Hospitalization twice in 1 year. Acute giant U with edema of the glottis after eating pizza</td>
<td>Skin test to Mus antigen extracted from black and white Mus caused intense wheal reaction followed by shock and glottic edema.</td>
<td>SPT IgE: RAST</td>
<td>Small amount Mus contaminating the pizza</td>
<td>Mustard not used in preparation of the pizza reaction to cross-contamination with Mus</td>
<td></td>
</tr>
<tr>
<td>Meding, 1985 Sweden</td>
<td>1 female</td>
<td>Atopy Rxn to Mus</td>
<td></td>
<td>SPT black &amp; white mustard, rape seed &amp; others Cruciferae</td>
<td></td>
<td></td>
<td>Negative SPT for allylisothiocyanate Positive SPT for Mus, rape seeds</td>
</tr>
<tr>
<td>Widstrom and Johansson, 1986 Sweden</td>
<td>1 female</td>
<td>Hx of allergy to egg and fish in childhood that cleared.</td>
<td>Acute Rxn to Mus/mayo U&amp;AE on face &amp; neck</td>
<td>IgE: RAST positive for white &amp; black Mus &amp; rape seed</td>
<td>One episode of possible cross-contamination of fast food</td>
<td>Fast food and mayonnaise</td>
<td></td>
</tr>
<tr>
<td>Vidal et al., 1991 Spain</td>
<td>2 females</td>
<td>Case 1: Hx pollen allergy Case 2: Hx of A and pollen allergy</td>
<td>Case 1: Sev U &amp; facial AE w/GI &amp; Res symptoms Case 2: U, facial &amp; throat edema &amp; chest tightness</td>
<td>Positive STP, Total serum IgE, Specific M IgE</td>
<td></td>
<td>Mayonnaise and Mus on sandwich and in a salad</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** A: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

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2. Severity of reaction: Refer to Methods (Brown, 2004).
### Table 4: Case Reports

#### 1. International Reports

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<th>Diagnostic Tests</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monreal et al., 1992, Spain</td>
<td>1 male &amp; 1 female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td>Case 1: family Hx of atopy, personal Hx of IgE dependent BA, allergy to molds and grasses, reoccurrent U/AE since age 5</td>
<td></td>
<td></td>
<td></td>
<td>Both reported as small amount</td>
<td>Both Mustard sauce</td>
</tr>
<tr>
<td>Case 1: 17y M</td>
<td></td>
<td>Case 2: family Hx of atopy, personal Hx of BA service</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2: 14y F</td>
<td></td>
<td>Case 1: Rxn to Mus edema lips/tongue, Dysphagia, Upper Res symptoms Required emergency service</td>
<td></td>
<td>Case 1: STP w/ Mus, aero-allergens positive Total IgE and specific IgE by RAST positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 2: 1 hr after physical exercise and ingestion of Mus; edema tongue, lips face, U, upper Res distress Required emergency service</td>
<td></td>
<td>Case 2: STP for Mus positive Total IgE and specific IgE by RAST positive</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malet et al., 1993, Spain</td>
<td>2 males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td>Case 1: Hypersensitivity to pollen, seasonal R</td>
<td></td>
<td>Case 1: AE of face, U, Dyspnea, after oral &amp; nasal exposure to Mus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1: 31 y</td>
<td></td>
<td>Case 2: No reported Hx of other allergies, Contact Hypersensitivity to Mus since age 10</td>
<td></td>
<td>Case 2: U, AE, pruritus, BA after accidental ingestion of Mus, requiring hospital treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2: 32 y</td>
<td></td>
<td>Case 1: AE of face, U, Dyspnea, after oral &amp; nasal exposure to Mus</td>
<td></td>
<td>Case 1: SPT, total IgE high, Specific IgE by RAST positive to Mus seed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 2: U, AE, pruritus, BA after accidental ingestion of Mus, requiring hospital treatment</td>
<td></td>
<td>Case 2: SPT, total IgE high, specific IgE by RAST to Mus seed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A: adult; CH: challenge; Ch: children; DBPCFC: double-blind placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old.

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2. Severity of reaction: Refer to Methods (Brown, 2004).

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<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>Cases</th>
<th>Clinical History</th>
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<th>Diagnostic Tests</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorro et al., 1995 Spain</td>
<td>2 male &amp; 1 female</td>
<td>Case 1: 43y M, Case 2: 17y F, Case 3: 19y M</td>
<td>Case 1: Hx of severe Rxn to Mus &amp; AE to shellfish, Case 2: Hx of BA, R, U, Case 3: Hx of R</td>
<td>All cases positive SPT &amp; IgE to Mus</td>
<td>Case 1 &amp; 3: Mus sauce, Case 2: Mus in salad</td>
<td>No oral challenge to Mus due to severity of Rxn, Oral challenge to others Cruciferae negative</td>
<td></td>
</tr>
<tr>
<td>Valero et al., 1995 Spain</td>
<td>3 male &amp; 2 female</td>
<td>Case 1: 34y F, Case 2: 31y M, Case 3: 25y M, Case 4: 52y F, Case 5: 33y M</td>
<td>4 cases w/ Hx of Aero-allergy, 3 cases w/ Hx of food allergies, 2 cases w/ Hx of reaction when present in areas where Mus was manipulated</td>
<td>All 5 cases positive to Mus by SPT &amp; IgE, 18.4% of the 86 subjects tested by SPT were positive to Mus</td>
<td>Mus sauce</td>
<td>The two cases described earlier in Malet et al., (1993) appear to be included in this case report</td>
<td></td>
</tr>
<tr>
<td>Kanny et al., 1995 France</td>
<td>1 female</td>
<td>Age: 38 y</td>
<td>Hx allergy to mustard, Hx of exposure to Mus powder in pharmacy where she worked</td>
<td>Positive SPT/ IgE: RAST</td>
<td>Chicken dip Hidden source of Mus allergen</td>
<td>Mus is the most allergenic of the commonly used spices, it is able to induce severe A in very small amounts</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A: adult; CH: challenge; Ch: children; DBPCFC: double-blind, placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind, placebo-controlled food challenge; SUB: subjects; w/: with; (y) years old

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2. Severity of reaction: Refer to Methods (Brown, 2004)

# Table 4: Case Reports

## 1. International Reports

<table>
<thead>
<tr>
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<th>Diagnostic Tests&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Eliciting Dose</th>
<th>Eliciting Allergen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frémont et al., 1996 France</td>
<td>1 female</td>
<td>Age: 22 m</td>
<td>Family Hx atopy Hx of Celiac Disease &amp; allergy to milk at age 11 month</td>
<td>Failure to thrive Diarrhea Vomiting Coughing fits AE</td>
<td>SPT/IgE positive for Mus, although the culprit food in this case was not Mus</td>
<td>Milk proteins in flour</td>
<td>Emphasis on Hidden allergens Case demonstrates Sen to Mus in young baby</td>
</tr>
<tr>
<td>Asero et al., 2002 Italy</td>
<td>1 male</td>
<td>Age: 54 y</td>
<td>Hx of A Rxn after ingestion of sunflower seed in bread</td>
<td>STP positive to Sunflower seeds and Mus IgE specific positive Cross-reactivity of Mus and sunflower seeds</td>
<td></td>
<td>Sunflower seeds in bread</td>
<td>Objective of the report was to demonstrate the Cross-reactivity of Sunflower seeds and Mus</td>
</tr>
<tr>
<td>Lingelbach et al., 2003 Germany</td>
<td>1 female</td>
<td>Age: 40 y</td>
<td>Hx of food and pollen allergies 11 years Hx of EIA</td>
<td>At least two episodes of EIA requiring emergency medical assistance.</td>
<td>STP, Specific IgE (RAST)</td>
<td></td>
<td>Fast food EIA associated to Mus in food Patient Rejected Oral challenge</td>
</tr>
<tr>
<td>Aygencel et al., 2007 Turkey</td>
<td>1 female</td>
<td>Age: 53 y</td>
<td>AE of tongue and oropharynx, Requiring hospital Emergency assistance</td>
<td></td>
<td></td>
<td>Wild mustard: raw leaves of Brassica (Sinapis) Arvensis in salad</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A: adult; CH: challenge; Ch: children; DBPCFC: double-blind placebo-controlled food challenge; F: female; Hx: history; LFC: labial food challenge; M: male; max: maximum; min: minute; mod: moderate; Mus: mustard; OC: occupational; OFC: oral food challenge; Res: respiratory; Rxn: reaction; Sen: Sensitization; SEV: severity; sev: severe; SBPCFC: single-blind placebo-controlled food challenge; SUB: subjects w/; with; (y) years old.

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2 Severity of reaction: Refer to Methods (Brown, 2004).

Appendix 1: Mustard Allergy: Search Strategies (MEDLINE, EMBASE & FSTA)

Databases searched:
- Ovid Medline (R) In-Process & Other Non-Indexed Citations and Ovid Medline (R) 1950 to Present
- Ovid Medline (R) In-Process & Other Non-Indexed Citations – November 18, 2008
- Ovid Embase 1980 to 2008 Week 46
- FSTA Direct (Last updated 17 Nov 2008)

Records exported to Reference Manager (to main database: ‘mustard_allergy’):
- Medline = 139 + 13 = 152
- Embase = 178
- FSTA = 28
- TOTAL = 358 references

Search Strategies
1. Mustard Plant/
2. Sinapis/
3. (mustard* or sinapis).tw.
4. (brassica adj3 (alba or juncea or nigra)).tw.
5. or/1-4
6. exp Hypersensitivity/
7. exp Urticaria/
8. (allerg* or hypersensi* or intoleran* or anaphyla* or urticaria* or hive*).tw.
9. ((sensiti* or toleran*) adj3 (mustard* or sinapis or brassica alba or brassica juncea or brassica nigra)).tw.
10. (food adj2 (challenge or rechallenge)).tw.
11. or/6-10
12. 5 and 11
13. limit 12 to humans

OVID MEDLINE (R) In-Process & Other Non-Indexed Citations – November 18, 2008
1. Mustard Plant/
2. Sinapis/
3. (mustard* or sinapis).tw.
4. (brassica adj3 (alba or juncea or nigra)).tw.
5. or/1-4
6. exp Hypersensitivity/
7. exp Urticaria/
8. (allerg* or hypersensi* or intoleran* or anaphyla* or urticaria* or hive*).tw.
9. ((sensiti* or toleran*) adj3 (mustard* or sinapis or brassica alba or brassica juncea or brassica nigra)).tw.
10. (food adj2 (challenge or rechallenge)).tw.
11. or/6-10
12. 5 and 11

EMBASE
1. Brassica/
2. Brassica Extract/
3. sinapis/
4. (mustard* or sinapis).tw.
5. (brassica adj3 (alba or juncea or nigra)).tw.
6. or/1-5
7. exp Hypersensitivity/
8. exp Urticaria/
9. (allerg* or hypersensi* or intoleran* or anaphyla* or urticaria* or hive*).tw.
10. ((sensiti* or toleran*) adj3 (mustard* or sinapis or brassica alba or brassica juncea or brassica nigra)).tw.
11. (food adj2 (challenge or rechallenge)).tw.
12. or/7-11
13. 6 and 12
14. limit 13 to human
15. limit 14 to yr="1988–2009"

FSTA
((KE = ‘MUSTARD’ OR KE = ‘MUSTARD GREENS’ OR KE = ‘MUSTARD SEED OILS’ OR KE = ‘MUSTARD SEEDS’) OR (CONTAINS(AF,’mustard* or sinapis or “brassica alba” or “brassica juncea” or “brassica nigra”’))) AND ((KE = ‘ALLERGIES’ OR KE = ‘INTOLERANCE’ OR KE = ‘ANAPHYLAXIS’ OR KE = ‘URTICARIA’) OR (CONTAINS(AF,’allerg* or hypersensi* or intoleran* or anaphyla* or urticaria* or hive*’)) OR (CONTAINS(AF,”food challenge*” or “food rechallenge”’)))
PY: 1988-now
Appendix 2: Detailed Summaries of Pivotal Studies for the determination of the Allergenicity of Mustard (Table 1)

1. Double-Blind, Placebo-Controlled Food Challenge (DBPCFC)

   a. Randomized

   Figueroa et al. (2005) conducted a prospective, randomized, double-blind, placebo-controlled, food challenge (DBPCFC) study in 38 subjects (age: 21.9 ± 8.6 years old) who had a reported history of allergic reactions to mustard. The control group consisted of subjects paired for age and sex, who had dust-mite allergies. The study included a clinical questionnaire, skin prick tests (SPT) with panels of aero-allergens and foods, serum extraction for in vitro tests, analysis of total IgE and specific IgE to mugwort pollen, mustard, cabbage, cauliflower, broccoli, and other foods and a double-blind placebo-controlled food challenge followed by an open challenge.

   A commercial yellow mustard sauce was masked in a natural yoghurt-based vehicle, containing a mixture of vanilla, lemon juices, sugar and yellow colouring. The mustard sauce was composed of water, S. alba seeds (14% w/v), vinegar, salt, turmeric, paprika and cloves, and the sauce was free of sulphites. Apart from mustard, all patients tolerated all of the ingredients in both the mustard sauce and the vehicle. Subjects were randomly assigned to either the mustard or placebo (vehicle) group.

   Subjects were challenged with increasing doses, 80, 240, 800, 2400 and 6480 mg of mustard sauce, during 15-min intervals until symptoms appeared or a cumulative dose of 10 g of mustard sauce was administered. Subjects who had negative results during the blind challenge were free to participate in an open feeding study of up to 25 g of mustard sauce. There was a 2-hour interval between the first (blind) and second (open) part of the oral challenges. A mustard allergy was considered positive if the subject had symptoms after a challenge with mustard and not after a challenge with the placebo.

   Of the 38 patients included in the study, 11% had a previous history of systemic anaphylaxis. Food challenges were not performed when a subject had a history of severe anaphylaxis to mustard (adverse reaction involving at least three target organs or with demonstrated vascular collapse). Fourteen patients did not undergo DBPCFC because of either severe symptoms (n = 4) or because of denial of consent (n = 10). Therefore, the DBPCFC was performed in 24 of the 38 patients. Of the 24 subjects who underwent the DBPCFC, 14 subjects (58%) were considered to have responded positive for a mustard allergy.

   Oral allergy syndrome (OAS) was the most frequent symptom observed (71%) among subjects, characterized by pruritus and mild angioedema of the lips, tongue, palate and throat, followed by a rapid resolution of symptoms. One patient showed angioedema and bronchial asthma after mustard ingestion and another subject reacted with systemic anaphylaxis. All patients completely recovered within 90 minutes after symptomatic treatment.

   The mean cumulative reactive dose of mustard sauce (±SD) was 891.4 ± 855.2 mg, equivalent to 124.8 ± 119.7 mg of mustard. Patients with positive outcomes showed significantly greater mustard SPT results than those with negative outcomes (8.2 ± 3.7 vs 5.3 ± 2.4 mm, P < 0.05). The receiver-operating characteristic (ROC) curve analysis yielded a cut-off value for commercial mustard SPT of 8 mm, with a specificity of 90% (95% CI, 55.5– 98.3) and a sensitivity of 50% (95% CI, 23.1–76.9).

   A significant association between mustard hypersensitivity and mugwort pollen sensitization was found (97% of patients) with a partial cross-reactivity demonstrated by in vitro inhibition assays. All patients showed sensitization to other members of Brassicaceae family and cross-reactivity among them was also confirmed. Moreover, significant
associations with nut (97%), leguminous (95%), corn (79%), and Rosaceae fruit (90%) sensitizations were also observed. Approximately 40% of these food sensitizations were symptomatic, including food-dependent exercise-induced anaphylaxis in six patients (2%).

Conclusions by the authors indicated that “mustard allergy is a not-uncommon disorder that can induce severe reactions” and that the significant associations with mugwort pollinosis and several plant derived food allergies suggests that a “new mustard–mugwort allergy syndrome” may exist. They also point out a relationship between this syndrome and food-dependent exercise-induced anaphylaxis.

b. Non-Randomized

Morisset et al. (2003) conducted the first DBPCFC prospective study to assess the allergenicity of mustard. Thirty subjects (28 children and two adults) were included in this study, subjects ages ranged from 3–20 years and 11 females and 19 males participated. Twenty-seven (27/30) subjects were screened for mustard-specific immunoglobulin E (IgE) using radioallergosorbent test (RAST). Twenty-four subjects participated in a DBPCFC and 6 subjects participated in a single-blind, placebo-controlled, food challenge (SBPCFC). A history of food allergies were suspected among the subjects based on a history of urticaria and angioedema, atopic dermatitis, asthma, abdominal pain, and episodes of diarrhea. For inclusion to the study, subjects were selected on the basis of a positive prick test (SPT) to ground mustard seeds (Brassica nigra), mustard flour (B. juncea), metabisulfite-free strong mustard seasoning (B. juncea) and a commercialized extract (B. nigra).

For the oral challenges mustard seasoning was used which contained 33% mustard seeds and was free of metabisulfite or other spices and flavoring. The taste of mustard was masked in very cold soft drinks and the soft drinks were used as the placebo. The amount of mustard seasoning tested was selected on the basis of routine consumption. Increasing doses of 10, 30, 100, 300, and 900 mg of mustard seasoning were administered every 20 minutes until a cumulative dose of 1340 mg of mustard seasoning was obtained. There was a 24-h interval between the administration of the mustard seasoning and the placebo.

Seven of the 30 subjects (23%) who participated in food challenges were considered positive. The mean skin prick test results in the positive and negative subjects were as follows: 5.5 mm vs 5.9 mm for the commercialized extract; 10.9 mm vs 5.8 mm for B. nigra ground seeds (P < 0.01), 9.9 mm vs 7.1 mm for B. juncea flour (n.s. P > 0.25) and 11.5 mm vs 9.1 mm for the metabisulfite-free mustard seasoning (n.s. P > 0.1). The mean specific IgE values determined by RAST were higher but not significantly different. The SPT conducted with different mustards showed increasing reactivity in the following order: 5.8 mm (1.5–15) for the commercialized extract, 6.9 mm (0.5–18) for B. nigra ground seeds, 7.9 mm (1–20) for B. juncea flour, and 9.7 mm (3–20) for the metabisulfite free strong mustard seasoning. The difference in mean diameters of wheals induced by SPT with the commercialized extract and the strong mustard seasoning were statistically significant (P < 0.005). The mean of mustard specific-IgE values was 8.7 KU/l (0.35–72.4).

The authors concluded that positive skin prick tests and the presence of specific IgE were not predictive of the mustard allergy and suggested that positive results from a SBPCFC or a DBPCFC should be required before recommending avoidance diets. They also noted that the seriousness of certain reactions argues for an informative labeling because mustard is often a masked in many manufactured sauces.
2. Single-Blind, Placebo-Controlled Food Challenge (SBPCFC)

a. Non-Randomized

Rancé et al. (2000 & 2001) investigated 36 children, 22 males and 14 females, aged 10 months to 15 years (average age 5.5 years) with a positive mustard SPT using an open or single-blind, placebo controlled food challenge (SBPCFC). The initial presenting clinical features among subjects were atopic dermatitis (52%), urticaria and/or angioedema (37%), and asthma (9%). In one subject laryngeal edema with oral allergy syndrome and rhinoconjunctivitis (2%) was observed. No anaphylaxis was reported among subjects. First-degree family atopy was reported for 29 of the 36 children (81%). Subjects were compared to 22 control subjects who did not have a history of food allergies.

Children were tested with increasing doses of 1, 5, 10, 20, 50, 100, 250, and 500 mg of mustard. The administration of the mustard and the placebo were separated by a 4-hour interval. The type, source or protein concentration of mustard used in the challenge was not specified. Symptoms that developed within a few minutes to 2 h after the last dose of the food challenge were considered for the diagnosis.

Fifteen children of 36 entered in the study (42%) were confirmed to be allergic to mustard (positive SBPCFC) and 21 children were non-allergic (negative SBPCFC). The cumulative reactive dose by open challenge or SBPCFC varied from 1 to 936 mg. No reactions to placebo were observed. The mean cumulative reactive dose was 153 mg. The reactions observed during the SBPCFC were urticaria (14 cases), rhinoconjunctivitis (three cases), angioedema (one case), oral allergy syndrome (one case), and eczema (one case). There was no significant difference in the food allergies and associated inhalant allergen sensitizations between the two groups. In the allergic group, the mean wheal diameter for mustard SPT was 8.8 mm and the median concentration of mustard serum (s) IgE 14.8 kU/l.

The authors noted that 8 of the 15 positive reactions to mustard (53%) reported a history of symptoms after mustard ingestion starting under 3 years of age. Authors suggest that sensitization to mustard may occur in utero or during lactation.
Appendix 3: Glossary of Medical Terms

The medical terms used throughout the document and tables are listed below alphabetically. The source of the definition is captured in the endnotes.

Anaphylaxis – A systemic allergic reaction that can be fatal within minutes, either through swelling that shuts off airways or through a dramatic drop in blood pressure. Contact with or ingestion of an allergen may set off a chain reaction in a person's immune system that may lead to swelling of the airways, loss of blood pressure, and loss of consciousness, resulting in anaphylactic shock. Some anaphylactic reactions involve only one organ system, such as the respiratory tract or skin. However, in anaphylaxis, several systems are usually affected simultaneously, including the upper and lower respiratory tracts, cardiovascular system, and gastrointestinal tract.¹

Asthma – A common disorder in which chronic inflammation of the bronchial tubes (bronchi) makes them swell, narrowing the airways. Asthma involves only the bronchial tubes and does not affect the air sacs (alveoli) or the lung tissue (the parenchyma of the lung) itself.¹

Atopic dermatitis – Dermatitis is an umbrella term for local inflammation of the skin; Atopic Dermatitis refers to the underlying inflammation being dominated by an IgE-antibody associated reaction.²

Atopy – A personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and develop typical symptoms such as asthma, rhinoconjunctivitis or eczema/dermatitis.²

Angioedema – Characterized by locally diffuse and painful soft-tissue swelling that may be asymmetric, especially on the eyelids, lips, face, and tongue but also on the back of hands or feet and on the genitals. Edema of the upper airways may cause respiratory distress. Complete airway obstruction may occur.³

Bronchial Asthma – Refer to the definition of asthma.

Bronchospasm – Spasmodic contraction of the muscular walls of the bronchial air passages to the lungs, as in asthma, which makes breathing difficult.⁴

Conjunctivitis – Inflammation of the mucous membrane lining the inner surface of the eyelids and covering the front part of the eyeball.⁴

Cardiovascular – The heart and the blood vessels as a unified body system.⁴

Diaphoresis – Perspiration, especially when profuse.⁴

Dyspnea – Shortness of breath.⁴

Eczema – A non-contiguous skin disorder, characterized by inflammation, itching, and the formation of scales.⁴

Erythema – An abnormal redness of the skin caused by various agents, as sunlight, drugs, etc., that irritate and congest the capillaries.⁴

Exercise induced Anaphylaxis – Exercise can induce an allergic reaction to food. The usual scenario is that of a person eating a specific food and then exercising. As the individual exercises and their body temperature increases, they begin to itch, gets lightheaded, and soon develops the characteristic allergic reactions of hives, asthma, abdominal symptoms, and even anaphylaxis. Refer to the definition of anaphylaxis or systemic reaction.¹
Glottis – The opening between the vocal cords in the larynx.

Gastrointestinal – The stomach and the intestines.

Hypotension – Abnormally low blood pressure.

Hypoxia – An abnormal condition resulting from a decrease in the oxygen supplied to or utilized by body tissue.

Incontinence – Inability to restrain a natural discharge of urine from the body.

Oral Allergy Syndrome – Oral allergy syndrome is a type of cross-reactivity. This syndrome occurs in people who are highly sensitive, for example, to ragweed or birch pollen. During the seasons that these allergens pollinate, the affected individual may find that when he or she tries to eat fruits, chiefly melons and apples, a rapid onset of itching is experienced in the mouth and throat, and the fruit cannot be eaten. The symptoms of this allergy, which is caused simply by the direct contact of the food with the lining of the mouth and throat, resolve rapidly.

Rhinitis – Hypersensitivity symptoms from the nose, eg, itching, sneezing, increased secretion, and blockage.

Rhinoconjunctivitis – Allergic conjunctivitis, is also called “rhinoconjunctivitis,” it is the most common allergic eye disorder. The condition is usually seasonal and is associated with hay fever. The main cause is pollens, although indoor allergens such as dust mites, molds, and dander from household pets such as cats and dogs may affect the eyes year-round. Typical complaints include itching, redness, tearing, burning, watery discharge, and eyelid swelling. To a large degree, the acute (initial) symptoms appear related to histamine release.

Stridor – A harsh, high-pitched whistling sound, produced in breathing by an obstruction in the bronchi, trachea, or larynx.

Syncope – The temporary loss of consciousness followed by the return to full wakefulness; fainting.

Systemic reactions – Several systems within the body are affected simultaneously, including the upper and lower respiratory tracts, cardiovascular system, and gastrointestinal tract. Refer to Anaphylaxis.

Urticaria – An allergic skin condition characterized by itching, burning, stinging, and the formation of smooth patches, or wheals, usually red; hives.
(Endnotes)


3 Merk Manuals: Online Medical Library, Last full review/revision September 2008 website: http://www.merck.com