SPECIAL GUEST EDITOR SECTION: FOOD ALLERGENS SURVEYS

Action Levels for Food Allergens: An Approach for Official Food Control in Germany

HANS-ULRICH WAIBLINGER
Chemisches und Veterinäruntersuchungsamt Freiburg, Bissierstrasse 5, 79114 Freiburg, Germany
GESINE SCHULZE
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Eggenreuther Weg 43, 91058 Erlangen, Germany

Official food control laboratories in Germany have established internal action values for the assessment of analytical results of food allergens especially obtained from samples without declaration of the specified allergen. A pragmatic approach was chosen considering the current situation for European food information legislation. Accordingly, when a positive result is obtained for an unlabeled allergen, it is not necessarily an irregularity if it can be demonstrated that the result was caused by cross-contamination. Action values take into account current analytical experiences as well as published allergologic reference doses. They are considered as internal de minimis thresholds by food control authorities that are used to support laboratories in the decision-making process and when a written expert opinion is requested by an enforcement authority. If only minor traces are detected at concentrations below the action values, further investigation of the issue and inspections at the location of manufacture can be abandoned. The present report includes a collection of results from official food control laboratories in Germany that have been evaluated in line with the aforementioned system of action levels.

Analysis of allergens in food is an important task for official food control in Germany. According to Regulation (EC) No. 1169/2011 on the provision of food information (1), ingredients derived from allergens listed in Annex II of this regulation have to be labeled in prepacked and non-prepacked foods.

However, to date, there is no obligation in the European Union (EU) to label any food allergens present in foods that are not part of the recipe, but present due to cross-contamination; for example, as introduced during manufacturing or packaging processes. Therefore, constituents of food allergens may still be present in food even without labeling. Precautionary allergen labeling (i.e., "may contain …" language), which indicates possible allergen residues, is primarily used by many food producers for product-liability reasons. Industrial food manufacturers especially have commonly established internal allergen management systems for risk evaluation and reduction of cross-contaminations. In this context, the Voluntary Incidental Trace Allergen Labelling (VITAL) concept (2) provides so-called action levels for the labeling of allergens in food caused by, for example, cross-contact or traces of allergens in ingredients. The VITAL concept is based on clinical oral challenge threshold data to which statistical models are applied. In 2014, the VITAL expert panel published reference doses for important food allergens deduced from an eliciting dose of the allergen at which a proportion of the allergic population would likely react. The reference doses are presented as a milligram protein level (total protein from an allergenic food) below which only the most sensitive individuals in the allergic population (between 1 and 5%, depending on the quality of the data set available) are likely to experience an adverse reaction (2, 3).

If by consumption of a nonlabeled allergen-containing food—depending on its serving size—the individual reference dose is exceeded, precautionary allergen labeling is recommended. For official control purposes, to date, threshold values concerning allergen residues do not exist in the EU. On the other hand, allergenic ingredients present in foods because of the recipe, even at very low trace levels (e.g., components of composite ingredients of a foodstuff or carriers of food enzymes), are mandatorily required to be labeled. Due to this fact, official food control laboratories find it rather difficult to provide correct interpretation of results when performing allergen analyses. A positive result obtained for an allergen without any declaration of this allergen on the product does not necessarily represent irregularity in terms of food information legislation.

In addition to an allergenic ingredient, whose labeling is mandatory, for traces of allergens due to cross-contact that may have caused a positive analytical result, labeling is not mandatory. Given that analytical discrimination between ingredient and unavoidable allergenic residue is not possible, inspections at the place of manufacture must be performed for further clarification. Within these inspections, for example, recipes of the foodstuffs, including specifications of each ingredient, are examined thoroughly.

Internal Action Values for Official Food Control in Germany

As a pragmatic approach to handle the current situation, official food control laboratories in Germany have set internal action levels (4). The reasons for such values are as follows:

(a) *Uniform evaluation of analytical results.*—The results of the official control laboratories concerning allergen analysis should be evaluated on an equal basis at least within Germany and ideally within the EU. A reliable basis for

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Corresponding author's e-mail: hans-ulrich.waiblinger@cvuafr.bwl.de

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action can be given to the enforcement authorities for the different German federal states in such a way to enable them to treat the analytical findings equally.

(b) *Calibration and validation of the analytical methods.*—At present, generally accepted well-characterized reference materials spiked or incurred with defined amounts of allergenic constituents are not available (5). Such materials would allow the laboratories to uniformly calibrate methods, leading to comparable results. If uniform target levels (given as a concentration in milligrams per kilogram) are set, e.g., providers of analytical proficiency tests and organizers of collaborative trials would be enabled to produce materials spiked in the relevant concentration levels, the analytical methods could subsequently be calibrated and validated specifically. In addition, such defined thresholds set target values, e.g., in terms of the sensitivity of the methods.

(c) *De minimis threshold.*—In a formal interpretation of current labeling provisions, no de minimis threshold value is foreseen. Even positive results at the detection limit level of the most sensitive analytical methods would make further measures of enforcement necessary. However, technically unavoidable contaminations not falling under the current labeling requirements may have result in the positive (trace) result. In this context, a de minimis threshold for traces of allergenic constituents seems justifiable, especially if it does not raise health concerns for most respectively allergic persons.

In 2014, a joint working group of experts from all German federal states (Federal Office of Consumer Protection and Food Safety and Working Group, "Food Hygiene and Food of Animal Origin") updated previous internal action values (4). At the same time, current experiences from an analytical point of view (5), as well as the reference doses published by the VITAL expert panel, were considered (*see* Table 1). From an analytical perspective, available experiences from collaborative trials and proficiency tests were especially considered. Currently, such experiences exist for (commercial) ELISA and real-time PCR methods (5).

Action Values in Detail: Egg Example

Using the example of the allergen, egg, the deduction of the action values according to Table 1 is explained in detail herewith:

(a) *"Analytically determined as."*—Currently, egg and egg proteins are mostly detected and quantified using an ELISA technique. The commercial kits used are calibrated with whole-egg powder (WEP) standard material. Therefore, the results are frequently reported in milligrams of WEP per kilogram of food.

(b) *"Basis of evaluation."*—(*1*) *"Reference dose in milligrams of protein."*—The reference doses published by Taylor et al. (3) refer to milligrams of the respective allergenic protein. In the case of egg, the reference dose is 0.03 mg egg protein.

(*2*) *"Reference dose in milligrams of food."*—The reference dose of 0.03 mg egg protein is converted into the analytically determined measurement unit, which is WEP here. The conversion factors published in ref. 3 are used. For the egg example, 0.03 mg egg protein is equal to 0.066 mg WEP.

Table 1. Food allergens: internal action values for official food control in Germany[a]

Allergens	Analytically determined to be[b]	Basis of evaluation (3)			Analytical result, mg/kg[d]
		Reference dose, mg protein	Reference dose, mg food	Reference concn, mg/kg[c]	
Cereals containing gluten (wheat, rye, barley, oats, spelt, kamut, or their hybridized strains)	Gluten	1.0 (wheat)	10	100	>80
Eggs and products thereof	Whole-egg powder	0.03	0.066	0.66	>1[e]
Peanuts and products thereof	Peanut	0.2	0.8	8	>5
Soybeans and products thereof	Soy flour	1	2.5	25	>20
Milk and products thereof (including lactose)	Defatted milk powder (NFDM)[f]	0.1	0.28	2.8	>2.5[e]
Nuts and products thereof	Whole hazelnut, almond etc.				
Hazelnuts		0.1	0.64	6.4	>5
Cashews		2	10.6	106	>50
Almonds, walnuts, pecan nuts, Brazil nuts, pistachio nuts, and macadamia or Queensland nuts		Not specified			>20[g]
Sesame seeds and products thereof	Whole seeds	0.2	1.18	11.8	>10
Lupine and products thereof	Lupine	4	10	100	>50
Celery and products thereof	Celery seeds	Not specified			>20[g]
Mustard and products thereof	Mustard seeds	0.05	0.19	1.9	>5[e]

[a] For a detailed explanation, *see Action Values in Detail: Egg Example*.

[b] Conversions to the specified type of material may be necessary, especially when using commercial ELISA kits (e.g., specified in the kit or available protein reference values for the foodstuff from the literature).

[c] The reference concentration is the concentration of allergenic constituent in the foodstuff in milligrams per kilogram that is needed to equal the reference dose if 100 g foodstuff is consumed.

[d] Mandatory labeling according to Article 7 of Regulation (EC) No. 1169/2011 (1) required? (inspection necessary).

[e] When using available commercial ELISA kits, depending on the degree of processing (especially heating) of the foodstuff, the real allergen concentration may be underestimated.

[f] NFDM = Non-fat Dry Milk.

[g] Currently no reference dose available; preliminarily value only based on analytical feasibility.

(*3*) *"Reference concentration."*—For the deduction of action values, an average consumption amount of 100 g (allergen-containing) foodstuff is assumed. For example, a reference dose of 0.066 mg WEP is obtained by consuming 100 g foodstuff containing 0.66 mg WEP/kg. Especially from an analytical point of view, it is beneficial to work with uniform concentrations for each allergen. From an allergologic point of view, consideration of real consumption might be more reasonable in some cases. For example, consumption of spices or instant soup powder is far below 100 g, and consumption of beverages mostly exceeds this value. However, given the above-mentioned practicability reasons, an approach with a fixed reference concentration was favored.

(c) *"Analytical result."*—For the egg example, a deduced reference concentration of 0.06 mg WEP/kg is very low. By using current ELISA methods, this concentration can indeed be detected, but frequently not reproducibly quantified (LOQ). Therefore, a 1 mg/kg preliminary action value was set, which is slightly higher than the value originally deduced value (i.e., thus the "basis of evaluation").

Comments on Other Action Values

Cereals Containing Gluten

The current action value is geared toward analytical feasibilities. To date, in most cases, gluten is analyzed in terms of celiac disease. Even for gluten-free foods, a maximum level of 20 mg gluten/kg is allowed according to Commission Implementing Regulation (EU) No. 828/2014 (6). The action value of 80 mg gluten/kg is equal to the upper calibration point of frequently used commercial ELISA kits. The consumption of 100 g foodstuff containing 80 mg gluten/kg would result in an uptake of 8 mg gluten. Catassi et al. (7) reported that symptoms characteristic of celiac disease appear with uptakes of 50 mg and higher.

With regard to the allergenicity of wheat, a reference dose of 1 mg wheat protein has been described (3). Recently, we published a method and its interlaboratory validation for the detection and quantification of cereals containing gluten by real-time PCR (8). Therefore, in the near future, it seems to be appropriate to establish preliminary action levels also on the basis of different cereals containing gluten.

Peanut, Hazelnut, Mustard, and Sesame

The individual action values of these four allergens are equal to or below the deduced reference concentrations and can be controlled by means of current commercial ELISA kits. In addition, sensitive real-time PCR methods can be used, at least for qualitative purposes; however, quantification in the concentration range of 5–10 mg respective allergenic constituent per kilogram is mostly not possible using real-time PCR (5).

Soybean

Compared with the above-mentioned allergens, the reference dose for soybean is slightly higher. This allows the use of real-time PCR for analytical control purposes because at least semiquantitative results can be obtained from the concentration level of 20 mg/kg and above using this technique. Evaluations of proficiency tests demonstrate that presently only a few ELISA methods are able to detect such low levels, e.g., as in heated or other processed foodstuffs.

Milk

Due to the very low reference dose, similar to egg, the action value of milk is based on analytical feasibilities. Lower concentrations might be detectable in some cases. However, reproducibility and quantifiability remain questionable.

Almonds, Walnuts, Pecan Nuts, Brazil Nuts, Pistachio Nuts, Macadamia or Queensland Nuts, and Celery

Due to a lack of reference doses, analytical criteria were used. For almonds and other nuts, a concentration level of 20 mg/kg can analytically be controlled using ELISA methods as well as real-time PCR, at least semiquantitatively. The action value for celery can only be monitored using real-time PCR; at present, there is no ELISA method available.

Cashew and Lupine

Due to the higher reference doses, action values of 50 mg/kg were considered to be justifiable. These values can also be checked using ELISA and PCR.

Fish, Molluscs, and Crustaceans

So far, only a few analytical experiences exist, especially in terms of method standardization (collaborative trials) and proficiency tests. This is due to the fact that these very heterogeneous groups each include a multitude of relevant species. Setting first action levels will be possible as soon as analytical proceedings are carried out.

Practical Use of Action Values

The action values presented in this report represent internal values of official food control laboratories. They cannot be equated with legal threshold values. They are primarily applied in the case of positive results obtained for an allergen without declaration of the allergen. In some cases, they may be applied to foodstuffs exhibiting precautionary allergen labeling of the detected allergen. Action values must support laboratories in the process of evaluation and decision when a written expert opinion is required by an enforcement authority.

This expert laboratory report provides recommendations for further investigations at the place of food manufacture. The aim of these inspections is to clarify whether the presence of the allergenic constituent is caused by a nondeclared ingredient (and consequently, the legal labeling requirements has not been fulfilled), by cross-contact, or unavoidable input. If only minor traces at concentrations below the action values are detected, further investigation of the issue can be abandoned.

Figure 1 shows the procedure for a case in which a food is not in any way labeled (not even with may-contain precautionary allergen labeling) with the allergen to be analyzed. Generally, it is worthy to note that these action values must be updated regularly. As soon as either new analytical expertise or data for the allergologic assessment becomes available, the values have to be reviewed.

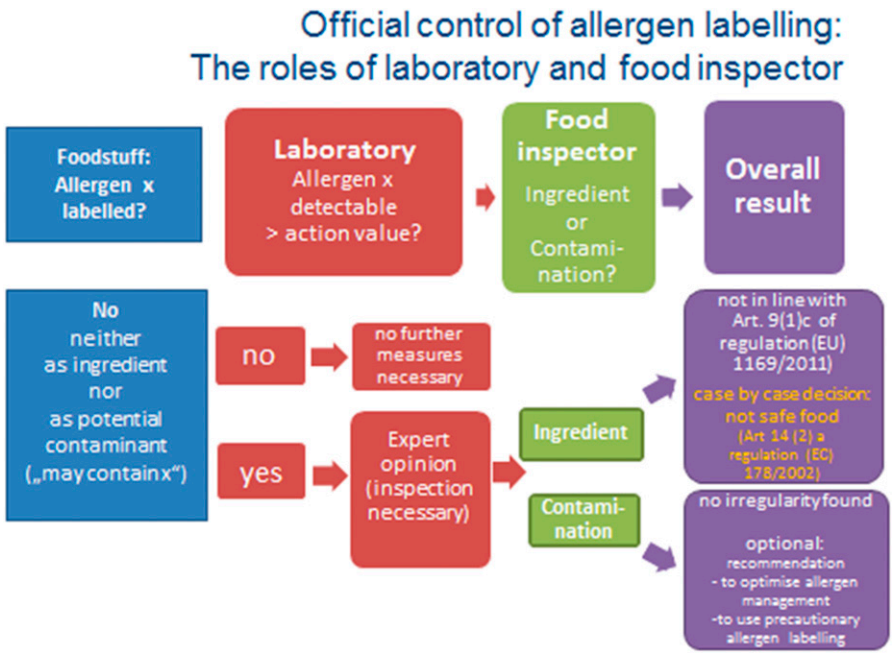

Figure 1. Official control of allergen labeling: the roles of laboratory and food inspector.

Results of Allergen Analyses Within Official Food Control in Germany

Figures 2–4 show the results of allergen analyses carried out in two German federal states, Baden-Württemberg (9) and Bavaria, considering the system of action values. The results include only food samples without ingredient or precautionary allergen labeling of the target allergen. Figure 2 summarizes the overall results from 2016, specifying prepacked and non-prepacked foods.

Analyses that had the detected allergenic constituents above the action level made up 9% of the total 6029 analyses. For non-prepacked foods, the value was twice as high as was for

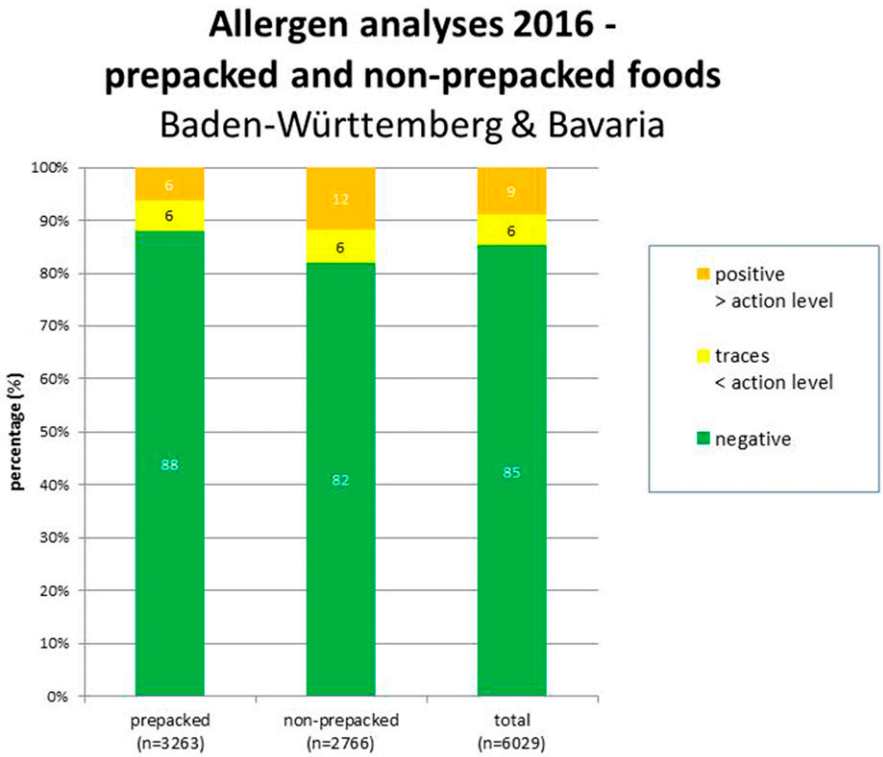

Figure 2. Allergen analyses of 2016 of prepacked and non-prepacked foods. Food samples without any declaration (whether ingredient declaration or precautionary labeling) of the allergen to be tested. Results of the allergen analysis by official food control in Germany. Data are from the federal states of Baden-Württemberg and Bavaria. Presented are the analyses (in percent) of allergenic constituents that were not detectable, detectable in traces below internal action values, and detectable in amounts above the action values.

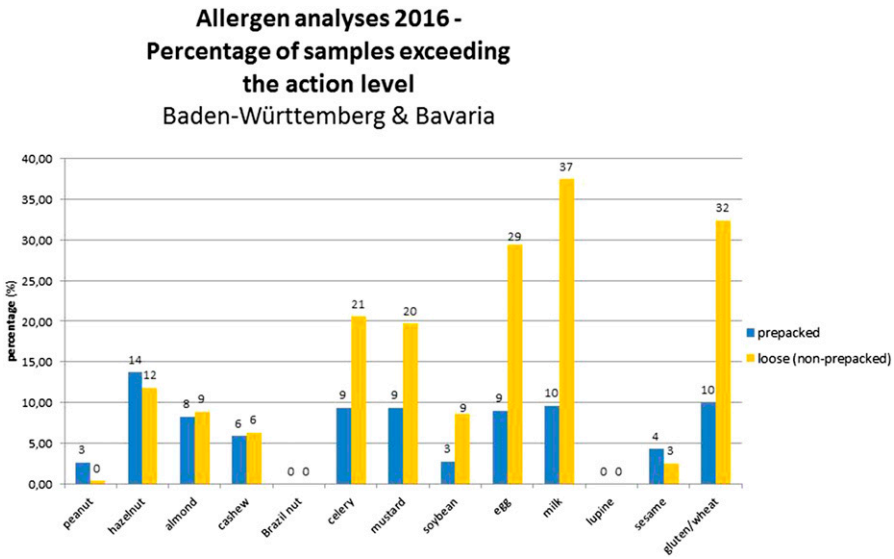

Figure 3. Allergen analyses of 2016 of prepacked and non-prepacked foods. Food samples without any declaration (whether ingredient declaration or precautionary labeling) of the allergen to be tested. Results of the allergen analysis by official food control in Germany. Data are from the federal states of Baden-Württemberg and Bavaria. Presented are the analyses (in percent) of samples/analyses exceeding the action levels specified for each allergen (only allergens with more than 50 analyses/samples are shown).

prepacked foods (12% versus 6%). Traces of allergenic constituents below the action levels were found in 6% of all analyses, with no differences between prepacked and non-prepacked foods.

In non-prepacked foods, results above the action values were most frequently found for milk (37% of all analyses), cereals containing gluten (32%), and egg (29%) and in prepacked foods for hazelnut (14%), milk (10%), and cereals containing gluten (10%; *see* Figure 3).

Compared with 2015, the percentage of analyses of detected, but not declared, allergenic constituents decreased in 2016 (Figure 4). This was the case for the results above the action level (12% instead of 15%) and for traces below the action level (7% instead of 10%). Mandatory allergen labeling of non-prepacked foods was introduced in the EU at the end of 2014.

Action Values and "Free from" Labeling

Action values have limited suitability in terms of assessment of food samples promoted as "free from allergen *x*."

Allergic persons are directly addressed by such claims and may, therefore, expect that the respective allergen is not at all

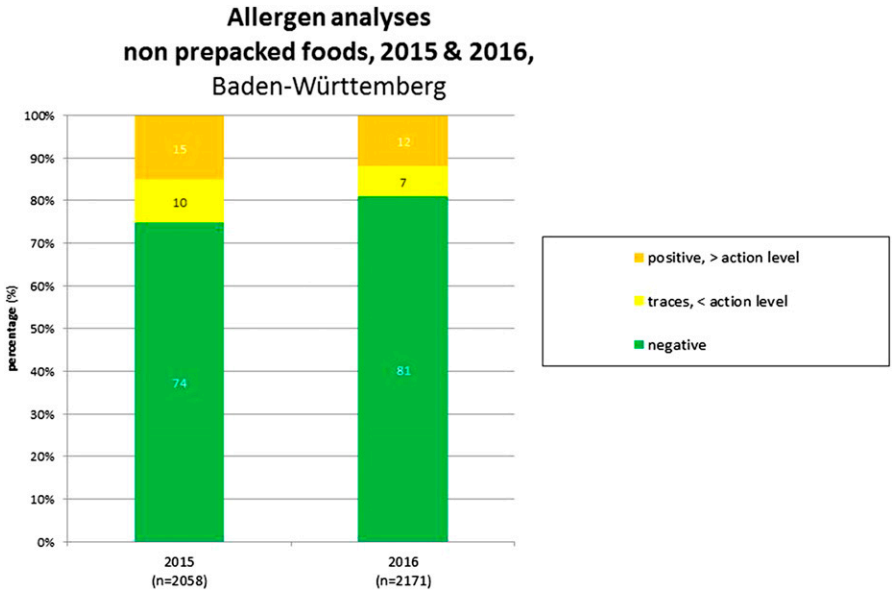

Figure 4. Allergen analyses of 2015 and 2016 of non-prepacked foods. Food samples without any declaration (whether ingredient declaration or precautionary labeling) of the allergen to be tested. Results of the allergen analysis by official food control in Germany. Data are from the federal state of Baden-Württemberg. Presented are the analyses (in percent) of allergenic constituents that were not detectable, detectable in traces below internal action values, and detectable in amounts above the action values.

present in the product. Apart from this fact, such information is misleading if the allergen can be detected; assessment of whether a product is unsafe and injurious to human health, according to Article 14(1) of Regulation (EC) No. 178/2002 (10), can be decided on a case-by-case basis using the principles of risk assessment. In accordance with Article 14(4) lit. c of the regulation, the particular health sensitivities of a specific category of consumers (here, allergic persons) have to be considered when the food is intended for that category of consumers (here, the claim of "free from allergen *x*").

For the assessment of a quantifiable positive result of an allergenic constituent in a product intended for an allergic person, the absolute intake of the allergen is more relevant than its concentration. Therefore, the amount of consumption (serving size) of the respective foodstuff and the reference dose of the allergen have to be considered.

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