

The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges

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Summary

Background The risk for allergic reactions depends on the sensitivity of individuals and the quantities of offending food ingested. The sensitivity varies among allergic individuals, as does the threshold dose of a food allergen capable of inducing an allergic reaction.

Objective This study aimed at determining the distribution of minimum provoking doses of hazelnut in a hazelnut-allergic population.

Methods Thirty-one patients with a history of hazelnut-related allergic symptoms, a positive skin prick test to hazelnut and/or an elevated specific IgE level, were included. Double-blind, placebo-controlled food challenges (DBPCFC) were performed with seven increasing doses of dried hazelnut (1 mg to 1 g hazelnut protein) randomly interspersed with seven placebo doses.

Results Twenty-nine patients had a positive challenge. Itching of the oral cavity and/or lips was the first symptom in all cases. Additional gastrointestinal symptoms were reported in five patients and difficulty in swallowing in one patient. Lip swelling was observed in two patients, followed by generalized urticaria in one of these. Threshold doses for eliciting subjective reactions varied from a dose of 1 mg up to 100 mg hazelnut protein (equivalent to 6.4–640 mg hazelnut meal). Extrapolation of the dose–response curve showed that 50% of our hazelnut-allergic population will suffer from an allergic reaction after ingestion of 6 mg (95% CI, 2–11 mg) of hazelnut protein. Objective symptoms were observed in two patients after 1 and 1000 mg, respectively.

Conclusion DBPCFCs demonstrated threshold doses in half of the hazelnut-allergic patients similar to doses previously described to be hidden in consumer products. This stresses the need for careful labelling and strategies to prevent and detect contamination of food products with hazelnut residues.

Keywords double-blind placebo-controlled food challenge, food allergy, hazelnut allergy, minimum provoking dose, threshold dose

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Introduction

It is important for food-allergic patients to avoid the offending food. The success of elimination diets, however, depends on the recognition by the consumer of the offending food in food products. The development of new food products and the global food marketplace have led to a variety of untraditional and unexpected ingredients and foods being present in the diet. Due to several factors, including unsatisfactory labelling regulations, undeclared common allergenic food residues can be present in consumer products [1–3]. According to the FAO/WHO, manufacturers are not obliged to specify ingredients if they are part of a food component that constitutes less than 25% of the

total product [4]. For example, if cheese on a pizza constitutes less than 25% of the total product, the ingredients of the cheese (i.e. milk) need not to be declared on the label. Amendments to the current rules for declaration are being discussed worldwide, and in some countries, such as the USA and Canada, very strict regulations are already in effect [3–5]. Apart from loopholes in labelling regulations, the unintended presence of hidden ingredients in food products due to contamination during food processing also constitutes a matter of concern [2, 6].

Several case reports have been described concerning food-allergic patients developing severe allergic reactions after ingestion of pre-packaged foods, either contaminated or supplemented with the offending food, but without (clear) declaration on the label [2, 6–8]. From these case reports and additional immunochemical analysis of the offending food, knowledge is gained about the minute quantities of food allergens capable of eliciting allergic reactions in some allergic patients [2, 9].

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Risks of developing allergic reactions depend on the individual's sensitivity and the (level of) exposure to the allergen [5]. For risk assessment, information about the exposure of food-allergic patients to certain doses of the offending food needs to be combined with information about the distribution of threshold levels for eliciting allergic reactions in the allergic population [10]. In a previous study aiming at determining threshold doses for peanut, the minimum threshold dose capable of inducing (subjective) allergic reactions appeared to be as low as 100 µg of peanut protein [11]. There is little information concerning threshold doses in hazelnut-allergic patients. Yet, hazelnut allergy is common in Western Europe, mainly as part of the para-birch syndrome [12]. Symptoms vary from the oral allergy syndrome (OAS) to gastrointestinal symptoms and wheezing but even fatal reactions have been described, mainly in children and adolescents [13–16]. Doses of about 7 and 10 mg of hazelnut protein have been described to induce respiratory or gastrointestinal symptoms in allergic patients [2, 17]. The ubiquitous use of both roasted and dried hazelnuts in pre-packaged foods further emphasizes the need to evaluate the sensitivity of allergic patients to hazelnut.

In the present study, patients underwent double-blind, placebo-controlled food challenges (DBPCFCs) with dried hazelnut meal to determine the distribution of threshold doses in a hazelnut-allergic population.

Methods

Patients

Adult patients of the outpatient department of Allergology of the University Medical Centre Utrecht with a history of adverse reactions to hazelnut and a positive skin prick test (SPT) and/or an elevated specific IgE level to hazelnut were included in the study. Hazelnut-specific IgE levels were determined using CAP system FEIA (fluoro-enzyme immunoassay from Pharmacia & Upjohn, Uppsala, Sweden) and RAST [18, 19]. IgE levels of ≥ 0.7 kU/L or IU/L were regarded positive. Exclusion criteria were: pregnancy, use of β -blocking agents, angiotensin-converting enzyme (ACE)-inhibitors or immunosuppressive agents and any relevant diseases. Before the challenge, all patients were examined regarding their general health condition. Furthermore, coexisting allergies or sensitizations were investigated to obtain information about possible cross-reactivity patterns between pollen, hazelnut and other plant foods, that may play a role in the included patients [13]. Additional food-related symptoms to apple, almond and peanut were reviewed and documented. SPTs were performed for birch pollen, grass pollen and hazelnut in a concentration of 10 000 BU/mL, almond in a concentration of 1:20 g/v and peanut in a concentration of 1:20 w/v using commercial extracts (ALK ABELLÓ, Nieuwegein, the Netherlands) and for apple using the prick-to-prick method with fresh fruit (Granny Smith variety). SPTs were performed and documented according to Dreborg [20], and were regarded positive when the mean weal diameter was at least half of the histamine weal. Total IgE and specific IgE levels for the above-mentioned allergens were determined using CAP system FEIA (Pharmacia & Upjohn). Patients entered the study when physical examination, serum chemistry, haematology and an electrocardiogram

revealed no abnormalities. Antihistamines were stopped for 1 week, short-acting β -agonists for 8 h and long-acting β -agonists for 48 h before challenge. All patients completely avoided the offending food for at least 2 weeks before the challenge.

Ethical considerations

This study was reviewed and approved by the Ethics Committee of the University Medical Centre Utrecht. All patients gave written informed consent before enrolment in the study.

Hazelnut source

Raw, dried hazelnuts (Barcelona variety) were purchased from Westnut (Dundee, OR, USA). The whole nuts were ground to a meal and stored at -20°C until use. The protein content of the hazelnut meal was 15.73% determined using Kjeldahl analysis [21]. Dried hazelnuts were chosen instead of roasted because Cor a 1 was shown to lose its IgE-binding capacity above a temperature of 80°C [22].

Challenge meals

The hospital pharmacy weighed out seven doses: 1 mg, 3 mg, 10 mg, 30 mg, 100 mg, 300 mg and 1 g of hazelnut protein (corresponding to 6.4, 19, 64, 190, 640, 1900 and 6400 mg of hazelnut meal, respectively). These doses were completed with a mixture of dry oatmeal flakes 0.67 g/g, brown sugar 0.33 g/g and salt 0.0033 g/g up to a total weight of 15 g per test meal. The placebo doses consisted of oatmeal flakes, brown sugar and salt alone. Just before administering, the test meal was mixed with water of a temperature of 40 – 50°C to obtain oatmeal porridge. All patients were noseclipped while eating the test meals. The increasing hazelnut doses were randomly interspersed with an equal number of placebos by the pharmacy so that the investigator could stay blinded when administering doses to the patient.

DBPCFC

Challenges were performed only when patients were symptom-free. On the day of the challenge only a light breakfast (crackers with tea or coffee) was allowed (> 1 h before the first test meal). The challenges were performed in a clinical research setting equipped for resuscitation and monitoring of vital signs. Intravenous access was established before the first test meal and rescue medication was present in the room. During the challenge the investigator or a nurse permanently accompanied the patient. Before and at regular intervals during the challenge, blood pressure, heart rate and the forced expiratory volume in 1 second (FEV₁) were measured for safety reasons and as parameters for detecting allergic reactions. A decrease in mean arterial pressure (MAP) with ≥ 15 mmHg or a fall in FEV₁ with $\geq 20\%$ were regarded clinically significant. Other objective symptoms indicative for an allergic reaction that were assessed during the challenge included skin and oral cavity-symptoms, wheezing, gastrointestinal symptoms like vomiting and diarrhoea, and rhinoconjunctivitis (scored according to Lebel) [23]. The skin and oral cavity were inspected before the challenge for pre-existing lesions and during the challenge when symptoms like itching or a feeling of swelling occurred. Subjective allergic reactions like itching of the skin or the oral cavity (referred to as the oral allergy syndrome, OAS) [24], nausea, abdominal pain and dyspnoea (without wheezing or decrease in

FEV₁) were scored using visual analogue scales (VAS). An interval of 30 min was allowed between each test meal when no reaction occurred, or longer if (subjective) symptoms were still present. A challenge was discontinued when objective symptoms occurred or when subjective symptoms lasted for more than 1 h. A challenge was regarded positive when objective symptoms were observed or when subjective symptoms were reported for at least three times after active doses [25]. The minimum provoking dose was determined as the lowest dose of hazelnut protein eliciting a convincing allergic reaction, but it is important to realize that the minimum provoking dose can also lie somewhere between the highest observed hazelnut dose not eliciting an allergic reaction (the 'no observed adverse effect level, NOAEL') and the 'lowest observed adverse effect level (LOAEL)' [10].

Patients were kept for observation for at least 2 h after ingestion of the last test meal. All patients were contacted by phone the day after the challenge to document any late reactions.

Statistics

Exact 95% confidence intervals (CI) for the range of threshold levels were calculated according to a binomial distribution (Clopper-Pearson) [26].

Results

Patients

Thirty-one adult patients with a mean age (range) of 32 years (18–55) were challenged. The characteristics concerning their hazelnut allergy and accompanying sensitizations are summarized in Table 1. All patients had a positive SPT for hazelnut. However, hazelnut-specific IgE levels were < 0.7 kU/L in 19 out of 31 patients using CAP system FEIA and < 0.7 IU/mL in 12 out of 31 patients using RAST. Apart from immediate type symptoms, as listed in Table 1, two patients mentioned a worsening of their atopic dermatitis the day after ingestion of hazelnut.

Sixteen patients suffered from asthma (well controlled), 19 from atopic dermatitis and 29 patients had symptoms of seasonal rhinoconjunctivitis. Accompanying food allergies (determined by case history and positive SPT and/or RAST) mainly concerned apple (81%) and almond (58%). Peanut allergy was present in 11 out of 31 patients (35%). According to case history, the first hazelnut-related symptoms were noticed after the development of birch pollinosis in all except seven patients (1, 13, 18, 19, 24, 30 and 31). Five of these patients already had hazelnut-related symptoms from childhood and two other patients did not have birch pollinosis.

Table 1. Patient characteristics

No.	Sex	Age (year)	Pollen sens	Hazelnut-related symptoms (case history)	From the age of	Skin prick test hazelnut	Cap hazelnut (kU/L)	RAST hazelnut (IU/mL)	Total IgE	Accompanying food-allergies
1	M	55	–	OAS, RC	44	3+	< 0.35	< 0.3	40	Al
2	F	46	B	OAS	30	2+	< 0.35	0.8	27	Al, ap
3	F	39	B	OAS	36	2+	< 0.35	0.4	132	Al, ap
4	M	50	B	OAS	47	3+	< 0.35	1	126	Al, ap
5	M	52	B/G	OAS	42	3+	0.95	1.9	69	Ap
6	M	35	B/G	OAS	30	3+	< 0.35	< 0.3	97	Al, ap
7	M	23	B/G	OAS	21	3+	4	6.2	> 5000	Al
8	F	23	B/G	OAS	30	3+	0.6	0.3	428	
9	M	51	B/G	OAS	23	3+	< 0.35	0.4	89	Al, ap
10	F	35	B/G	OAS	28	3+	7.6	7.9	311	Pe, al, ap
11	F	24	B/G	OAS, nausea	21	3+	0.38	1.3	555	Al, ap
12	F	25	B/G	OAS, RC	12	3+	0.63	2.1	914	Ap
13	F	33	B/G	OAS, AD	4	4+	3.9	1.7	> 5000	Pe, ap
14	M	33	B/G	OAS	30	3+	2.34	1.2	297	Pa, ap
15	F	25	B/G	OAS	14	2+	9.2	6	> 5000	Pe, al, ap
16	F	31	B/G	OAS, urticaria	29	2+	1.28		174	Pe, ap
17	M	28	B/G	OAS	27	2+	< 0.35	< 0.3	554	Al, ap
18	F	22	G	OAS, urticaria, AE, AS	21	3+	< 0.35	< 0.3	81	
19	M	22	B/G	OAS	3	2+	4.3	3.9	752	Pe
20	F	23	B	OAS	21	3+	< 0.35	0.3	26	Pe, al, ap
21	F	42	B/G	OAS, urticaria	20	3+	< 0.35	0.3	154	Pe
22	F	23	B/G	OAS	17	2+	< 0.35	< 0.3	19	Al, ap
23	F	30	B/G	OAS, nausea	25	3+	< 0.35	0.5	86	Pe, al, ap
24	M	19	B/G	OAS	1	3+	1.1	1.2	125	Al, ap, pe
25	M	18	B/G	OAS, AD	15	3+	0.45	0.7	196	Pe, al, ap
26	M	46	B	OAS	23	3+	0.5	1.3	92	Al, ap
27	M	30	B/G	OAS	24	3+	3.5	4	349	Pe, ap
28	F	19	B/G	OAS	16	3+	2.24	2.3	409	Ap
29	M	25	B/G	OAS	21	2+	0.51	1.1	151	Ap
30	F	35	B/G	OAS	6	3+	< 0.35	< 0.3	88	Al, ap
31	F	19	B/G	OAS	3	4+	6.2	2.2	1331	Ap

OAS, oral allergy syndrome; B, birch pollen; G, grass pollen; RC, rhinoconjunctivitis; AE, angio-oedema; AD, worsening of atopic dermatitis; AS, anaphylactic shock; al, almond; ap, apple; pe, peanut.

Twenty-six patients mentioned accidental ingestion of hazelnut in the previous year, due to hidden or undeclared hazelnut, mainly in pastry or chocolate products. The symptoms consisted of OAS in all patients except for patient 18. This patient experienced OAS, generalized urticaria, angio-oedema and hypotension after ingestion of two chocolate cookies. She was treated at the hospitals emergency department for anaphylactic shock. For five patients the last known ingestion of hazelnuts was more than 1 year ago.

Symptoms as observed during DBPCFCs

Of 31 challenged patients, 29 patients did react during the hazelnut challenges, confirming their hazelnut allergy. One patient (no. 15) had symptoms of OAS after several active doses but also had mild subjective symptoms after all placebo doses. So, independently from the question as to whether this patient is hazelnut-allergic or not, it was not possible to determine a reliable threshold dose, and therefore this patient was omitted from further analysis in this study. One patient (no. 21) did not react during the challenge.

Reactions as observed during the 29 positive challenges all started with the typical itching or feeling of tightness in the oral cavity or tingling of lips (OAS) within 5–10 min after ingestion of the test meal. Five patients also suffered from gastrointestinal symptoms and one patient also reported difficulty in swallowing. All subjective reactions were reported repeatedly (> 3 times) after active doses in all but one patient. One patient (no. 9) became anxious after the first (subjective) symptom of tightness of his throat and difficulty in swallowing, resulting in premature discontinuation of the challenge. However, because of his convincing reaction after an active dose without any placebo responses, it was still possible to determine a reliable threshold dose in this patient. The severity of subjective symptoms (OAS and gastrointestinal symptoms like abdominal pain and nausea) increased after higher hazelnut doses in most patients as revealed by increasing VAS-scores (not shown).

Two patients (18 and 31) exhibited additional objective symptoms. Patient 18 experiencing OAS from a dose of 100 mg of hazelnut protein, developed generalized urticaria and lip swelling 1 h after the highest dose of 1 g of hazelnut protein. Patient 31 developed slight lip swelling after the lowest dose of 1 mg of hazelnut protein in addition to OAS.

Nine challenges were discontinued because of consistent subjective symptoms for longer than 1 h ($n = 7$), anxiety ($n = 1$) or objective symptoms ($n = 1$). Oral antihistamines were required in six patients. Patient 18 developed generalized urticaria and received clemastine and prednisolone intravenously. The reported or observed symptoms, the minimum provoking doses, the highest ingested dose and the required therapies are summarized in Table 2. No delayed or late reactions (> 2 h after ingestion) were reported.

During the 29 positive challenges, mild aspecific placebo responses were reported in 5% (nine out of 180 given placebos), with a maximum of one placebo response per patient against three or more reactions to active doses.

Minimum provoking doses

Of 29 positive challenges, four patients already reacted to the lowest dose of 1 mg hazelnut protein. Threshold doses for subjective reactions appeared to vary from 1 mg up to a dose

between 30 and 100 mg hazelnut protein. A dose-response curve is shown in Fig. 1. As can be extrapolated from this Figure, 50% of a hazelnut-allergic population will suffer from an allergic reaction after ingestion of 6 mg (95% CI, 2–11 mg) hazelnut protein, and after ingestion of 100 mg hazelnut protein all hazelnut-allergic patients (95% CI, 88%–100%) will have suffered a kind of allergic reaction.

Discussion

In this study, DBPCFCs were performed in 31 patients sensitized to hazelnut and with a history of adverse reactions to hazelnut. Twenty-nine patients had a positive challenge, confirming their hazelnut allergy. One patient had a negative challenge, leaving the question whether this patient is allergic to hazelnut or not or whether her threshold level is higher than 1 g of hazelnut protein (6.4 g of hazelnut meal). Although the DBPCFC is considered to be the gold standard for diagnosing food hypersensitivity [25, 27], there is no international standardization how to perform such challenges [28]. It has been suggested that once an amount of 8–10 g of dried food is tolerated during DBPCFC, food hypersensitivity can be ruled out, but should be confirmed by a negative open challenge [25, 27]. Ortolani et al. performed open challenges after negative DBPCFCs with hazelnut using a maximum cumulative dose of seven hazelnuts [14]. In this study, no open challenges were performed, but the patient who tolerated a cumulative dose of more than eight hazelnuts (> 8 g of hazelnut meal) was believed not to be allergic to hazelnuts. This presumption was stressed by the observation that all other patients already reacted after a dose of 100 mg of hazelnut protein, a dose 10 times below the highest used dose.

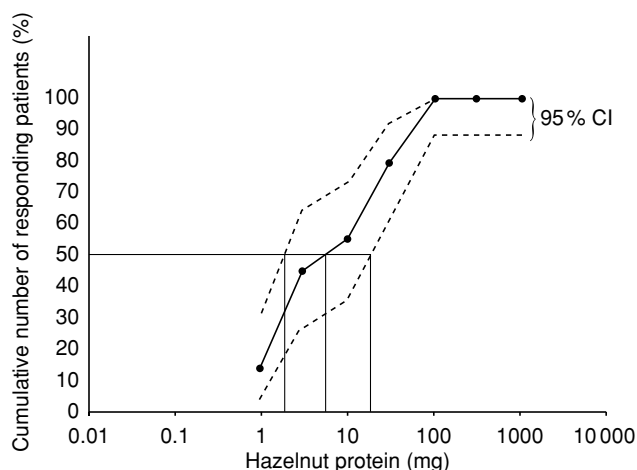
With respect to the tests used to determine sensitizations in patients included in this study it was observed that in contrast to the 100% sensitivity of SPTs with commercial hazelnut extract, the sensitivity of CAP-system FEIA appeared to be very low (35% using a cut-off level of 0.7 kU/L). In a recent multicentre study, however, a sensitivity of 74.6% was calculated for hazelnut using this CAP system [14]. Perhaps differences in patient characteristics and, accordingly different allergen recognition profiles, can explain this observation. Nevertheless, the detection of hazelnut-specific IgE using RAST demonstrated a higher sensitivity (59% using a cut-off level of 0.7 IU/mL).

Little is known about the stability of hazelnut allergens. Immunoblotting and IgE inhibition experiments showed that heating hazelnuts with temperatures of 100–185 °C resulted in a decrease in IgE-binding activity to hazelnut allergens apart from one allergen of < 14 kDa that appeared to be very heat-stable [29]. Investigation of mediator-release from basophils sensitized with antibirch pollen IgE showed a decrease in immunoreactivity after roasting hazelnuts, pointing towards a heat lability of the birch pollen-related major hazelnut allergens [30]. In fact, in an earlier report we demonstrated a decrease in IgE-binding activity of Cor a 1 after heating to temperatures of 80 °C and higher [22]. Most patients in this study are sensitized to birch pollen so it is possible that a substantial part of the hazelnut sensitizations in this study can be explained by IgE to (heat-labile) birch pollen-related hazelnut-allergens [13]. Indeed, most patients suffered from OAS after ingestion of raw (dried) nuts, hidden in pastry or chocolate. Four patients

Table 2. Symptoms and minimum provoking doses of 29 patients

No.	Reaction (s) during challenge	Minimum provoking dose (mg hazelnut protein)		Highest received dose during challenge (mg hazelnut protein)	Therapy required
		Subjective reaction	Objective reaction		
1	OAS	100		1000	Oral antihistamines
2	OAS	3		300	
3	OAS	30		1000	Oral antihistamines
4	OAS	100		1000	
5	OAS	30		1000	Oral antihistamines
6	OAS	30		1000	
7	OAS	3		1000	Oral antihistamines
8	OAS	30		1000	
9	OAS, DS	100		100	Oral antihistamines
10	OAS	3		300	
11	OAS	30		1000	Oral antihistamines
12	OAS	3		1000	
13	OAS	1		100	Oral antihistamines
14	OAS	10		300	
16	OAS	30		1000	Oral antihistamines
17	OAS, GI	3		300	
18	OAS, LS, UR	100	1000	1000	Intravenous antihistamines + prednisolone
19	OAS	30		1000	
20	OAS	100		1000	Oral antihistamines
22	OAS, GI	10		1000	
23	OAS, GI	3		100	Oral antihistamines
24	OAS	1		1000	
25	OAS	10		1000	Oral antihistamines
26	OAS	3		1000	
27	OAS	3		1000	Oral antihistamines
28	OAS	100		1000	
29	OAS	1		1000	Oral antihistamines
30	OAS, GI	3		300	
31	OAS, GI, LS	1	1	30	

OAS, oral allergy syndrome; DS, difficulty in swallowing; GI, gastrointestinal symptoms; LS, lip swelling; UR, urticaria (generalized).

**Fig. 1.** The cumulative number of responding patients to increasing doses of dried hazelnut ($n = 29$).

mentioned allergic symptoms after eating chocolate cookies (hiding heated hazelnut meal). This study confirmed the relevance of dried hazelnuts as it can induce allergic symptoms. However, as patients with IgE directed to stable

non-pollen-related hazelnut allergens may suffer from more severe allergic reactions compared to patients with pollen-related food allergy [31, 32], minimum provoking doses could be different in both groups and attention needs to be paid to the different allergen recognition profiles of patients to elucidate this.

Because we used dried hazelnuts, the dark brown testa could not be removed from the raw nuts and were visible as observed by some patients and the investigator in the highest two doses of 300 and 1000 mg of hazelnut protein. This could have endangered the double-blind character of the challenges, but as the highest threshold dose appeared to be 100 mg it is unlikely that it did interfere with establishing reliable threshold doses.

Threshold levels for subjective reactions found in this study (using raw, dried hazelnuts) varied between a dose lower than 1 mg hazelnut protein (equal to 6.4 mg of hazelnut protein) up to a dose between 30 and 100 mg of hazelnut protein (i.e. between 190 and 640 mg hazelnut meal). The lowest dose inducing objective symptoms was 1 mg of hazelnut protein. These low values resemble the threshold values demonstrated by Hourihane et al. in peanut-allergic patients [11]. After a dose between 30 and 100 mg of hazelnut protein (corresponding to one-third of a nut), all patients had developed an allergic reaction. Figure 1 shows that 55% (95% CI, 36–74%) of a

hazelnut-allergic population will react to a dose between 3 and 10 mg of dried hazelnut protein, assuming that the included patients are representative for the general population. However, as cross-reactivity with birch pollen may be involved in some areas, like in Western Europe, these observations can not just be extrapolated to other parts of the world.

In summary, threshold levels, as found in 29 hazelnut-allergic patients, varied between a dose of 1 mg up to a dose between 30 mg and 100 mg of hazelnut protein. Because these threshold levels equal doses of hidden hazelnut sometimes present in consumer products, a more precise declaration of hazelnuts and strategies to prevent and detect contamination of food products with hazelnut residues are recommended.

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