



Risk of consuming products with or without precautionary wheat or gluten labelling for persons with coeliac disease

Sisse Nygaard Rasmussen^{a,b}, Håkan Vigre^a, Charlotte Bernhard Madsen^{a,*}

^a National Food Institute, Technical University of Denmark, Denmark

^b New Nordic Manufacturing ApS, Denmark

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ABSTRACT

Persons with Coeliac disease (CD) need to keep a lifelong diet without gluten and therefore need to know which foods are safe to eat. The objective of this study was to analyse ordinary foods products without gluten containing ingredients (PWG) with or without the precautionary allergen statement (PAL) "May contain wheat/gluten" or similar wording and use these data to perform a probabilistic risk assessment. Foods with and without PAL were analysed for gluten. Inputs for the risk assessment were analytical results, daily food consumption and data from a 90-days challenge study in coeliac patients (Catassi et al., 2007). 20/77 products with PAL and 10/51 without PAL had gluten ≥ 5 mg/kg 3/77 with PAL and 3/51 without PAL had gluten ≥ 20 mg/kg. A coconut cookie brand was outlier with 421 mg gluten/kg. The likelihood of developing mucosal damage in the CD population when eating the same PWG for 90 days in addition to certified gluten-free products was low (0.2–3.2%) with little difference between with or without PAL. Including the coconut cookie results increased the risk to 3.8–9.2%.

Caution should be taken in connection to foods where there is an obvious chance of contamination.

1. Introduction

Persons with Coeliac disease (CD) need to keep a lifelong diet without gluten and therefore need to know which foods are safe to eat (Fasano and Catassi, 2001).

Coeliac disease (CD) is an immune-mediated response triggered by ingestion of gluten in wheat, rye and barley. The disorder only develops in genetically susceptible individuals and is characterised by T-cell mediated inflammation of the small bowel mucosa accompanied by intestinal malabsorption (Catassi et al., 2007, Sabatino and Corazza 2009).

The international regulation of the composition and labelling of foodstuff suitable for persons with CD changed in 2008 (Codex Alimentarius Standards for foods special dietary use for persons intolerant to gluten, 1979) and was adopted by the European Union in 2009 (Regulation (EC) 41/2009) (Regulation (EU) No. 828/2014). The regulation specifies that products labelled "gluten-free" cannot exceed 20 mg gluten/kg. In addition to food products having the gluten-free claim there are ordinary food products without gluten containing ingredients. These may be contaminated with gluten and may or may not be labelled with a precautionary allergen labelling (PAL). PAL may be

used if there is a risk of unintended allergen presence (UAP) in a product. It was introduced by the industry, making it possible to communicate to allergic consumers that products may not be suitable for them, due to UAPs. PAL is voluntary and not explicitly regulated (Remington et al., 2015; DunnGalvin et al., 2019). PAL is used frequently where equipment or facilities are shared between formulations, which do and do not contain allergens. The widespread use of PAL limits the food choices of allergic consumers (Remington et al., 2015).

To date, probabilistic risk assessment of gluten in pre-packed food products in relation to coeliac disease has only been conducted using data from certified gluten-free (CGF) products. It was found that the mean percentage of the European population with CD at risk of mucosal damage resulting from consumption of these products ranged from 0.01 to 0.15%, and was <1% even in the worst case scenario where a threshold for intestinal damage at 10 mg/d was used (Gibert et al., 2013).

To maintain a varied food supply and a balanced diet, persons with CD also consume products that are not certified gluten-free. These products do not have gluten-containing ingredients in the list of ingredients. Some of these have PAL stating "May contain wheat or May contain gluten" others have not. The question for the person with CD is,

* Corresponding author. National Food Institute, Technical University of Denmark, Kemitorvet, Building 202, DK, 2800, Kgs. Lyngby, Denmark.

E-mail address: charm@food.dtu.dk (C.B. Madsen).

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whether it is safe to eat these products and whether products with PAL constitute a higher risk than products without PAL.

The aims of this study were i) to investigate gluten contamination of ordinary food products without gluten (PWG) containing ingredients both with and without the PAL warning “May contain wheat/gluten”, and ii) to assess the risk of adverse response of the CD population triggered by consumption of these products.

2. Materials and methods

2.1. Gluten contamination in food products without gluten (PWG) containing ingredients

2.1.1. Gluten products for analysis

One hundred twenty-eight ordinary food products without gluten as an ingredient, with or without PAL were purchased from Danish supermarkets. A sampling plan was developed based on results from The Nordic co-operation's report *Undeclared allergens in food – Food control, analyses and risk assessment* and UK Food Standards Agency (FSA) report *Survey of Allergen precautionary allergen Labelling* (Bolin and Lindeberg, 2016; Hirst, 2014). The following product categories were included based on expected risk of gluten contamination.

- Spices
- Snacks
- Ready meals
- Cereal & cereal products
- Dried sauces, gravies & mixes
- Confectionery

2.1.2. Gluten analyses

From each food item two 30 g samples were homogenised and analysed separately. From each 30 g sample 0.25 g was extracted giving 256 samples in total. Samples were extracted with the Ridascreen Cocktail (R7006/R7016).

The extraction procedure depended on the food matrix. For spices and spice mixes, 0.25 g of the sample and 0.25 g of skimmed milk powder was mixed with 2.5 ml cocktail. Skimmed milk powder was added to bind tannin and polyphenols. For other food samples, 0.25 g of the sample was mixed with 2.5 ml of the cocktail. Positive control: 0.25 g of the positive sample, was mixed with 2.5 ml of the cocktail.

Samples were extracted 40 min at 50 °C, cooled down before adding 7.5 ml 80% ethanol and extracted while shaken upside down for 1 h at room temperature and centrifuged at 2500 g for 10 min. One ml of the supernatant was transferred to a screw top vial and stored in dark at room temperature until ELISA was performed.

After purchase of foods, samples were extracted in a running process and stored until the ELISA test could be performed according to the instruction from the kit manufacturer. Each ELISA plate included a positive control to control for variation.

The gluten content was measured by the sandwich R5 ELISA method from RIDASCREEN (Gliadin ELISA art. no. R7001; R-Biopharm AG). The limit of quantification (LOQ) was 5 mg/kg gluten, and the limit of detection (LOD) was 1 mg/kg gluten.

2.1.3. Positive control

A positive control containing a mean amount of 8.8 mg gluten/kg (L93), kindly provided by Swedish National Food Agency, was used on every ELISA plate. The ELISA results were accepted if the result of the positive control was between 5.39 mg/kg (m-3sd) and 12.21 mg/kg (m+3sd). If not, the ELISA results were invalid and should be replicated. No ELISA plates were replicated.

2.2. Quantitative risk assessment of gluten in food products without gluten (PWG) containing ingredients

A probabilistic model, estimating the average likelihood of developing mucosal damage in the small intestine, in a population of patients with CD when eating food products without gluten containing ingredients was developed.

To take into account the individual variation in daily consumption of different food groups; variation in the gluten contamination; and individual variation in likelihood of developing mucosal damage in the intestine given exposure to gluten, these sources of variation were included in a Monte Carlo simulation model as probabilistic distributions. Each iteration represents the likelihood for one random patient with CD to develop mucosal damages given a random selected daily consumption over a period of 90 days of different products with randomly selected concentration of gluten. Each simulation included 100,000 iterations, and the population average of likelihood of developing damages was calculated as the mean of the likelihoods obtained in the iterations.

The Monte Carlo model was executed in the software ModelRisk, version 6.1.46.

The input parameters for each iteration were.

- 1) Total daily consumption of the relevant food groups [gram/day]
- 2) Concentration of gluten in the relevant food group [mg gluten/gram]
- 3) A likelihood function describing the of likelihood of mucosal damage given exposure [probability/mg gluten]

2.2.1. Daily food consumption

The amount of food consumed in a meal was taken from (Biro et al., 2017). In this paper food consumption data from the Danish National Food Consumption Survey are grouped for use in food allergen risk assessments. The tested food products were categorised with the best describing groups from Biro, giving the groups described in Table 1.

In the model, the daily intake from each food group was defined as a normal distribution utilizing the values presented in Table 1, where negative values were truncated to zero (meaning no intake).

To simulate a daily consumption, the amount consumed in one meal from one product from each food group was included. This did not apply to Soups, Mashed potato powder or Composite dishes. Only one of these were included per day to represent one warm meal. This resulted in a mean food consumption of PWG food of 0.6 kg/day (SD 0.2) used in all analyses.

In the assessment it was assumed that a person was eating the same food and the same amounts of food every day for 90 days.

Table 1

Food groups from Biro et al. (2017) including mean and standard deviation of consumption used in risk assessment.

Food Group	Mean (g)	Standard deviation (g)
Herbs and spices mixes, bouillon cubes yeast extract	18	32
Soups	396	170
Mashed potato powder	151	170
Composite dishes	205	122
Cookies	38	26
Cereal bars	27	24
Peanuts, nuts and dried fruit	24	29
Legumes	110	70
Potato and other starch-based chips (including salty sticks)	42	39
Small sweets	41	39
Breakfast products eaten unprocessed (e.g., müsli, oats, and maize flakes)	44	28

2.2.2. Determination of gluten threshold distribution

A quantitative model describing the likelihood of adverse response given exposure to gluten was estimated utilizing previously published data describing the effect of gluten challenge in persons with coeliac disease (Catassi et al. 1993, 2007).

In the studies by Catassi et al. the response to gluten was measured as change in small intestinal morphology before and after challenge, in Catassi et al., (2007) in adults after 90 days challenge and in Catassi et al., (1993) in children after 28 days challenge. In our study, we used the data describing ratio between villus height and crypt depth (Vh/Cd) in the small intestine, because it is reported to be the coeliac disease parameter that best reflects the effects of gluten micro challenge (Catassi et al., 2007).

In both studies, patients were challenged with gliadin or gluten in addition to the gluten content of the gluten free diet. In Catassi et al. (2007), three groups with thirteen participants each, were exposed daily to placebo, 10 or 50 mg gluten. Vh/Cd from each patient before and after the intervention was reported as plots. We used the program Web-PlotDigitizer (Rohatgi, A. 2020) to obtain the quantitative values of Vh/Cd before and after exposure of each person included in the study. Thereafter, the adverse effect of the exposure was calculated as the percentage decrease in the Vh/Cd ratio. These data could be compared with the text stating how many patients had improved Vh/Cd during intervention. The 200 mg data on Vh/Cd was taken from Table I in Catassi et al. (1993) (see Table 2).

The observed proportional decrease (%) in Vh/Cd in each person in each exposure group is presented in Table 2. A decline above or equal to 10% was defined as “adverse effect”, whereas decline smaller than 10% or an increase was defined as “no adverse effect”. The 10% cut of, named Catassi 1, was chosen because we had to derive the data from a figure and in addition based on the assumption that, taking the morphometric method into account, a decline in Vh/Cd of 10% is a real change. The other, named Catassi 2, where any change in Vh/Cd was interpreted as an effect of treatment, is in accordance with the interpretation by the authors in Catassi et al., (2007) (see Table 3).

Table 2

Decrease in Vh/Cd after 90 days challenge with gluten from Catassi et al., (2007) and after 28 days challenge (200 mg) in children (Catassi et al., 1993). For the data from Catassi et al., (2007) the program WebPlotDigitizer was used to read the position on the Y-axis on the figure and turn it into a corresponding number. Negative numbers describe an increase i.e., improvement in Vh/Cd.

Placebo	10 mg	50 mg	200 mg
% decrease	% decrease	% decrease	% decrease
31.9	18.9	3.0	19.0
7.8	2.9	21.9	35.9
−1.4	25.0	19.4	12.9
−15.3	29.0	20.0	12.2
−10.6	9.1	13.8	16.9
−5.2	23.8	24.1	3.7 ^c
−10.8	−100.0	−12.5	10.4
−36.4	−68.4	20.8	−14.0 ^b
−16.6	−27.8	9.1	16.3
−7.1	−53.3	19.1	11.1
−16.5	6.7	15.0	
−3.5	−15.8	20.0	
−15.4	−x.x ^a	−50.0	

^a It was only possible to identify 12 pairs of data in the 10 mg group. As the authors describe that 6 of the 13 patients improve, the Vh/Cd of the unidentified patients must be in the improve group.

^b This child had a very low Vh/Cd before challenge and an increase in IEL during challenge, so the child is excluded because the improvement in Vh/Cd is disregarded as a result of challenge.

^c Although the Vh/Cd decrease was less than 10% we included this child in both groups in Table 3 as the IEL count nearly tripled (from 12 to 31 IEL x100) after challenge.

Table 3

Data for distribution of adverse effect of gluten challenge on Vh/Cd.

	Placebo	10 mg	50 mg	100 mg ^b
Decrease in Vh/Cd $\geq$ 10% ^a (Catassi 1)	1/13	4/13	9/13	9/9
Any decrease in Vh/Cd included (Catassi 2) ^c	2/13	7/13	11/13	9/9

^{a, b} Estimated. See text for explanation.

^c According to text in Catassi et al., (2007): 11/13 (placebo), 6/13 (10 mg), 2/13 (50 mg) improve Vh/Cd ratio.

In the 50 mg group, two patients had a clear improvement of the Vh/Cd ratio. This means that for these persons being on a well-controlled gluten free diet and in addition challenged with 50 mg had no deleterious effect. The level giving histological changes in all CD patients is therefore higher than 50 mg. In Catassi et al. (1993) children were challenged with gliadin for 28 days and there was an effect in all children in the 100 mg gliadin group (200 mg gluten) (Table 2). This means, that the dose that gives histological changes in the small intestine in all participants after chronic challenge lies somewhere between 50 and 200 mg/day. To be conservative and, to account for the shorter duration of challenge in the children, we chose a dose of 100 mg gluten/day as the estimated dose giving reaction in 100% of coeliac patients (Table 3).

Based on the data presented in Table 3, we built a logistic model with the exposure to gluten as explanatory variable with linear effect and the logit of the likelihood of adverse effect as dependent variable. The estimation was performed in the software R (version 3.5.1). The confidence interval of the effect (the slope) of exposure to gluten on the likelihood of adverse effect was calculated by the Wald method. The estimated model parameters (intercept and slope) were subsequently used to predict the likelihood of adverse effect given the simulated exposure to gluten. This was done by inverting the predicted value of logistic model given the exposure.

In addition to the above distributions, we performed analyses with the distribution used by Gibert et al., (2013). They also developed a distribution derived from Catassi et al., (2007). Here the 10 mg group was the “no observed adverse effect group” based on lack of statistically significant difference between the 10 mg and the placebo group. The 50 mg group was taken as the “lowest observed adverse effect level” based on statistically significant difference between the 50 mg and the placebo group. They used a normal distribution with a mean of 30 mg $\pm$ SD of 10 mg/day, assuming that the adverse effects can be described by a normal distribution.

2.2.3. Scenarios used in the risk assessment

The risk assessment was performed using three different scenarios of exposure.

- 1) Consumption of PWG,
- 2) Consumption of PWG with PAL
- 3) Consumption of PWG without PAL.

The input variables required for the implementation of the probabilistic approach were as follows:

- 1) the distribution of the consumption of the compiled food groups from Birot et al. (2017)
- 2) the distribution of gluten contamination obtained from the analysis made in the present study using LOD or LOQ as cut-off
- 3) the three distributions of gluten thresholds among the CD population as described above

A probabilistic risk assessment was conducted to evaluate the probability that CD patients will develop mucosal damage due to daily consumption of a compilation of products without gluten containing

Table 4

Overview of the model variables and input parameters.

	Distribution	Assumption	Data
Food consumption	Normal distribution	Negative set to zero One product from each food group One warm meal ¹	Mean and SD (Biro et al., 2017)
Gluten concentration	Uniform distribution		Present study (Table 5)
Frequency of gluten contamination	Binomial distribution		Present study
Gluten contamination	Zero-inflated uniform distribution		Present study
Gluten threshold distribution (Catassi 1)	Logistic distribution	$\geq 10\%$ decrease in Vh/Cd was included as an adverse effect	Catassi et al. (2007)
Gluten threshold distribution (Catassi 2)	Logistic distribution	Any decrease in Vh/Cd was included as adverse effect	Catassi et al. (2007)
Gluten threshold distribution (Gibert)	Normal distribution 30 ± 10 mg/d	Truncated at zero	Gibert et al. (2013)

Warm meal includes Soups, Mashed potato powder or Composite dishes. For further description see text.

ingredients that may be contaminated with gluten with or without PAL. The probabilistic approach uses distributions of the consumption, gluten concentration, and gluten thresholds (Table 4).

2.2.3.1. Consumption. The consumption data was presented as normally distributed on the mean and SD from Biro et al. (2007) (Biro et al., 2017) and organised as described in section 2.2.1.

2.2.3.2. Gluten contamination. Gluten contamination was based on the results from the gluten analyses giving levels as well as frequency of contamination. The concentration was presented as uniform distribution. Since not all products were contaminated, a binomial distribution of the frequency of contamination was used. The two distributions were combined into a zero-inflated uniform distribution of the contamination.

The distributions of the consumption and concentration formed the distribution of the exposure to gluten and the data from the challenge studies the distribution of the adverse effect on CD individuals after 90 days exposure to gluten. The probabilistic approach compared the

distribution of gluten exposure with the distribution of the adverse effect, thereby determining the probability of an adverse effect. For each iteration, which represents an individual, an exposure and sensitivity to gluten were drawn for the distributions. The comparison resulted in either a reaction or not.

2.2.3.3. Estimation of the effect of PWG. In every simulation, 100,000 iterations were used where each iteration represents one person's exposure to gluten from PWG and the likelihood of developing CD-related changes in the gut at that exposure. The increased likelihood of CD was calculated as the difference between the likelihood of reaction to exposure to gluten from PWG and the likelihood of reaction to placebo. The likelihood of reaction to placebo is equal to the logit inverse of the intercept in the regression model. The likelihood of reaction to PWG is equal to the logit inverse of (intercept + exposure*effect) in the regression model. The average increased likelihood of developing CD lesions when consuming PWG was estimated as the mean of the 100,000 values of increased likelihood of CD obtained in the simulation.

2.2.3.4. Statistical analyses. Association between PAL and gluten concentration ≥ 5 mg/kg (LOQ) or ≥ 20 mg/kg was performed as a Fishers-test. 20 mg/kg was tested since it is the regulatory limit for certified gluten free products.

3. Results

The logistic regression model describing the relation between gluten intake in addition to a gluten free diet and likelihood of developing CD, diagnosed as $>10\%$ change in the Vh/Cd ratio, is presented in Fig. 1a (Catassi 1). The estimated likelihood of developing CD related lesions at placebo (equivalent to a strict gluten free diet) was 13%, and there was a significant increase in the likelihood with increasing intake. In this model, at an intake of 32–33 mg pr day, 50% of the population with CD will develop morphological changes.

The logistic regression model describing the relation between gluten intake in addition to a gluten free diet and likelihood of developing CD, diagnosed as any change in the Vh/Cd ratio, is presented in Fig. 1b (Catassi 2). The estimated likelihood of developing CD related lesions at placebo was 26%, and there was a significant increase in the likelihood with increasing dose. In this model, at a dose 16–17 mg pr day, 50% of the population with CD will develop morphological changes.

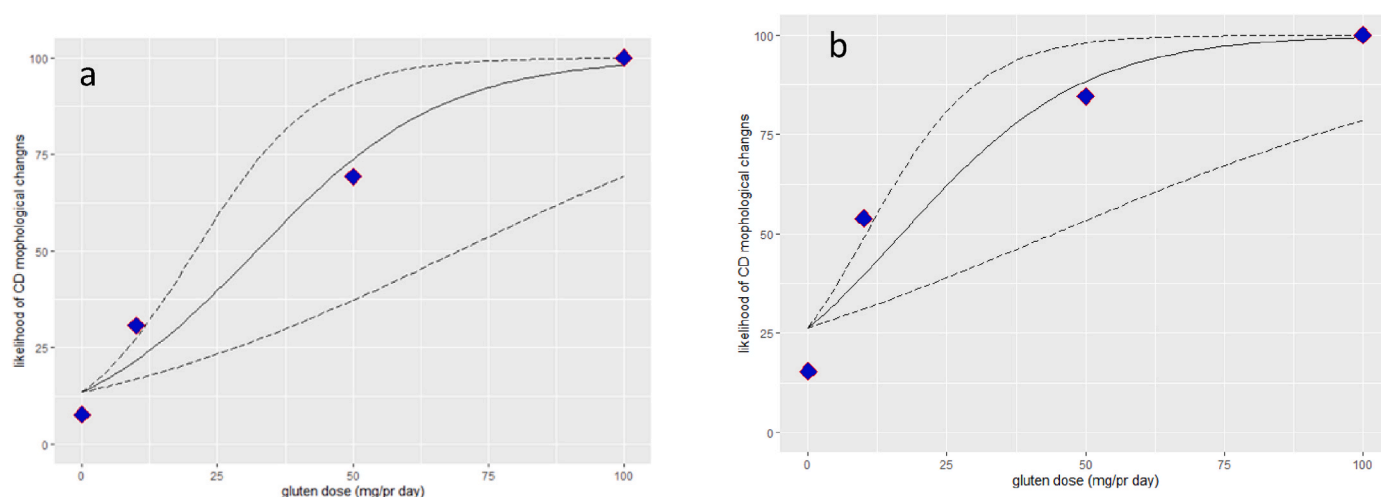


Fig. 1. The relationship between daily exposure to gluten (mg/pr day) and likelihood of CD morphological changes. Squares is observed data by Catassi et al. (data presented in see Table 3). a. Catassi 1 using 10% decrease in Vh/Cd as indication of CD. b. Catassi 2 using any % decrease in Vh/Cd as indication of CD. The solid line represents the estimated function of the relationship using the most likely value of the effect parameter estimated in the logistic model. The dashed lines represent the function using the upper and lower values of the 95% confidence interval of the effect parameter.

Table 5

Total number (N) of products tested in each food group allocated into the group without PAL (NO PAL) or with PAL (PAL). For each food group ELISA results above level of quantification (LOQ) are shown.

Food groups	TESTED			CONCENTRATION mg gluten/kg	
	N	N (NO PAL)	N (PAL)	NO PAL > LOQ	PAL > LOQ
Herbs and spices mixes, bouillon cubes yeast extract	56	21	35	12.5, 18.4, 18.4, 8.3, 6.3, 10.9	7.0, 9.0, 13.2, 20.5, 10.0
Soups	3	2	1	5.74	13.4
Mashed potato powder	4	0	4		6.7, 7.3
Composite dishes	7	3	4		5.8, 6.5
Cookies ^a	3	3	0	39.8, 421	
Cereal bars	5	1	4		6.5, 13.2
Peanuts, nuts and dried fruit	13	7	6		6.5, 25.4
Legumes	11	1	10		24.6, 14.6, 9.4, 6.6,
Potato and other starch based chips	5	2	3	21.2	7.4
Small sweets	16	8	8		5.2

^a The three cookies analysed were coconut cookies traditionally made using coconut flakes, egg white and sugar.

3.1. Gluten concentration

The results of the gluten analysis of the 128 pre-packed products with and without PAL is shown in Table 5. The gluten concentration was measured in two separate samples from each product. The two measurements showed good accordance in 119 products, which had a difference less than 10 mg/kg. The largest variation, in absolute numbers, were the coconut cookie giving 421 and 258 mg/kg respectively. The data shown below and used in the analyses are the highest value measured from each product. 30/128 (23.4%) products had quantifiable traces of gluten ≥ 5 mg/kg, 20/77 (26.0%) with PAL and 10/51 (19.6%) without PAL. The gluten concentration ranged from LOD to 421 mg gluten/kg for all products. Taking all products into account gluten concentration ranged from LOD to 25.4 mg gluten/kg with PAL and LOD to 421 mg gluten/kg without PAL. Thirty products corresponding to 23.4% of all products had a gluten concentration above LOQ in one or both extractions. Out of the 30 products above LOQ, 24 products had a concentration between 5 and 20 mg gluten/kg and six products had a concentration >20 mg gluten/kg. This corresponds to 18.8% of all products having a concentration between 5 and 20 mg gluten/kg gluten and 4.7% having a concentration ≥ 20 mg gluten/kg gluten. Thereby, 76.6% of all tested products had a gluten concentration <5 mg gluten/kg (LOQ) and 95.3% a gluten concentration <20 mg/kg 57 products had a gluten concentration between LOD and LOQ (≥ 1 and < 5 mg/kg).

3.2. Association of gluten contamination and PAL

Out of 30 products with a gluten concentration $\geq$ LOQ, 16% stated a PAL and 8% did not (Table 6 A). Of the 98 products below LOQ 42% had

Table 6

Association of gluten contamination and PAL in relation to LOQ (5 mg/kg) or to the regulatory limit for gluten free food (20 mg/kg).

A)		≥ 5 mg/kg	<5 mg/kg	Total
PAL	20	57	77	
No PAL	10	41	51	
Total	30	98	128	
B)		≥ 20 mg/kg	<20 mg/kg	Total
PAL	3	74	77	
No PAL	3	48	51	
Total	6	122	128	

no PAL and 58% had PAL (Fishers-test p-value 0.52).

Three products with PAL and three without PAL had a concentration ≥ 20 mg/kg (Table 6 B). (Fishers-test p-value 0.68).

3.3. Distribution of gluten intake

Fig. 2 shows the probability of gluten intake based on the total daily consumption of the relevant food groups [gram/day] and the concentration and frequency of gluten contamination in the relevant food group [mg gluten/gram].

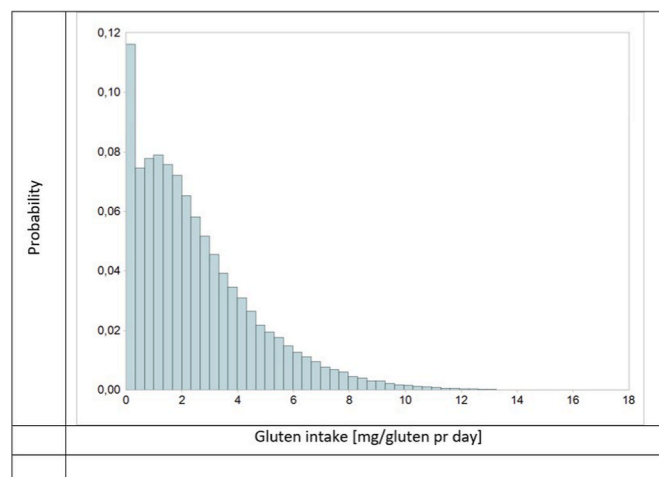


Fig. 2. Histogram of the probability of gluten intake from PWG. X-axis shows the distribution of gluten intake based on 1) the daily consumption of the relevant food groups [gram/day] from Birot et al., (2017) and 2) concentration and frequency of gluten contamination in the relevant food group [mg gluten/gram] found in the present study. Y-axis shows the probability based on the 100,000 iterations.

3.4. Percentage of CD population at risk

To estimate the additional risk of eating PWG on top of a gluten free diet we subtracted the risk in the placebo group. Thus, the effect of gluten intake from PWG were estimated as the likelihood of developing CD related gut lesions when including PWG, subtracted the likelihood of developing CD related gut lesions on a strict gluten free diet, representing the risk on a strict gluten free diet in Italy before 2007.

The probabilistic risk assessment was performed including all products, products with or without PAL, cut-off at LOQ or LOD, and with the three different distributions of gluten sensitivity (Catassi 1, Catassi 2, Gibert).

Table 7 shows that when using the “Catassi 1” distribution, the risk of consuming all PWG products with or without PAL in addition to consuming CGP is similar and low, when the product with the extreme result is excluded. Including the contamination level between 1 and 5 mg/kg increases the risk moderately in all groups. The higher risk connected to the group ALL is caused by the larger number of product groups with detectable gluten when combining the PAL and no PAL group. Including the coconut cookie (with 421 mg/kg and “no PAL”)

Table 7

Predicted (additional) risk [of CD] from consuming products without gluten containing ingredients (PWG) in addition to a gluten free diet based on Catassi 1. ALL (all products), PAL (products with PAL), No PAL (products without PAL). Probabilistic risk assessment was performed with or without the 421 mg/kg coconut cookie result.

100,000 iterations	ALL %	PAL %	No PAL %
Cut-off LOQ (5 mg/kg) Not including coconut cookie	1.9	1.5	1.3
Cut-off LOD (1 mg/kg) Not including coconut cookie	2.8	2.2	2.4
Cut-off LOQ (5 mg/kg) Including coconut cookie	6.7	1.5	6.0

Table 8

Predicted (additional) risk [of CD] from consuming products without gluten containing ingredients (PWG) in addition to a gluten free diet based on Catassi 2. ALL (all products), PAL (products with PAL), No PAL (products without PAL). Probabilistic risk assessment was performed with or without the 421 mg/kg coconut cookie result.

100,000 iterations	ALL %	PAL %	No PAL %
Cut-off LOQ (5 mg/kg)	3.2	2.6	2.3
Cut-off LOD (1 mg/kg)	4.7	3.8	4.1
Cut-off LOQ (5 mg/kg) Including coconut cookie	10.0	2.6	9.2

Table 9

Predicted (additional) risk [of CD] from consuming products without gluten containing ingredients (PWG) in addition to a gluten free diet based on Gibert et al. (2013). ALL (all products), PAL (products with PAL), No PAL (products without PAL). Probabilistic risk assessment was performed with or without the 421 mg/kg coconut cookie result.

100,000 iterations	ALL %	PAL %	No PAL %
Cut-off (5 mg/kg) Not including coconut cookie	0.2	0.2	0.2
Cut-off (1 mg/kg) Not Including coconut cookie	0.4	0.3	0.3
Cut-off (5 mg/kg) Including coconut cookie	4.3	0.2	3.8

increases the risk in the ALL and no PAL group considerably. It is worth noting that this is the risk when eating the same products in the same amount for 90 days.

Using the “Catassi 2” distribution where all decreases in Vh/Cd are included, increases the risk further in all groups (Table 8).

Using the distribution from Gibert et al. (2013) gives a very moderate additional risk when eating PWG although it increases when the extreme coconut cookie is included (Table 9). The distribution used here with a mean of 30 mg±SD of 10 mg/day implies that there are very few CD patients getting mucosal damage below the 10 mg dose and very few not getting mucosal damage above the 50 mg dose. In both cases 2.2%.

4. Discussion

This is the first study to quantify the risk of mucosal damage from ordinary food products without gluten containing ingredients in relation to CD. To do this we have used data from our own survey of gluten contamination, consumption data from the Danish National Food Survey (Biro et al., 2017) and challenge data from two studies (Catassi et al., 1993, 2007).

We used the R5 sandwich ELISA as this is a recommended method (Codex Alimentarius). We tested 60 g of product, two samples of 30 g each, homogenised and extracted separately. For financial reasons and according to the manufacturer's instruction, only 0.25 g of the homogenised sample was extracted. This small extraction size may explain the relatively large variation of contamination seen in the two samples (Sharma et al., 2020).

Gluten containing ingredients are an important part of many staple foods e.g., bread, pasta, cake making it a challenge to substitute these foods in the diet. For these food categories, persons with CD largely rely on certified gluten free foods. It is understandable, that the amount of gluten from certified gluten free food in the diet of CD persons has drawn much attention, but the daily diet also includes other pre-packaged foods without gluten containing ingredients. Verma et al. (2017) tested naturally gluten free products such as legumes, cereals, and nuts with or without PAL and found that 6% of products without PAL and 2% of products with PAL had gluten contents >20 ppm.

Two recent studies of food allergen contamination analysed composite foods and included gluten, as a measure for wheat contamination. A Nordic study analysed 167 products for gluten contamination 3.3% (1/30 products) with PAL contained gluten (6.4 ppm) and 4.4% (6/137 products) without PAL contained gluten (6.6–27 ppm) (Bolin and Lindeberg, 2016). A study from UK analysed estimated 404 products for gluten contamination. 6.1% (8/131 products) with PAL contain gluten (17.5–262.5 ppm) and 3.3% (9/273 products) without PAL contained gluten (13.75–62.5 ppm) (Remington et al., 2015).

Based on the Nordic and the UK study we selected foods where we expected the highest chance for contamination. As examples, based on the UK study we included spices and cereal bars and based on the Nordic study we did not include meat products and chocolate products because the Nordic study did not find any gluten contamination in these two food groups. None the less, we found that the large majority of PWG had gluten concentrations below LOQ of 5 mg/kg (76.6%) and nearly all (95.3%) a gluten concentration <20 mg/kg. We also found that PAL cannot be used as a guidance for CD persons, as there were no statistically significant differences in number of products contaminated between the PAL and the non-PAL group, and the level of contamination was comparable. The three cookie products investigated poses a special problem. They are all traditional Danish coconut cookies. These cookies are in their original recipe gluten free and may be produced in traditional bakery factories without special emphasis on cross-contamination with gluten. Apart from the two brands of coconut cookies, the highest levels of contamination in other products were close to 20 ppm.

We assume that the low level of gluten contamination is a result of focus on allergen control in food production. From 2004 when EU legislation on food allergens were implemented there has been an

increasing awareness and an increasing knowledge on how to manage food allergen contamination (DIRECTIVE, 2003/89/EC, [Food Drink Europe, 2013](#)). This is reflected in the results from Bustamante et al. (2017) showing that the level of gluten contamination in cereal based CGF and PWG has decreased in the period from 1998 to 2016. In addition, [Guennouni et al. \(2021\)](#) found that the prevalence of gluten contamination in all types of gluten free foods decreased between the time periods 2000–2008 and 2015–2020.

In food allergy risk assessment, it is assumed that if an allergic person includes a product in the diet, the amount consumed is the same as in the general population. We made the same assumption in this study and used data from the Danish national food survey grouped to be suitable for food allergy risk assessment ([Biro et al., 2017](#)). In contrast to food allergy, the risk assessment in CD must be based on consumption over a longer period. We assumed that the CD consumer will consume one portion from each food group each day although only a portion from one of the categories: soups, mashed potato powder or composite dishes. We chose this approach realizing that we would probably overestimate the daily consumption and thereby the risk. The total daily consumption of PWG in our model was 0.6 kg/day (SD 0.2). In addition to this, persons with CD are expected to eat certified gluten free food. In [Catassi et al., 2007](#) the mean consumption of CGF is estimated to be 332 g/day (SD 98) and in [Gibert et al. \(2006\)](#) the median intake is 173–268 g/day. The total consumption in Danish adults of solid food, including processed and unprocessed food is 1.17 kg/day (SD 0.38) (Christensen personal communication from the Danish national food consumption database). When we add CGF consumption of 0.2–0.3 kg/day to the intake of PWG of 0.6 kg/day there is still room for consumption of the food groups we did not include e.g., meat and chocolate product as well as unprocessed food such as fruit and vegetables.

Probabilistic risk assessment in food allergy is considered the best approach for assessing the risk at a population level ([Madsen et al., 2009](#)). It is now well established and described and relies on a large number of challenge data ([Madsen et al., 2020](#)).

Getting similar data from challenge studies in CD patients demands far more resources as the deleterious effects may be caused by long term exposure to small amounts of gluten and the end point needs to be established after gut biopsies. There is unfortunately only one study using placebo-controlled long-term challenge with gluten ([Catassi et al., 2007](#)). In this study CD patients were challenged daily with gluten on top of a gluten free diet. The amount of gluten in the gluten free background diet was estimated and assumed to be < 5 mg gluten/day based on data from another survey on gluten free food and on analytical data. The study in children ([Catassi et al., 1993](#)) had a similar set up, but without placebo group. The amount of gluten in the gluten free background diet was estimated to be 1 mg/day based on available information. In both studies patients were asked to maintain a strict gluten free diet during the study, but the gluten content in food without gluten as ingredient, seems not to be included in the estimate. These studies show the effect of a given gluten dose added to a normal GF diet.

We used the most predictive parameter (Vh/Cd) ([Catassi et al., 2007](#)) and data from each individual. This allowed us to create distributions reflecting the actual data. In the model “Catassi 1” patients having a decrease in Vh/Cd ratio after challenge of $\geq 10\%$ was included. [Taavela et al. \(2013\)](#) has suggested a difference of 0.4 in Vh/Cd to be likely clinically relevant. This criterion is in accordance with the cut-off of 10% in all but one patient from the 50 mg group, that had a 15% reduction in Vh/Cd ratio, corresponding to a decrease of 0.3. Had we used the 0.4 absolute change as criterion the 50 mg group would have had 8/13 with adverse effect instead of 9/13. This would most likely not have changed the results more than marginally. The “Catassi 2” model used the raw data without setting limits for degree of change in Vh/Cd. This is in accordance with the interpretation from the authors. The number of patients we identify with a decrease in Vh/Cd is complementary to the description in the paper of patients improving Vh/Cd after challenge ([Catassi et al., 2007](#)).

We used the reactions in the placebo group as starting point showing that for some persons the background diet gave changes in the gut. This may be a reflection of the possibilities of living GF in Italy before 2007. It is easier to avoid gluten in the diet in 2022 as described above. We subtracted the background from the results in order to see the added effect of including PWG in the diet. In our opinion this analysis allows maximum use of the available data.

The probabilistic risk assessment using the most stringent criteria for the distribution (Catassi 1) showed a small additional likelihood of developing mucosal damage in the CD population (1.3–1.9%) when eating PWG in addition to CGF. The study shows very little difference between the risk of eating food with or without PAL. We also made the analyses including data between LOQ and LOD as e.g., a soup with a contamination of 4 mg gluten/kg could add considerably to the risk. The results showed that with the contamination levels and frequencies we found, adding data below LOQ only increased the risk slightly. Including the coconut cookie with the extreme result (421 mg gluten/kg) increased the risk in the ALL and no-PAL group considerably (6.7 and 6.0%). This indicates that it is important to advice coeliac patients to consider likelihood of contamination when they choose which PWG food to eat. Here cookies that by tradition is PWG, but that may have been produced together with ordinary gluten containing cookies in a bakery factory.

Using the less stringent criteria for the distribution (Catassi 2) showed a larger, but still small additional likelihood of developing mucosal damage in the CD population (2.3–3.2%) when eating PWG in addition to CGF, with the same trends when products with gluten contamination between LOD and LOQ added or with the coconut cookie added.

In addition to using data from individual patients we performed the probabilistic analysis using the distribution as described by [Gibert et al. \(2013\)](#). Here the 10 mg/kg challenge was considered a no effect level and the 50 mg/kg the lowest effect level, described in a normal distribution (30 mg/day $\pm$ SD 10 mg). This model predicted a very low additional likelihood of developing mucosal damage in the CD population when eating PWG of 0.2%. Including the contamination between LOD and LOQ only increased the risk marginally (0.3–0.4%). When performing the analysis including the coconut cookie with 421 mg gluten/kg, the risk increases to 4.3% in the ALL and 3.8% in the no-PAL group. These results reflects that the “Gibert” distribution predicts very few adverse reactions around 10 mg gluten/day and the majority of reactions (68%) between 20 and 40 mg/day.

All three challenge data distributions rely on very limited data. These are the same data that has been decisive for establishing the 20 mg gluten/kg limit for CGF products. It may seem hazardous to use so few data as the basis for a probabilistic risk assessment. On the other hand, the alternative is to make no analyses at all, as the data we used are the best data available. Although the limited amount of data, the estimated 95% confidence interval of the relationship shows a statistically significant relationship between the exposure to gluten and likelihood of morphological changes. Hopefully there will be new studies in the future that may strengthen the knowledge base, and thereby increase the precision of the estimated risks. In the two “Catassi” distributions we have used the data differently than previously. The lack of significance between the placebo and the 10 mg group has led to the conclusion that 10 mg is a no effect level. In contrast to this approach, we use the adverse effects in individuals to form the distribution. As a consequence, the probabilistic risk assessment predicts a daily gluten intake around 10 mg as an adverse event and contributes to the increase in risk. This makes our model more conservative than the model described by [Gibert et al. \(2013\)](#).

The results of the risk assessment rely, apart from the challenge data, heavily on the snapshot of gluten contamination in PWG products in Danish supermarkets. The prevalence of gluten contamination in the Danish data is comparable with the data from UK and the Nordic countries although the level of contamination is lower in the Nordic

survey (Bolin and Lindeberg, 2016) and higher in the UK survey (Remington et al., 2015).

5. Conclusion

We have shown that the level and frequency of gluten contamination in ordinary food products without gluten containing ingredients found in Danish supermarkets is low. A probabilistic risk assessment, assessing the likelihood of developing mucosal damage in the CD population from 90 days exposure, shows that when CD patients eat these products, there is a low increased likelihood of mucosal damage in addition to the risk from eating certified gluten free food. The risk related to products with or without the warning “May contain wheat/gluten” is similar. We found one product, a traditional coconut cookie, that when included in the assessment, increased the likelihood of mucosal damage significantly. This indicates that it is important to advice coeliac patients to consider the possibility of contamination when they choose which PWG food to eat.

Declaration of competing interest

The authors declare no financial interests/personal relationships which may be considered as potential competing interests.

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References

- Biot, S., Madsen, C.B., Kruizinga, A.G., Christensen, T., Crépet, A., Brockhoff, P.B., 2017. A procedure for grouping food consumption data for use in food allergen risk assessment. *J. Food Compos. Anal.* 59, 111–123.
- Bolin, Y.S., Lindeberg, L., 2016. Undeclared Allergens in Food. <http://norden.diva-portal.org/smash/record.jsf?pid=diva2:934651&dsid=1499>.
- Catassi, C., Fabiani, E., Iacono, G., D'Agate, C., Francavilla, R., Biagi, F., Volta, U., Accomando, S., Picarelli, A., De Vitis, I., Pianelli, G., Gesuita, R., Carle, F., Mandolesi, A., Bearzi, I., Fasano, A., 2007. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am. J. Clin. Nutr.* 85, 160–166.
- Catassi, C., Rossini, M., Ratsch, I.M., Bearzi, I., Santinelli, A., Castagnani, R., Pisani, E., Coppa, G.V., Giorgi, P.L., 1993. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. *Gut* 34 (11), 1515–1519.
- Codex Alimentarius Standards for foods special dietary use for persons intolerant to gluten, 1979. Standard CXS 118. Adopted in 1979. Amended in 1983 and 2015. Revised in 2008.
- DunnGalvin, A., Roberts, G., Schnadt, S., Astley, S., Austin, M., Blom, W.M., Baumert, J., Chan, C.H., Crevel, R., Grimshaw, K., Kruizinga, A.G., Regent, L., Taylor, S., Walker, M., Mills, E., 2019. Evidence-based approaches to the application of precautionary allergen labelling: report from two iFAAM workshops. *Clin. Exp. Allergy : J. Brit. Soc. Allergy Clin. Immunol.* 49 (9), 1191–1200.
- Fasano, A., Catassi, C., 2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* 120 (3), 636–651.
- Food Drink Europe, 2013. Guidance on Food Allergen Management for Food Manufacturers.
- Gibert, A., Kruizinga, A.G., Neuhold, S., Houben, G.F., Canela, M.A., Fasano, A., Catassi, C., 2013. Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation. *Am. J. Clin. Nutr.* 97 (1), 109–116.
- Guenouni, M., Admou, B., El khoudri, N., Bourrouat, A., Zogaam, L.G., Elmoumou, L., Hilali, A., 2021. Gluten contamination in labelled gluten-free, naturally gluten-free and meals in food services in low-, middle- and high-income countries: a systematic review and meta-analysis. *Br. J. Nutr.* 1–15. <https://doi.org/10.1017/S0007114521002488>.
- Hirst, B., 2014. Survey of allergen advisory labelling and allergen content of UK retail pre-packed. Project. In: Project Code FS241038 (T07067). FSA, 241038, 2014.
- Madsen, C.B., Hattersley, S., Buck, J., Gendel, S.M., Houben, G.F., Hourihane, J.O.'B., Mackie, A., Mills, E.N.C., Nørhede, P., Taylor, S.L., Crevel, R.W.R., 2009. Approaches to risk assessment in food allergy: report from a workshop “developing a framework for assessing the risk from allergenic foods.”. *Food Chem. Toxicol.* 47, 480–489.
- Madsen, C.B., van den Dungen, M.W., Cochrane, S., Houben, G.F., Knibb, R.C., Knulst, A. C., Ronsmans, S., Yarham, R., Schnadt, S., Turner, P.J., Baumert, J., Cavandoli, E., Chan, C.H., Warner, A., Crevel, R., 2020. Can we define a level of protection for allergic consumers that everyone can accept? *Regul. Toxicol. Pharmacol.* 117, 104751.
- r biopharm. Ridascreen®gliadin, art. nr. r7001, 2021-07-09. <https://food.r-biopharm.com/products/ridascreen-gliadin/>.
- Regulation (EC) No 41/2009 Of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten Official J. Eur. Union 16/3L.
- Regulation (EU) No. 828/2014, 2014. Commission implementing regulation (EU) No. 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. *Off. J. Eur. Union* L228 (828), 5–8.
- Remington, B.C., Baumert, J.L., Blom, W.M., Houben, G.F., Taylor, S.L., Kruizinga, A.G., 2015. Unintended allergens in precautionary labelled and unlabelled products pose significant risks to UK allergic consumers. *Allergy* 70, 813–819.
- Rohatgi, A., 2020. WebPlotDigitizer (Version 4.4) [Computer Software]. Retrieved from [Http://Arohatgi.Info/WebPlotDigitizer](http://Arohatgi.Info/WebPlotDigitizer).
- Di Sabatino, A., Corazza, G.R., 2009. Coeliac disease. *Lancet* 373 (9673), 1480–1493. [https://doi.org/10.1016/S0140-6736\(09\)60254-3](https://doi.org/10.1016/S0140-6736(09)60254-3).
- Sharma, G.M., Pereira, M., Wang, S.S., Chirtel, S.J., Whitaker, T.B., Wehling, P., Arlinghaus, M., Canida, T., Jackson, L.S., Williams, K.M., 2020. Evaluation of sampling plans for measurement of gluten in oat groats. *Food Control* 114, 107241.
- Taavela, J., Koskinen, O., Huhtala, H., Lähdeaho, M.-L., Popp, A., et al., 2013. Validation of morphometric analyses of small-intestinal biopsy readouts in celiac disease. *PLoS One* 8 (10), E76163. <https://doi.org/10.1371/Journal.Pone.0076163>.

Glossary

- CD: Coeliac disease
 PAL: Precautionary allergen labelling
 UAP: Unintended allergen presence
 PWG: Products without gluten as ingredient
 CGF: Certified gluten free products
 ELISA: Enzyme linked immune sorbent assay
 LOQ: Level of quantification
 LOD: Level of detection
 Vh/Cd: ratio between villus height and crypt depth