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Presence of peanut and hazelnut in cookies and chocolates: the relationship between analytical results and the declaration of food allergens on product labels

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Abstract:

Accidental exposure to hazelnut or peanut constitutes a real threat to the health of hazelnut or peanut allergic consumers. Correct information regarding the ingredients of food products is of paramount importance to inform the consumer and thereby reducing the exposure to food allergens. For this study we have purchased 569 cookies and chocolates on the European market. All products were analysed to determine their peanut and hazelnut content allowing a comparison of the analytical results with the information provided on the label of those food products. Compared to cookies, chocolates are more likely to contain undeclared allergens, while in both food categories hazelnut traces were detected at higher frequencies than peanut. The presence of a precautionary label was found to be related to a higher frequency of positive test results. The majority of chocolates carrying a precautionary label tested positive for hazelnut, whereas in three quarters of the cookies carrying a precautionary label peanut traces could not be detected.

Keywords:

Peanut, Hazelnut, food labelling, cookies, chocolate, ELISA, dipstick, monitoring

Introduction

Food allergy is being recognised as a serious health problem and is estimated to affect up to 8 percent of children and up to 2 percent of the adult population (Ortolani et al. 2001; Sicherer et al. 2003A). The perceived prevalence of food induced allergies is even higher with a quarter of all adults believing that their children are afflicted with a food allergy (Sampson 2005).

Allergic reactions to foods are characterised by adverse reactions of the immune system that are triggered by the uptake of particular foods. Certain proteins that are natural components of foods can cause an allergic sensitization which results in the development of a hypersensitivity and the formation of allergen-specific immunoglobulin E (IgE) antibodies. Subsequent consumption of the food responsible for the sensitization triggers the immune system and can induce a plethora of clinical symptoms the type and variety of which varies widely between individuals and ranges from mild reactions like hives to life-threatening anaphylactic reactions.

Given the incurable nature of food allergy and it's potentially life-threatening consequences the management of food allergy concentrates on a strict avoidance of the offending food, which has to be implemented by the allergic individuals or their care givers. Next to it's impact on the life and behaviour of allergic individuals food allergy also has a significant impact on society, which constitutes next to the health care costs related to it, also of socioeconomic factors like parents having to give up time or work to look after children that are afflicted with a food allergy, or schools adapting to specific needs to ensure a better protection of the health of allergic pupils.

In order to protect allergic individuals and to enable the consumer to readily identify foods containing food allergens, an accurate and unambiguous labelling of food products is absolutely required. World-wide regulatory initiatives have been aimed at a mandatory declaration of the most important food allergens. Within the European Union Directives 2000/13/EC and 2003/89/EC (European Parliament and Council 2000; 2003) require the mandatory labelling of the eleven most commonly allergenic foods, being cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk, tree nuts, celery, mustard and sesame

and ingredients derived from those foods. In addition to those eleven foods, sulphites are also included in the labelling requirements.

The above mentioned legislation concerns known (allergenic) food ingredients, but also the inclusion of allergens in food products resulting from adventitious contamination can compromise the health of allergic consumers. Therefore Directive 2001/95/EC on product safety (European Parliament and Council 2001) as well as Regulation 2002/178/EC on food safety (European Parliament and Council 2002) are relevant since foodstuffs containing allergenic ingredients that are not indicated on the label are unsafe for a specific category of consumers (consumers with a food allergy) and therefore should not be placed on the market. Amongst all food allergens, peanuts and tree nuts are responsible for most of the severe anaphylactic reactions and deaths attributed to food allergies (Pumphrey 2001; Bock et al. 2001). In addition to this, peanut allergy seems to be increasing steadily over recent years and currently has a prevalence of 1 to 1.5% (Grundy et al. 2002; Kagan et al. 2003; Sicherer et al. 2003B), with doses as low as 10 to 300 mg peanut flour capable of eliciting an allergic reaction (Flinterman et al. 2006). Whereas some food allergies are largely outgrown during childhood (e.g. milk allergy), the resolution of peanut allergy in children is rare (Rangaraj et al. 2004; Skolnick et al. 2001; Spergel et al. 2000). All those factors stress the importance of providing information on the allergenic ingredients of food products to the consumer which will enable him or her to adhere to a strict elimination diet. In order to achieve this goal concerted action of regulatory bodies and food producers is required.

Shortly after implementation of the requirements of Directive 2003/89/EC we purchased 569 chocolates and cookies in ten European countries which we analysed for the presence of hazelnut or peanut traces. The objective of our study was to compare the analytical data with the information provided on the labels of the analysed food products. For this purpose a distinction was made between labels on which peanut or hazelnut were declared as ingredients, labels stating that the food product may contain either of those food allergens, and labels stating that the food product had been produced in an environment where peanut or hazelnut were present.

Materials and methods

Cookie and chocolate samples

A total of 315 different types of cookies and 254 different types of chocolates were purchased for analysis. The food products were obtained from shops and supermarkets in 10 European countries (Austria, Belgium, Bulgaria, Czech Republic, Germany, Hungary, Poland, Romania, Slovakia and The Netherlands).

Analysis of hazelnut content in cookie and chocolate samples

Each food product was ground in liquid nitrogen to obtain a fine powder. Aliquots of 1.0 g (± 0.1 g) of the ground food products were weighed out and analysed by using the RIDASCREEN®FAST Hazelnut assay kit from R-Biopharm (Germany), a sandwich-type enzyme-linked immunosorbent assay (ELISA). The analyses were performed in duplicate according to the manufacturers' instructions. Sample extraction was performed by adding 1 g of skim milk powder and 20 ml of preheated provided extraction buffer to the samples, this mixture was incubated for 10 min at 60 ºC in a water bath with continuous shaking. The extracts were centrifuged at 1730 g for 20 min at 4 ºC. The supernatant was collected and used in the immunoassay. The principle of the analysis is the detection of hazelnut proteins by specific antibodies. Quantitative estimates of hazelnut content were obtained by using a regression line that was established with the hazelnut standards supplied with this ELISA test kit. Quantitative assessments of food samples and standards were performed by measuring the optical density (OD) at 450 nm values using a spectrophotometer (1420 Multilabel Counter Victor^{3}V, Perkin Elmer, Singapore).

Analysis of peanut content in cookie and chocolate samples

Each food product was ground in liquid nitrogen to obtain a fine powder. Aliquots of 0.25 g were weighed out and analysed by using the *Biokits* RAPID peanut test from Tepnel *BioSystems* (UK), which is a lateral flow device (dipstick). The analyses were performed in duplicate according to the manufacturers' instructions. The principle of the analysis is the detection of Ara h 1, an allergenic peanut protein by specific antibodies. The presence of peanut protein within food extracts will lead to an immunological detection that can be read by the appearance of coloured lines on the device. The test provides a qualitative result.
In addition to this, aliquots of 1.0 g (± 0.1 g) of all the ground food products were weighed out and analysed by using the *Biokits* peanut assay kit from Tepnel *BioSystems* (UK), a sandwich-type ELISA. The analyses were performed in triplicate according to the manufacturers' instructions. Sample extraction was performed with 10 ml Tris-HCl buffer (0.6

% Tris, 1.17 % NaCl and 10 % gelatine; pH 8.2) for 15 min at 60 ºC in a water bath with continuous shaking. The extracts were centrifuged at 1730 g for 20 min at 4 ºC. The supernatant was collected and used in the immunoassay. The principle of the analysis is the detection of Ara h 1, an allergenic peanut protein by specific antibodies. Quantitative estimates of peanut content were obtained by using a regression line that was established with the peanut standards supplied with this ELISA test kit. Quantitative assessments of food samples and standards were performed by measuring the OD at 450 nm using a spectrophotometer (1420 Multilabel Counter Victor^{3}V, Perkin Elmer, Singapore).

Results

Food labels

Cookies and chocolates were purchased in ten European countries, four of those countries were Member States of the European Union before 2004 (Austria, Belgium, Germany and The Netherlands), while four countries became Member States in 2004 (The Czech Republic, Hungary, Poland and Slovakia), and another two countries, Bulgaria and Romania, were Candidate Countries during the time of this study, but have now joined the European Union. The majority of the food products did not declare either peanut or hazelnut as ingredients on their labels. However, most of them contained a precautionary warning implying that (traces of) these allergenic foods could be unintentionally present in the food products. Although the wording of such precautionary warnings is very variable they could be grouped in two major groups. The first group of precautionary warnings were so called "may contain"-type warnings (e.g. "This product may contain peanut"). The second group of precautionary warnings gives a more detailed explanation of the mechanism by which a contamination with allergenic foods can possibly have occurred and refers to the production environment (e.g. "This product is made on a line that also handles hazelnut" or "This product is made in a factory that also produces hazelnut-containing products). In this study the latter type of labelling is referred to as "present in environment"-type labelling.

Precautionary warnings can either refer to a specific allergenic compound like peanut or hazelnut, or alternatively it can refer to a more generic term like nuts (van Hengel 2007). For the purpose of this study we have differentiated between these two possibilities.

Table 1 shows an overview of the relevant information given on the food labels. Around half of all cookies purchased on which peanut, hazelnut or nuts were not declared as ingredients contained precautionary warnings referring to those allergenic compounds. Interestingly, on the cookies purchased in the countries that were members of the EU prior to 2004 ("old Member States") the precautionary warnings ("may contain" and "present in environment" - type labelling) refer mostly to nuts (18% + 7% = 25%) compared to the specific allergenic compound peanut (8% + 12% = 20%). For hazelnut this trend is even clearer with 36% (26% + 10%) carrying a reference to nuts compared to only 7% (2% + 5%) referring to hazelnut. The reverse was observed for products purchased in the countries that joined the EU after 2004 ("new Member States") with only 5% (5% + 0%) of cookies mentioning nuts compared to 44% (12% + 32%) referring to peanut, and 9% (9% + 0%) of cookies mentioning nuts compared to 28% (9% + 19%) referring to hazelnut.

Table 1 also shows an overview of the declarations on the labels of chocolates that did not declare peanut, hazelnut or nuts as ingredients. Just as was observed for cookies, on the labels of chocolates purchased in the "new Member States" the majority of precautionary labels refer to peanut or hazelnut compared to the more generic term nuts, whereas the opposite was observed on the labels of chocolates purchased in the "old Member States". In Candidate Countries labels referring to nuts were found to be rare.

Chocolates that do not contain peanut, hazelnut or nuts, and that do not carry a precautionary label referring to those allergenic compounds are likely to be identified as safe for consumption by vigilant consumers with a peanut or hazelnut allergy. In the "old Member States" this is the situation for every one in five (peanut) or one in six (hazelnut) chocolates that do not contain peanut or hazelnut as ingredients. In the "new Member States" this is reduced to every one in ten, and in the Candidate Countries the figure is every one in three. All food products were analysed for the presence of hazelnut and peanut to compare the analytical data with the information on the food label.

Hazelnut analysis

Samples were taken from all food products purchased and analysed by ELISA in order to determine whether this method would detect hazelnut in those food products. The limit of detection (LOD) had been set at 1.5 mg kg-1 by the ELISA kit producer. In all cookies and

chocolates that declared hazelnut as an ingredient on the food label hazelnut was indeed detected (35 cookies and 32 chocolates).

Analysis of cookies that did not declare hazelnut as an ingredient on their label identified 77 products (28%) in which hazelnut traces could be detected. Since this study focussed on the detection of hazelnut traces rather than on an exact absolute quantification of this allergenic ingredient, products that were found to test positive by employing the hazelnut specific ELISA test kit were ordered in the following three categories. The first category contains products with trace amounts in which the hazelnut content was estimated to be between 1.5 and 5 mg kg-1, the second category contains products where the hazelnut content was estimated to be higher than 5 mg kg-1, but below 20 mg kg-1, the highest point of the standard curve. The last category contains all products where the estimated hazelnut content exceeded 20 mg kg-1, no dilutions were made to obtain a better quantification of the hazelnut content in such samples. Table 2 gives an overview of the above mentioned 77 products and shows that the majority of them fall in the last category (> 20 mg kg-1). With regard to the different types of labelling that were taken into account, products that tested positive were found to carry precautionary labels referring to either nut or hazelnut, but also product without any reference to hazelnut or nut were found to yield positive test results. Table 3 shows that more than a third of all cookies carrying a "may contain" type label tested positive for hazelnut traces.

Analysis of chocolates that did not declare hazelnut as an ingredient on their label identified 162 products in which hazelnut traces could be detected. This accounts for three quarters (76 %) of the total. In comparison with the cookie data it is clear that chocolates usually contain relatively high levels of hazelnut since 139 samples were estimated to contain more than 20 mg kg-1 hazelnut (Table 2). The chocolate samples that tested positive fall into all the different classes of labelling that were differentiated. Table 3 shows that nearly 80 percent of the chocolates carrying a "may contain" type of label referring to either hazelnut or nut were indeed found to contain this allergenic compound. Surprisingly, for around half of the chocolates that did not contain any reference to (hazel)nut ELISA analysis yielded positive results.

The data presented in Table 1 already points at general differences in labelling practices that can be observed when "old Member States", "new Member States" and Candidate Countries

are compared. The differences in the use of the "present in environment"-type labelling and the specificity of the precautionary labelling (nut versus hazelnut) did not allow a detailed comparison of the analytical results differentiating between specific types of precautionary labels. Therefore, the comparison of the three groups of countries in Table 4 is focussed on the food products for which a positive test result was obtained. Table 4 shows the fraction of products that tested positive as a percentage of all products carrying either 1) a precautionary label, or 2) a label without a reference to either nut or hazelnut, or 3) as a percentage of all food products in which hazelnut is not declared as an ingredient. Cookies in which hazelnut traces were detected but not declared as an ingredient were found to be more common in the "new Member States" compared to the "old Member States", while for chocolate the results are very similar. Unfortunately the analytical results do not show differences between the likelihood of the presence of hazelnut traces in food products carrying precautionary warnings, compared to those where (hazel)nut is not mentioned on the label. One exception to this is the observation that only a low percentage of chocolates purchased in the two Candidate Countries on which no reference was made to (hazel)nut were found to test positive after ELISA analysis.

Peanut analysis

Samples were taken from all food products purchased and analysed by two different methods designed to detect peanut in food products. The first method, an ELISA test kit, was employed to analyse all samples in triplicate in order to quantify the peanut content within the range covered by the standards supplied with this test kit. The second method, a lateral flow device (dipstick), was employed to analyse all samples in duplicate. The latter method allows only qualitative analysis. The antibodies utilised in both methods are of the same origin which enables a direct comparison of the analytical results obtained with those two methods. Although the vast majority of samples purchased for this study were selected on the basis of absence of peanut in the list of ingredients, 15 cookies and 6 chocolates declaring peanut as an ingredient were included in the analysis. Surprisingly and in contrast to the results obtained with the hazelnut analysis where all such samples tested positive, amongst the samples that declared peanut as an ingredient 8 cookie samples and 1 chocolate sample tested negative with both the ELISA and the dipstick method, questioning the presence of this ingredient in those 9 food products.

Analysis of cookies that did not declare peanut as an ingredient on their label identified 68 products (23%) in which peanut traces could be detected by ELISA. Figure 1 shows that the majority of those 68 also tested positive by using the dipstick method. On the other hand, 20 samples tested positive with the latter method while analysis with the ELISA test kit did not result in detectable levels of peanut. Since both methods employ the same antibodies differences in methodology (e.g. the absence of washing steps in the dipstick analyses) are the most likely causes for this discrepancy. The limit of quantification (LOQ) for the ELISA test kit was set by the kit producer at two times the absorbance of the zero standard measured at 450 nm, this level was found to correspond to 0.7 mg kg-1.

Table 5 gives an overview of the 68 products that tested positive by ELISA and shows that nearly half of them (47 %) fell in the last category (>20 mg kg-1). It also shows that most of the samples in this category tested positive after dipstick analysis (88%), while the opposite was observed for samples containing peanut at levels below 5 mg kg-1 where only 37 % yielded positive results after dipstick analysis. With regard to the different types of labelling that were taken into account, products that tested positive were found to carry precautionary labels referring to either nut or peanut, but also products without any reference to peanut or nut were found to yield positive test results. Table 6 shows that around a quarter of the cookies carrying either a "may contain" or a "present in the environment" type of label were found to test positive after ELISA analysis. While for products without any reference to (pea)nut this figure was around 10 percent.

After analysis of chocolates that did not declare peanut as an ingredient on their food label we identified 92 products in which peanut traces could be detected. This accounts for 37 % of the total. Figure 1 shows that around two thirds of those 92 also tested positive after analysis with the dipstick method. Only a single sample that was found to test positive with the latter method tested negative with the ELISA test kit. Of the 92 products that tested positive by ELISA around half (47%) contain peanut at levels exceeding 20 mg kg-1 (Table 5). A comparison of the analytical results obtained by the two different methods shows that a positive result after dipstick analysis is almost always correlated to a positive result after ELISA analysis. But ELISA analysis identified more samples that tested positive. Table 5 shows that samples in which low levels of peanut were detected dipstick analysis more often results in negative readings.

With regard to the different types of labelling that were taken into account, chocolates that tested positive were found to carry precautionary labels referring to either nut or peanut, but also analysis of chocolates without any reference to peanut or nut were found to yield positive test results (Table 5). Table 6 shows that a large fraction of the chocolates carrying a "may contain" type of label were found to test positive after ELISA analysis. This figure is much lower for chocolates with a "present in the environment" type of precautionary warning, but it has to be noted that only 15 chocolates were carrying this type of label. Furthermore, a quarter of the chocolates without any reference to (pea)nut was found to contain detectable levels of peanut.

Table 7 shows a comparison of the analytical data obtained from products purchased in different groups of European countries. With regard to peanut detected in cookies the results are roughly comparable to the results of the hazelnut analyses (as presented in Table 4), with the exception that in the "new Member States" the percentage of cookies that tested positive was higher in the absence of a precautionary warning. Clear differences between the peanut and hazelnut data can be observed for chocolate. Whereas around 80 percent of all chocolates purchased in EU Member States tested positive for hazelnut, in the "old Member States" a clearly lower fraction (13%) was shown to contain peanut residues. Furthermore no peanut traces were detected in chocolates purchased in the "old Member States" that did not contain any reference to (pea)nut.

Limit of detection

As described above, the cut-off values as given by the ELISA test kit producers were used to differentiate between samples that tested positive for traces of allergens, and samples in which no such traces could be detected. The collection of analytical results of over 550 food products, as described in this study, allows a more detailed investigation into the performance of the ELISA test kits. Quantitative results obtained with ELISA are based on calibration curves. Therefore it is possible for measurement results obtained from samples where the (allergen) analyte is absent or present at very low levels to generate observations below zero. Such observations are not necessarily a problem (Eurachem 2000; Analytical Methods Committee 2001). Moreover, an unbiased measurement on blank samples, or a component at a very low level should be expected to generate approximately 50% negative values (Cowen and Ellison 2006). Theoretically, for all samples that do not contain the allergen the analytical results are expected to show a normal distribution around the mean value of zero. Therefore

for samples that contain no, or very low levels of analyte plotting all analytical results > 0 should result in a curve similar to that obtained by plotting the absolute values of all analytical results < 0. Such a representation of the data obtained after analysis with the hazelnut ELISA test kit is shown in Figures 2 A and B, in which the hazelnut content (as determined via the standards regression line) is plotted for all samples in which less than 5 mg kg-1 hazelnut was detected. In figure 2 A both curves are similar, indicating that the data for cookie samples indeed show a Normal distribution around the 0 value within the range of -0.6 mg kg-1 to 0.6 mg kg-1. Since no negative measurement result below 0.6 was obtained we assume that all food products in which the hazelnut content was estimated to be above 0.7 mg kg-1 (blue line in Fig 2 A) are likely to contain minor traces of hazelnut. All samples for which a value of 1.5 mg kg-1 or more was obtained (red line in Fig 2 A) had already been assigned positive on basis of the LOD of the ELISA test kit leaving 11 cookie samples for which the hazelnut analysis resulted in values between 0.7 and 1.5 mg kg-1 and that are therefore suspect of containing hazelnut traces. Figure 2 B shows that both curves are dissimilar and therefore the data obtained for the chocolate samples do not point at a Normal distribution around the 0 value. Nevertheless, also here no values below -0.7 mg kg-1 were obtained confirming that samples with a hazelnut content estimated between 0.7 and the LOD of 1.5 mg kg-1 are suspect of containing trace levels of the allergen. This concerns 6 chocolate samples. Figures 2 C and D show a representation of the data obtained after analysis with the peanut ELISA test kit. Only values below the LOD are plotted in the graphs. Figure 2 C shows that as was observed for the hazelnut data, the values around the 0 value point at a Normal distribution, at least in the range of -0.2 to 0.2 mg kg-1. In addition to this, we determined the LOD by analysing a dilution range of the 5 mg kg-1 standard supplied with the kit. A standard curve was obtained for standards of 0, 1, 2, 3, 4 and 5 mg kg-1 (nine independent measurements per concentration) and the Y-intercept was calculated. The Y-intercept plus 3x the standard deviation yielded an LOD of 0.2 mg kg-1, which supports our interpretation of the results above. The lowest value obtained for cookie samples analysed with the peanut specific ELISA was found to be -0.34 mg kg-1. We therefore assume that all food products in which the peanut content was estimated to be above 0.4 mg kg-1 (blue line in Fig 2 C) are likely to contain minor traces of peanut. This means that 7 cookies samples for which the peanut analysis resulted in values below the LOD of the test kit, but above 0.4 mg kg-1 are suspect of containing peanut traces.

Both curves in Figure 2 D do not overlap, indicating that the peanut ELISA results for chocolate samples do not point at a Normal distribution around the 0 value. In the range -0.4 to 0.4 a large number of negative values were obtained. Also here no values below -0.4 were obtained confirming that samples with a hazelnut content below the LOD of the test kit, but above 0.4 mg kg-1 are suspected to contain peanut traces. This was found to concern only a single chocolate sample.

Discussion

In this study we scrutinised the food labels of cookies and chocolates. Based on the frequency of precautionary labelling, the vast majority of chocolates and almost half of the cookies that do not declare peanut or hazelnut as an ingredient pose a risk to allergic consumers. Interestingly a higher frequency of precautionary labelling was found on chocolates purchased in "new Member States" compared to "old Member States". But, on the other hand, the precautionary labels on chocolates purchased in the "old Member States" were found to be of a more general nature. This can deter allergic consumers from purchasing products that could very well be identified as safe when more specific terms are used instead (van Hengel 2007). The undeclared presence of allergens in food products is a known cause of accidental exposure for people with a food allergy as apparent from consumer calls (Altschul et al. 2001). Previously it was shown that undeclared peanut and / or hazelnut traces could be detected in commercial food products (Vadas and Perelman 2003; Kiening et al. 2005). However, the limited number of samples analysed in those studies prevents an investigation into the relation between labelling and the frequency of food products containing peanuts or hazelnut traces. Our results show that hazelnut traces could be detected in more than a quarter of cookies and in almost three quarters of chocolates that were analysed. For peanut those figures were found to be 23 and 37 % respectively. Traces of those allergens were more often found in food products carrying a "may contain" type of label compared to food products without any reference to peanut or hazelnut. For chocolate higher levels of hazelnut (>20 mg kg-1) were generally found in products with a "may contain" type of labelling, which was not apparent for cookies. For peanut, higher levels (>20 mg kg-1) were not found to be related to a "may contain" type of labelling.

Some precautionary labels refer to the production environment which intends to provide the consumer with a graduated risk (van Hengel 2007). It was therefore of interest to compare the frequency of positive ELISA results between "may contain" and "present in the environment"

type of labels. The latter type of labelling was found to be relatively rare for chocolate samples but more general for cookies. With regard to cookies, products carrying a "present in the environment" type of label were found to be as likely to contain peanut as products without any reference to peanut, while products with a "may contain" type of label were found to be more likely to contain peanut. Surprisingly the opposite was observed with regard to hazelnut traces, where the analytical results for "may contain" and "present in the environment" type of labels were found to be very similar. The usefulness of two different types of precautionary labelling is therefore questionable.

The frequency of cases in which peanut or hazelnut traces can be detected relies on the analytical method used. Low levels of allergen can remain undetected. Whether such low levels are capable of triggering an allergic reaction depends on the amount consumed and the clinical threshold. A recent study reported that the no-observed-adverse-effect level for peanut is as low as 1 mg peanut flour (Flinterman et al. 2006), which was deduced from the observation that the population of peanut allergic individuals that took part in this study could safely consume 1 mg of peanut flour. But, unfortunately currently there is insufficient information available on clinical thresholds, and labelling regulations are not yet guided by threshold considerations. This stresses the importance of a low LOD for methods designed to detect allergenic ingredients.

Here we report positive analytical results for food products in which we could detect more than 1.5 mg kg-1 hazelnut or 0.7 mg kg-1 peanut. A deeper investigation into detection limits identified a number of additional food products that might contain hazelnut or peanut albeit at trace levels below the LOD of the ELISA test kits. It has to be stressed that it can not be claimed that those food products really contain hazelnut or peanut since this would be against the intended use of the ELISA test kits. This investigation also confirmed that the matrix effect of chocolate is different from that of cookies as had been shown previously (Poms et al. 2005). The difference in matrix effects was revealed by a Normal distribution around the 0-value for the cookie samples where a skewed distribution was observed for the chocolate samples. Our observation that a relatively large number of chocolates generated results below zero is in agreement with the negative intercept values for peanut detection in this particular food matrix as reported by Whitaker et al. (2005).

The robustness of the peanut ELISA test kit as well as that of the peanut dipstick has recently been investigated by collaborative trials (Poms et al. 2005; van Hengel et al. 2006). The analysis of cookie material prepared for those validation studies showed that the sensitivity of the ELISA is higher than that of the dipstick method. The current study allows a direct comparison of both methods by analysing a large number of different commercial food products, and it confirms the higher sensitivity of the ELISA method, since food products containing low amounts of peanut were less likely found to be positive after analysis by the dipstick method. The fact that both the ELISA and dipstick method used to detect peanut traces utilise the same antibodies, implies that differences in extraction (e.g. temperature of extraction solvent) or methodology (e.g. time allowed for antibody antigen binding) are the likely causes for the observed differences in sensitivity.

In conclusion, the concerted action of food producers, regulatory agencies and interest groups of allergic patients has resulted in a much improved transfer of information that is required by allergic individuals to identify food products that could endanger their health. Precautionary labelling can assist in this as a deterrent, provided that it is recognised that overuse of precautionary labelling results in an unduly restricted choice for allergic consumers and an erosion of the message. Furthermore, it can only be an effective deterrent when precautionary labelling identifies a heightened chance of allergen contamination. Our study indeed confirmed that food products carrying a precautionary warning show a higher frequency of contamination with hazelnut or peanut. Ideally the absence of a precautionary warning constitutes a guarantee that peanut or hazelnut traces can not be detected in a cookie or a chocolate. This indeed was found to be the case for chocolates purchased in the "old Member States" where peanut could not be detected in any of the products without a precautionary warning. But, unfortunately we identified allergen traces in a number of food products where on the label no reference was made to the allergen. A continued effort to achieve accurate labelling practises and the implementation of allergen management plans by the food industry is expected to further increase safeguarding the health of allergic consumers.

The views expressed are purely those of the writers and may not in any circumstances be regarded as stating the official position of the European Commission.

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Figure legends

Figure 1. Venn diagrams showing the number of food products yielding negative test results, or positive test results achieved by either ELISA or dipstick analysis. The intersect represents food products for which positive test results were obtained with both methods. A. Cookies, B. Chocolate

Figure 2. Analytical values of the allergen content of individual food samples as determined by ELISA and quantified using the standards supplied with the test kits. Blue diamonds show the allergen contents > 0, and red squares show the absolute value of samples for which the allergen content was < 0.

A. Hazelnut content of all cookie samples that were estimated to be below 5 mg kg-1. The red line shows the LOD of the test kit and the blue line indicates the lower limit for samples that are likely to contain minor traces of hazelnut.

B. Hazelnut content of all chocolate samples that were estimated to be below 5 mg kg-1. The red line shows the LOD of the test kit and the blue line indicates the lower limit for samples that are likely to contain minor traces of hazelnut.

C. Peanut content of all cookie samples that were found to be below the LOD of the test kit. The red line shows the LOD of the test kit and the blue line indicates the lower limit for samples that are likely to contain minor traces of hazelnut.

C. Peanut content of all chocolate samples that were found to be below the LOD of the test kit. The red line shows the LOD of the test kit and the blue line indicates the lower limit for samples that are likely to contain minor traces of hazelnut.

Table 1 Percentage of labelling types observed on cookies and chocolates

	"may contain" peanut	nut	"present in environment" peanut	nut	no reference to (pea)nut	"may contain" hazelnut	nut	"present in environment" hazelnut	nut	no reference to (hazel)nut
Cookies										
"old Member States"	8	18	12	7	55	2	26	5	10	57
"new Member States"	12	5	32	0	50	9	9	19	0	63
Candidate Countries	insufficient number of samples					insufficient number of samples				
Chocolates										
	"may contain" peanut	nut	"present in environment" peanut	nut	no reference to (pea)nut	"may contain" hazelnut	nut	"present in environment" hazelnut	nut	no reference to (hazel)nut
"old Member States"	27	41	2	10	20	9	61	3	9	16
"new Member States"	87	3	0	0	10	53	38	0	0	9
Candidate Countries	62	6	0	0	32	65	4	0	0	31

Table 2 Food products that do not declare hazelnut as ingredient on the label but tested positive with the hazelnut ELISA. Out of a total of 278 cookies and 248 chocolates

	"may contain"		"present in environment"		no hazel(nut) on label	
	hazelnut	nut	hazelnut	nut		total
Cookies						
1.5-5mg kg-1	3	5	2		13	23
5-20 mg kg-1	1		1		9	11
>20 mg kg-1	7	8	4	3	21	43
total	11	13	7	3	43	77
Chocolate						
1.5-5mg kg-1	3	9		1	4	17
5-20 mg kg-1	1	4	1			6
>20 mg kg-1	51	64	1	6	17	139
total	55	77	2	7	21	162

Table 3 Relation between labelling type and positive ELISA results for food products without any reference to (hazel)nut or with a precautionary type of labelling

	reference made to (hazel)nut on the label	total	percentage positive for hazelnut
Cookies	"may contain"	66	36
	present in environment	43	23
	no reference	169	25
	total	278	28
Chocolate	"may contain"	167	79
	present in environment	15	60
	no reference	40	53
	total	222	73

Table 4 Percentage of food products testing positive for hazelnut

	precautionary warning	(hazel)nut not mentioned	total
Cookies			
"old Member States"	20	14	17
"new Member States"	55	41	45
Chocolate			
"old Member States"	79	79	79
"new Member States"	82	60	80
Candidate Countries	66	19	51

Table 5 Food products that do not declare peanut as ingredient on the label but tested positive by dipstick and ELISA. Out of a total of 296 cookies and 248 chocolates

	"may contain"		"present in environment"		no peanut on label	total
	peanut	nut	peanut	nut		
Cookies						
Dipstick and ELISA positive						
0.7-5mg kg-1	1	1	2		7	11
5-20 mg kg-1	2				1	3
>20 mg kg-1	9		5		14	28
total	12	1	7		22	42
Dipstick negative / ELISA positive						
0.7-5mg kg-1	1		5		13	19
5-20 mg kg-1		1			2	3
>20 mg kg-1	2				2	4
total	3	1	5		17	26
Chocolate						
Dipstick and ELISA positive						
0.7-5mg kg-1	2	1				3
5-20 mg kg-1	12				3	15
>20 mg kg-1	33	1			8	42
total	47	2			11	60
Dipstick negative / ELISA positive						
0.7-5mg kg-1	12	5		1	1	19
5-20 mg kg-1	5	2			1	8
>20 mg kg-1	5					5

total	22	7	1	2	32

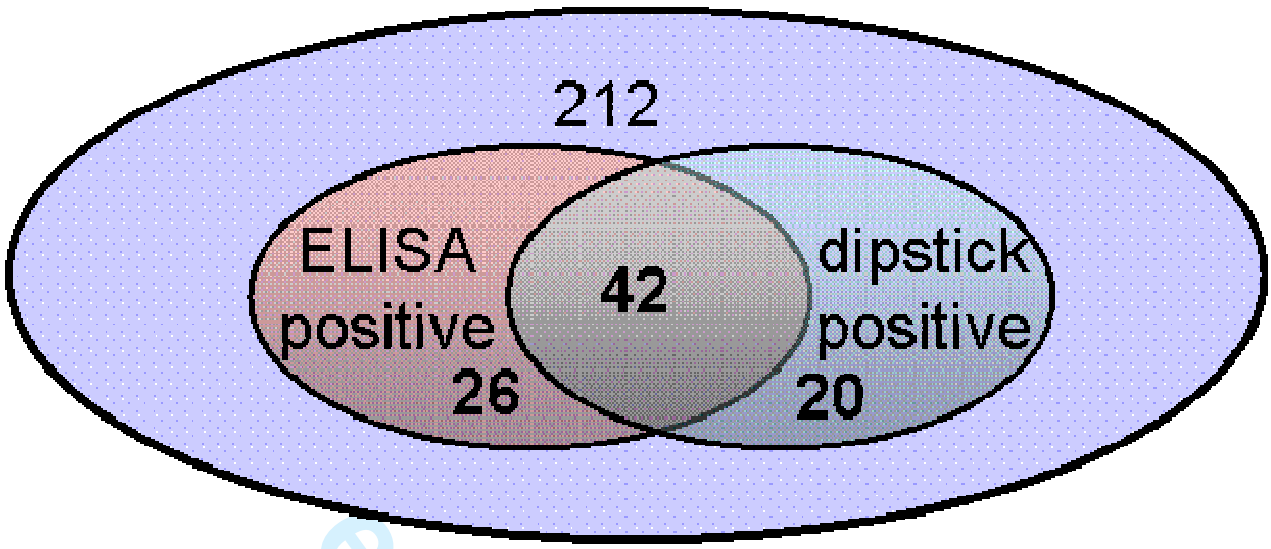
Table 6 Relation between labelling type and positive ELISA results for food products without a reference to (pea)nut or with a precautionary type of labelling

	reference made to (pea)nut on the label	total	percentage positive for peanut
Cookies	"may contain"	68	25
	present in environment	69	25
	no reference	159	11
	total	296	23
Chocolate	"may contain"	181	43
	present in environment	15	7
	no reference	52	25
	total	248	37

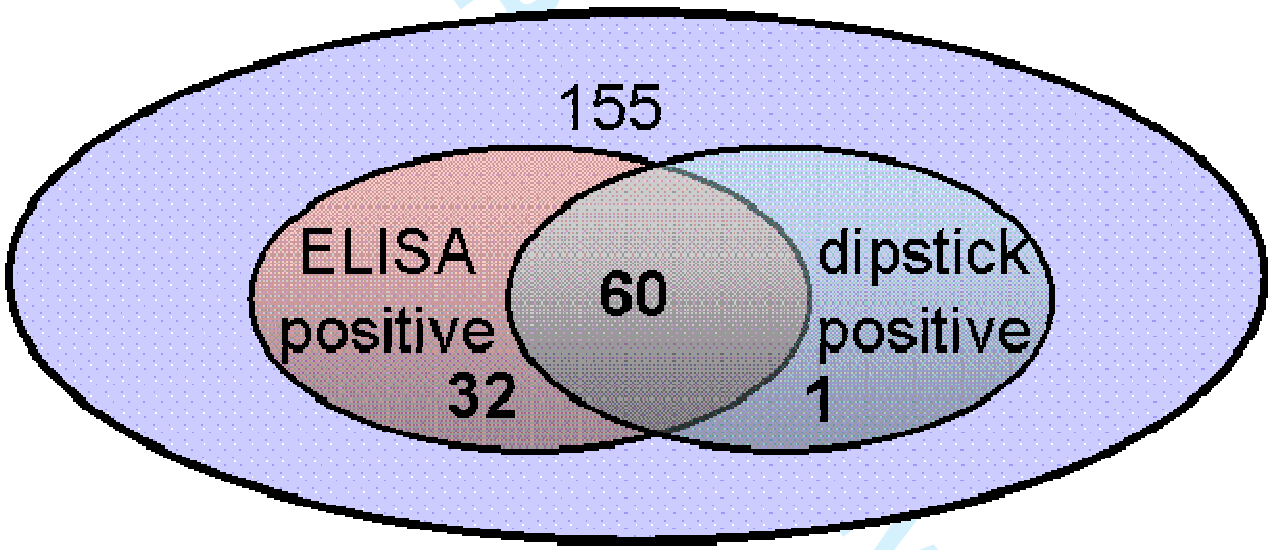
Table 7 Percentage of food products testing positive for peanut

	precautionary warning	(pea)nut not mentioned	total
Cookies			
"old Member States"	14	5	8
"new Member States"	30	56	43
Chocolate			
"old Member States"	16	0	13
"new Member States"	80	67	79
Candidate Countries	50	53	51

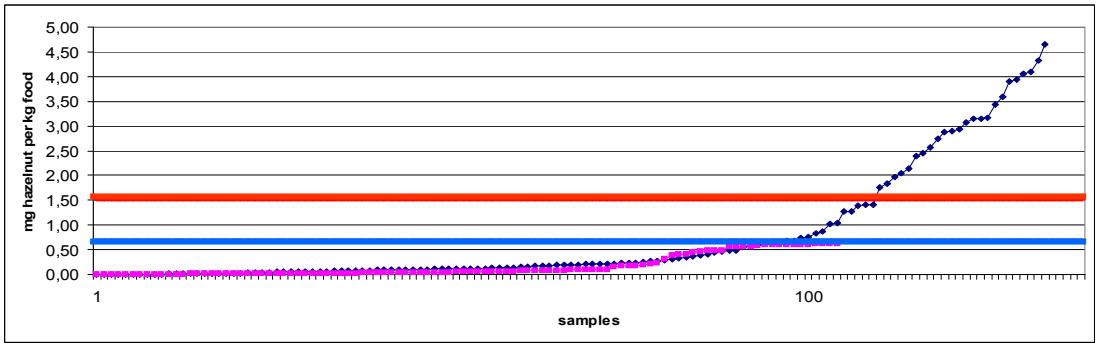
Cookies



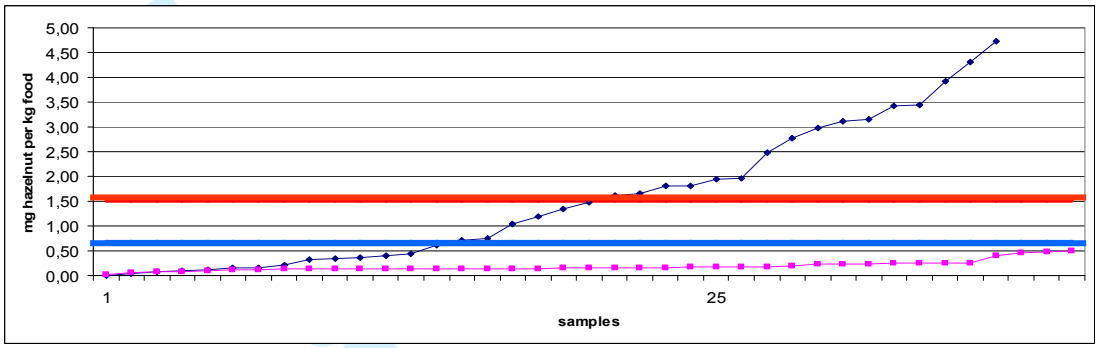
Chocolate



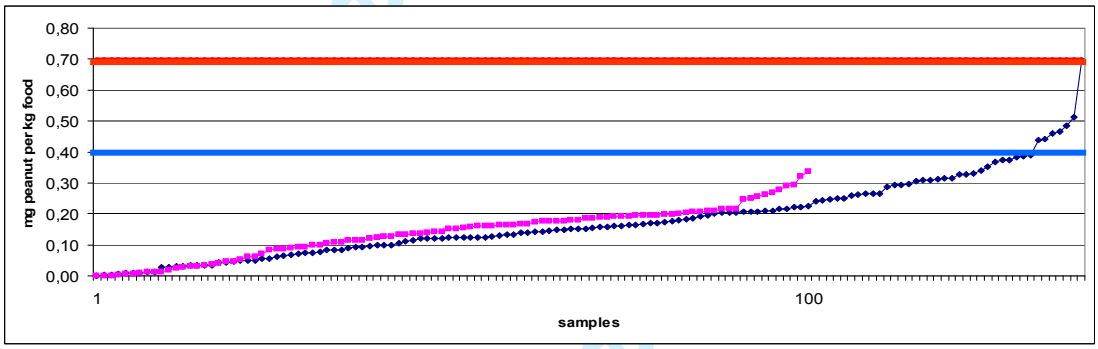
A


mg hazelnut per kg food
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
1
100
samples

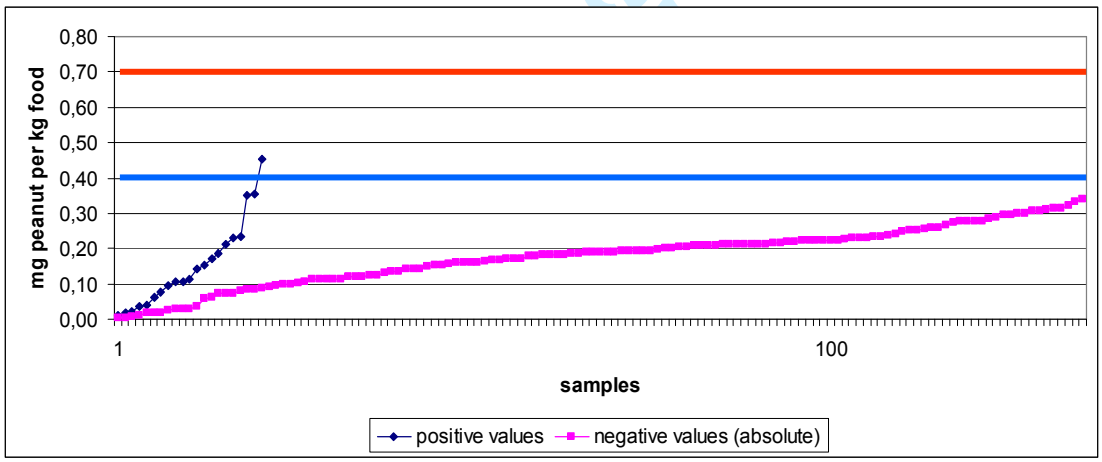
B


mg hazelnut per kg food
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
1
25
samples

C


mg peanut per kg food
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
1
100
samples

D


mg peanut per kg food
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
1
100
samples
positive values
negative values (absolute)