Health Canada Santé Canada

Canada

Mustard Allergy Review and Discussion of Mustard Data

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Mustard /canola – labelling considerations

- The mustard plant belongs to the *Brassicaceae* family (radish, various type of cabbage, broccoli, turnip, rapeseed,…).
- White/yellow (*Sinapis alba*), black (*Brassica nigra)* and brown/oriental mustard (*Brassica juncea*) are the main types of mustard seeds used in cuisine and food processing.
- Commercially available mustard powder is usually a mixture of ground white and black mustard seeds. White mustard seeds are the main ingredient in North American mustard, while the brown seeds are mainly used in Europe and China.

- Mustard is considered a priority allergen in Canada.
- Definition of mustard in the *Canadian Food and Drug Regulations*: ***Sinapis alba**, Brassica hirta Moench, **Brassica juncea** L. Cosson or **Brassica nigra**.*
- Canola can include 3 different species: *Brassica napus* (rapeseed), *Brassica rapa** (field mustard, turnip) and *Brassica juncea* (mustard seed) *from which the oil shall contain less than 2% erucic acid and less than 30 micromoles of total glucosinolates (Canola Council of Canada).*
  - ⇒ If the detected protein is from *B. napus* or *B. rapa* (canola species not defined in the FDR as a mustard seed source) then the allergen labelling Regulations would not apply and precautionary labelling for mustard would not be warranted
  - ⇒ if the detected protein is from a mixture of different sources of canola that included *Brassica juncea* (listed in the FDR as a source for mustard seed) then precautionary labelling for mustard would be warranted

* *Brassica rapa* subsp. *rapa*, (turnip); *Brassica rapa* subsp. *oleifera*, syn. *B. campestris* L. (wild turnip, field mustard)

Brassicaceae family and allergens of interest

Scientific name (common name)	Allergen	Biochemical name	Superfamily/family	Molecular Weight (kDa)
Sinapis alba (white/yellow mustard)	**Sin a 1**	2S albumin	Prolamin (Napin)	14
	Sin a 2	11S globulin	Cupin	51
	Sin a 3	nsLTP	Prolamin	12.3
	Sin a 4	profilin	Profilin	13-14
Brassica juncea (brown/oriental mustard)	**Bra j 1**	2S albumin	Prolamin (Napin)	14
Brassica napus	**Bra n 1**	2S albumin	Prolamin (Napin)	12-16
	Bra n 2	11S/12S globulin	Cruciferin	300
Brassica rapa	**Bra r 1**	2S albumin	Prolamin (Napin)	10-14
	Bra r 2	PR3- protein	PR protein	25

Canola

Cross-reactivities

- Seed proteins of rapeseed, turnip rape and mustard have a high amino acid sequence homology (e.g. 94% identity between rapeseed and white mustard 2S albumins, *UniProt database*) => IgE cross-reactions are conceivable. *Monsalve et al.* reported one case of allergy to both rapeseed flour and to mustard
  - *Puumalainen et al., 2005; Monsalve et al., 1997*

- Clinical cross-reactions between mustard and other *Brassicaceae* family allergens (radish, various type of cabbage, broccoli,…) are usually considered as rare. These cross-reactions have been described essentially in only adults.
  - *Caballero et al., 2002; Rancé et al., 2000, 2003; Morisset et al., 2003; Figueroa et al, 2005*

Mustard allergy

- Mustard allergy is usually life-long

- Common symptoms: oral allergy syndrome (OAS), rhinitis/conjunctivitis, urticaria/angioedema, dyspnea, abdominal pain/vomiting
  - *Caballero et al., 2002; Morisset et al, 2003; Figueroa et al., 2005; Poikonen et al, 2008*

- A few cases of severe incidents and/or anaphylaxis with mustard are clearly documented (*Panconesi et al.,1980; Jorro et al., 1995; Monreal et al., 1992; Rancé et al., 1998 & 1999; Caballero et al., 2002*)
  - Mustard implicated in 3% (n=17) of anaphylactic reactions in 580 patients (480 adults and 100 children) in France (*André, 1994*)
  - Health Canada report (2009): 22 individual cases of allergic reactions to mustard described in 13 international case reports; 15 of which reported anaphylactic-type reactions that required emergency medical intervention. Other severe reactions described in case reports included laryngeal oedema, generalised urticaria and bronchial asthma *http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/pubs/label-etiquet/mustard-moutarde/index-eng.pdf*

- More severe incidents in adults than in children
  - *Morisset et al., 2003; Figueroa et al., 2005*.

Mustard allergy: prevalence data

- Approx. 7% of Canadians self-report a food allergy (FA)
  - *Soller et al., 2012*

- In Canada, no prevalence data available in the general population for mustard allergy (mustard added to the list of priority allergens in 2012)

- Only one population-based study based on self-reported diagnosis of mustard allergy could be found in the literature where 3 % of teenagers (5-17 years old) in France reported adverse reactions to mustard
  - *Touraine et al., 2002*

- Reported cross-reactivities with pollens (e.g. mugwort) may influence the occurrence of oral allergy syndrome-like symptoms elicited by mustard

- Another factor contributing to a possible overestimation of mustard allergy as determined by allergy labial provocation challenge, is the presence of irritating substances (isothiocynanates, sinalbin,…) in mustard that may cause false positive allergy-like reactions

Thresholds

- **Individual threshold** can be defined as an individual's lowest level of exposure at which an allergic reaction has occurred and below which an adverse effect is normally not expected => would inform allergic individuals about the management of their food allergy.
  - Practically, clinicians do not provide a threshold level as part of the diagnosis of FA and allergic people do not really know what level of allergen will elicit a reaction.

- **Population threshold** is minimum dose of allergen that can elicit a reaction in an allergic population (not a single individual) => can help regulatory authorities to assess the public health risk and to guide risk management.

Thresholds

- **Two main approaches:**
  1. Lowest Observed Adverse Effect Levels (LOAELs)

     - LOAEL (also called Minimal Observed Eliciting Dose for allergens) is based on systematic evaluation of a population to determine the exposure levels (hazard) at which adverse effects can be observed.
     - To determine the lowest provoking doses (for each allergen) based on literature results (with DBPCFC)
     - Some reference values established by the FDA (FDA 2008), but does not cover mustard
     - This conventional approach is applied in Europe, USA and Canada for Health Risk Assessments.

Thresholds

- **Two main approaches (cont'd):**

  2. Dose-distribution modelling and derivation of reference doses

    - Statistical tool based on results from DBPCFCs and reported LOAELs in literature
    - Correspond to eliciting doses (EDp) of an allergen at which a proportion (p) of the allergic population (mustard) would be likely to react (EDp) = to estimate, on the basis of historical data, the probability of an event occurring in the (mustard) allergic population

      e.g. ED05 = dose at which 5% of the mustard allergic population would react.
    - Supported by FARRP (University of Nebraska)
    - Recommended by ILSI Europe (2012) and by the US National academies of Sciences, Engineering and Medicine (2017), used by VITAL (Australia)

Thresholds

1. LOAELs (mustard)

Study	Population (mustard allergy)	Age	Test material	Lowest dose tested (mg of protein)	LOAEL (mg of protein) observed (positive responses at this threshold)	Symptoms observed used to determine LOAEL	If dose response data, Threshold eliciting dose (% of population)	Onset of reaction for LOAEL (dose intervals)
Rancé et al. (2000)	15 children with positive SBPCFC	Mean=5.5 (10 months - 15 years)	Mustard seed powder (capsules)	0.3mg	0.3mg (2 patients)	2 objective (urticaria)	No	Immediate (>20 min)
Morisset et al. (2003)	7 patients with positive S+DBPCFC	Range=3-20 years	Mustard seasoning preparation	0.9mg	3.5mg (1 patient)	1 objective (rhinitis, urticaria)	No	Immediate
Figueroa et al. (2005)	14 patients with history of mustard allergy + positive DBPCFC	Mean= 21.9 +/- 8.6 years	Mustard sauce composed of Sinapis alba seeds (14% w/v)	2.9mg	11.7mg (7 patients)	1 objective (urticaria) 6 OAS	No	Immediate (15 min)

Oral challenge studies for MUSTARD

Thresholds

1. LOAELs (mustard)

- Only 3 studies have been found in the literature;
- The primary goal of the 3 studies was not to define a minimum eliciting dose for mustard allergen but to determine prevalence of mustard allergy among sensitized people (*Figueroa*), clinical features (*Morisset, Rancé*) and potential cross-reactivity (*Figueroa)*;
- There are a low number of participants in the 3 studies;
- 2/15 patients reacted at the first dose tested (0.3mg) (*Rancé*);
- Nature of challenge materials (mustard sauce vs. seasoning vs. capsules) and food matrix are different for each study.
- There is no information about potential correlation between the level of individual threshold doses for mustard and the severity of induced symptoms (as typically described for other allergens such as peanut)

The range of LOAELs for mustard (objective symptoms) = 0.3-11.7 mg of mustard proteins

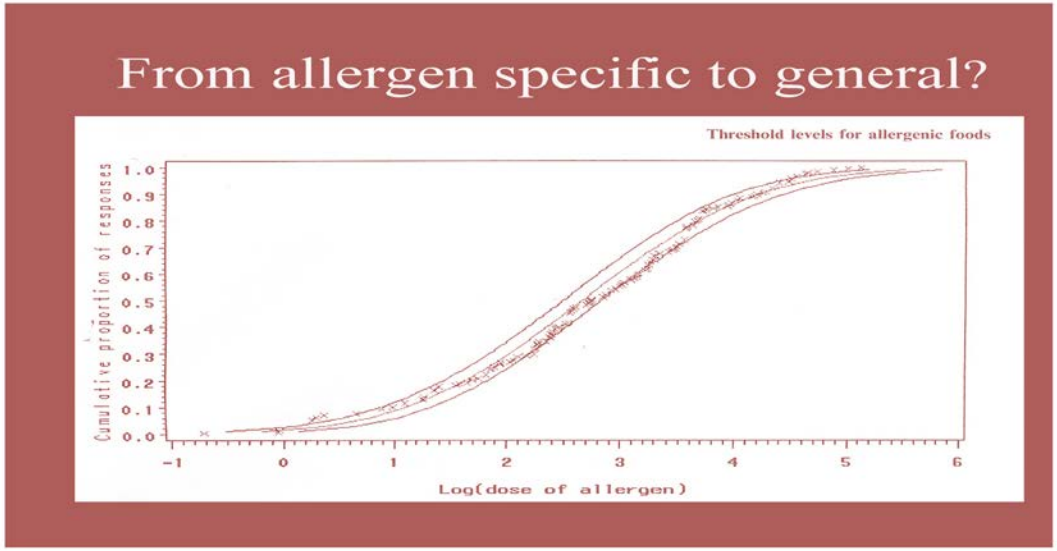
Thresholds

2. Dose-distribution modelling and derivation of reference doses

- Modelling is an accepted way of defining the probability of rare events with potentially severe consequences
- Very low doses of exposure can provoke allergic reactions in some sensitive individuals
- Severe reactions tend to occur at higher doses of exposure (depending on allergens)

However,

- Regulatory consensus does not exist on threshold doses



Source: *Bindslev – Jensen et al. (2002).* Probability distribution model for individual allergen thresholds

Thresholds

2. Dose-distribution modelling and derivation of reference doses (mustard)

- **ED_{05} = 0.32-0.46 mg mustard proteins** (*Taylor et al., 2014)* with ED_{05} = the dose that would be predicted to elicit allergic reactions in 5% of all mustard-allergic individuals.

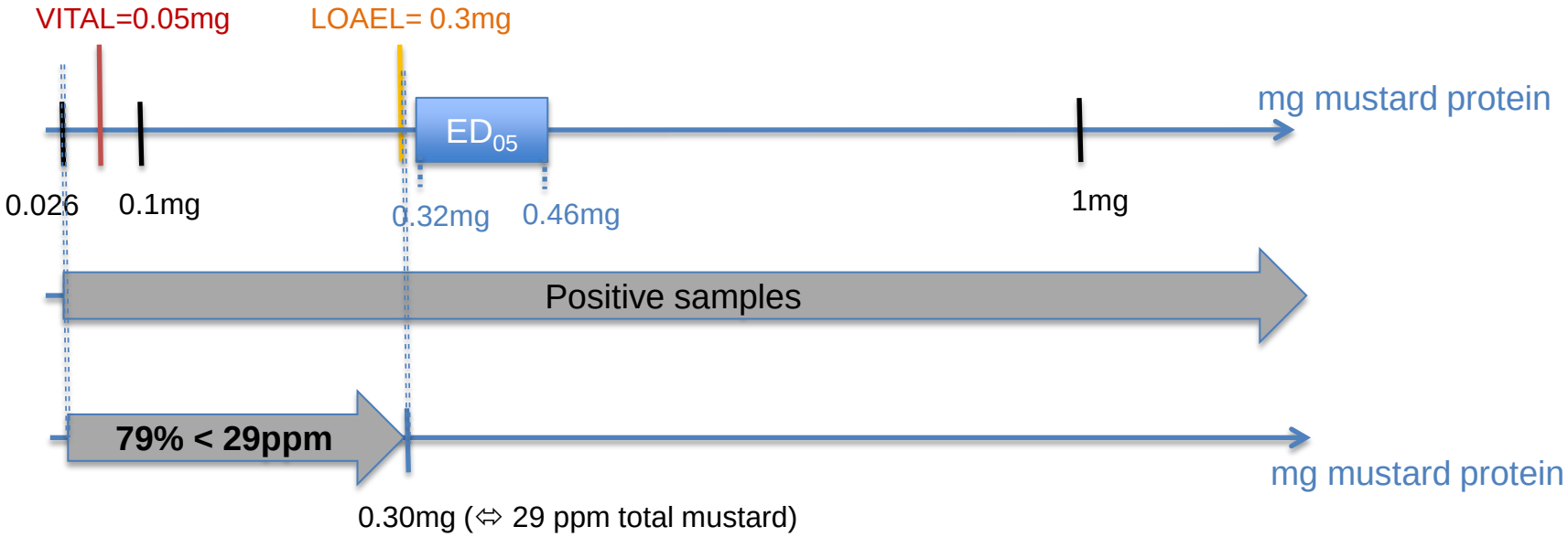
- ILSI Europe and VITAL have suggested **0.05 mg of total protein** (⇔ 95% lower CI of ED_{05}) as a reference dose for mustard. This value was obtained from modelling individual mustard thresholds for **36 individuals.**
  - Nature of the challenge material (mustard sauce vs. seasoning vs. capsules) with different matrixes
  - Low number of participants in these studies
  - Relevance of the statistical model used notably for the part of the curve with a low number of values

⇒ Very limited amounts of clinical data exist to estimate population-based risk levels such as ED_{05} with this approach.

Mustard thresholds and flours (Food Directorate data)

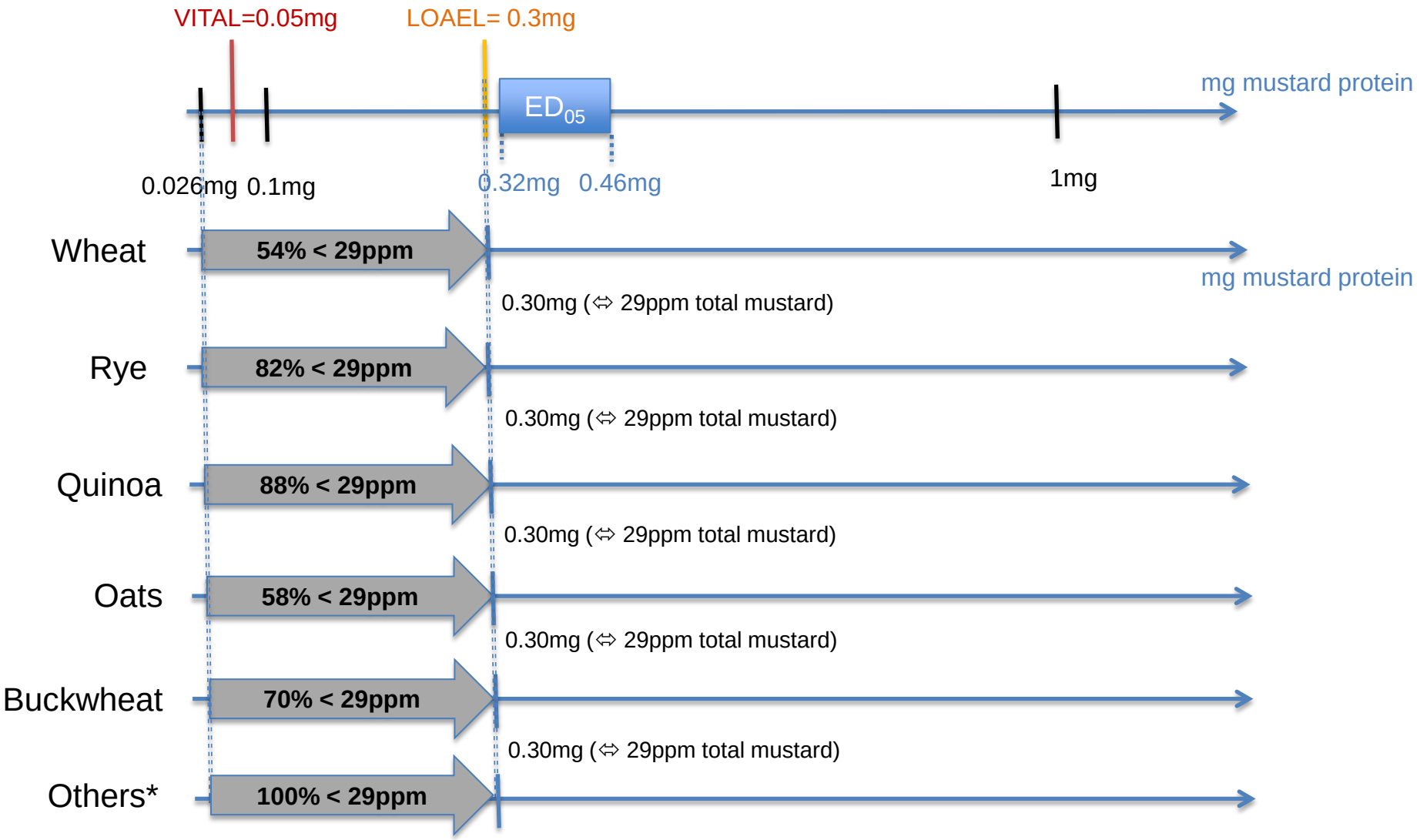
- **Flours :**

  40 g serving size (mean consumption per eating occasion, CCHS 2015)



=> 79% of the flour products would not be expected to trigger allergic reaction in more than 95% of mustard allergic individuals.

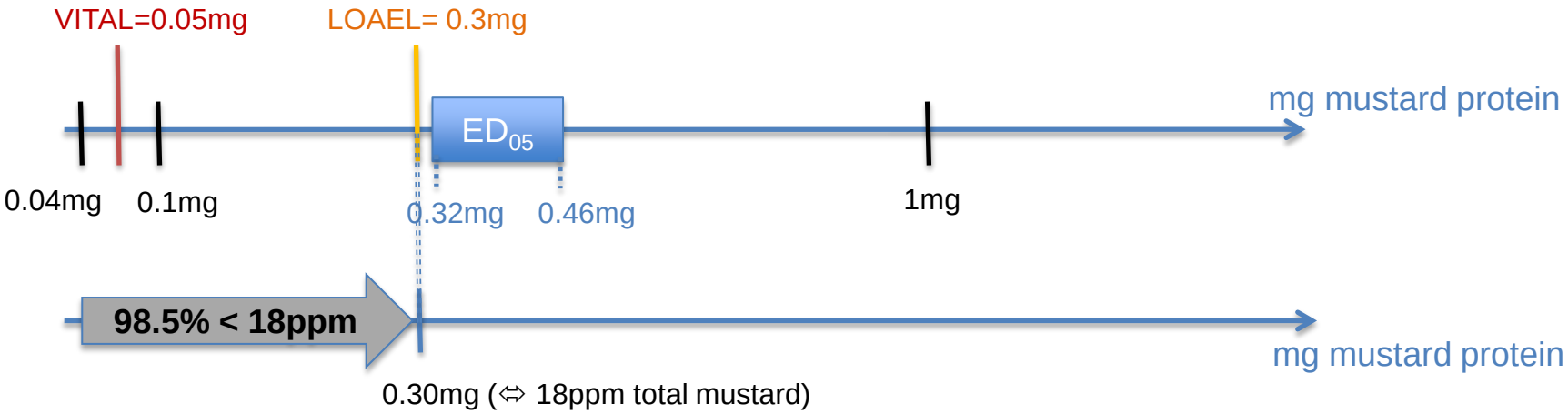
Mustard thresholds and flours (Food Directorate data)



*barley, corn, kamut, rice, sorghum, soy and spelt

Mustard thresholds and finished products
(Food Directorate data)

- **Finished products**:
  - 63g serving size (mean consumption per eating occasion, CCHS 2015)

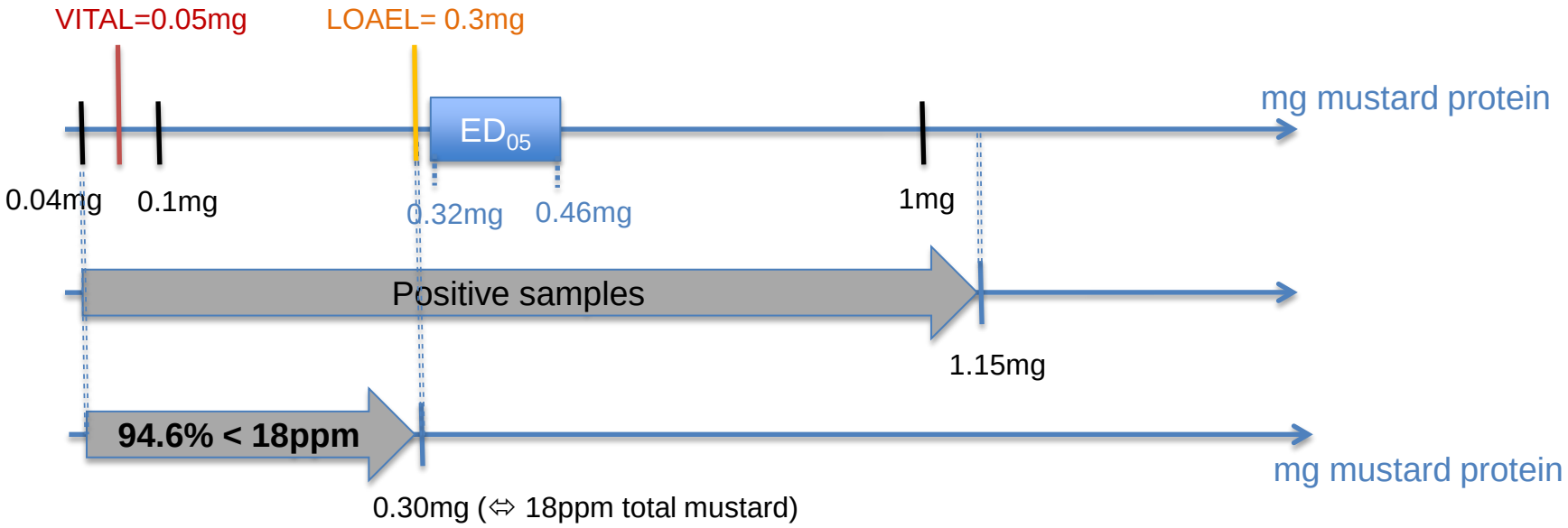


⇒ **98.5% of the finished products contained less than 18ppm total mustard (⇔ 0.30mg mustard protein).**

Mustard thresholds and Bread (Food Directorate data)

- **Bread**:

  63g serving size (mean consumption per eating occasion, CCHS 2015)



=> 95% of the bread would not be expected to trigger allergic reactions in more than 95% of mustard allergic individuals.

Conclusions

- Data collected by HC/FD were generated on products available on the Canadian market;
- Prevalence of mustard allergy in Canada is not known but likely rare
- Due to high amino acid sequence homology of rapeseed, turnip rape and mustard, canola seed proteins are presumed to be able to trigger allergic reactions in mustard allergic individuals (worst case scenario);
- 39% of the flours tested were positive for mustard
- High levels of mustard reported notably in wheat flours (46% >29ppm)
- In 79% of the flours tested (including wheat), based on a 40g serving size, the presence of mustard in flour is below the lower range of the mustard LOAEL available in the literature (0.3mg)

=> Relatively high levels of positive test results for mustard in flours

Conclusions (cont.)

However,

- Only 16% of the finished products tested were positive for mustard
- Among finished products, bread had the highest percent of products positive for mustard (41%) but with a low range;
- In 98.5% of the finished products tested (including bread), based on a 63g serving size, the presence of mustard is below the lower range of the mustard LOAEL (0.3mg)
- 98.5% of the finished products (including bread) would not be expected to trigger allergic reactions in more than 95% of mustard allergic individuals (63g serving size).

=> Based on this data, the use of precautionary statements in flours may be justified due to high frequency of detection and levels but the systematic carry-over to finished products to inform mustard allergic consumers of the adventitious presence of mustard may not be required.