**Scientific Committee on Consumer Safety**

**SCCS**

# OPINION ON

# Hydrolysed wheat proteins

## *- Sensitisation only -*

The SCCS adopted this opinion at its 6th plenary meeting

of 18 June 2014

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of six weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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## 1. BACKGROUND

Hydrolysed wheat proteins have a widespread use in cosmetic products. The use of hydrolysed wheat proteins in cosmetic products is not currently regulated in the Cosmetics Directive.

Several Member States have recently indicated safety problems in relation to cosmetic products containing hydrolysed wheat protein. Several cases of contact urticaria provoked by these cosmetic products, followed by anaphylactic shock after the ingestion of food containing wheat proteins, have been recently reported.

As hydrolysed wheat proteins can be part of several ingredients it is important to have a clear picture whether it is only the hydrolyzed wheat protein in itself or any combination containing hydrolysed wheat protein that causes the problems raised by Member States.

A public call for scientific data on the use of hydrolysed wheat proteins in cosmetic products was made by the Commission Service during autumn/winter 2009-10.

## 2. TERMS OF REFERENCE

1. *Does the SCCS consider the use of hydrolysed wheat proteins to be safe for consumers in cosmetic products on the basis of the provided scientific data?*

2. *And/or does the SCCS have any scientific concerns with regard to the use of hydrolysed wheat proteins in cosmetic products?*

# 3. OPINION

## 3.1. Chemical and Physical Specifications

### 3.1.1. Chemical identity

#### 3.1.1.1. Primary name and/or INCI name

Hydrolyzed Wheat Protein
INCI name: Hydrolyzed Wheat Protein

#### 3.1.1.2. Chemical names

Hydrolyzed wheat protein

#### 3.1.1.3. Trade names and abbreviations

Pronalen, Cropeptide, Gluadin, Glupal / Glupearl 19S, Tritisol

#### 3.1.1.4. CAS / EC number

CAS: 94350-06-8 / 222400-28-4 / 70084-87-6 / 100209-50-5
EC: 305-225-0

#### 3.1.1.5. Structural formula

/

#### 3.1.1.6. Empirical formula

Formula:

### 3.1.2. Physical form

Powder, white-yellowish to brown

### 3.1.3. Molecular weight

Molecular weight 0.1 – 90 kDa. Mostly between 25 and 90 kDa. (Note French Authorities 2009, EFfCI 2009-b)

### 3.1.4. Purity, composition and substance codes

Mixture of proteins with different molecular weights and structures, depending on the hydrolysation procedure and subsequent separation

### 3.1.5. Impurities / accompanying contaminants

/

### 3.1.6. Solubility

Soluble in water

### 3.1.7. Partition coefficient (Log Pow)

/

### 3.1.8. Additional physical and chemical specifications

/

### 3.1.9. Homogeneity and Stability

/

**General Comments to physico-chemical characterisation**

/

## 3.2. Function and uses

Hydrolysed Wheat Protein (HWP) is obtained from the grains of wheat (*Triticum aestivum* and other *Triticum* species). The major part of the grain is the albumen, which contains, besides polysaccharides, proteins. A large component of the proteins is the glutens, consisting of glutenins, gliadins and albumin-globulines.
The gluten proteins can be extracted by washing or by coagulation, and further hydrolyzed. This hydrolysis is aimed at cutting the large proteins in order to enhance solubility and to obtain the required technical characteristics. There are different types of hydrolysis: acid, enzymatic and alkaline hydrolysis. The enzymatic method is the most widely used technique in the cosmetics industry, obtaining hydrolysates of molecular weights between 25 and 90 kilodalton (Autorités Françaises / Note French authorities 2009). These hydrolysates can be further separated by, for example, centrifugation and ultrafiltration in order to obtain hydrolysates that are considerably smaller.

The use of HWP's in cosmetics is based on their function as surfactant, film-former, foaming agent, hydrating agent, antistatic and softener. HWP is used in a wide variety of products such as facial care products, make-up, manicure products, soaps and shampoos, shower gels and bath oils (CTFA 2008). In addition, HWP's can be present in food, for example in liver pâté and ham. According to information provided to the CIR Expert Pamel, the use concentration of HWP in various cosmetic products ranges from $2x10^{-5}$ to 1.7% (CIR 2014).

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

/

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1. Skin irritation

According to data submitted for a review (CIR 2013), acid- and enzyme-hydrolyzed HWP was not irritant in a primary dermal irritation study in 6 New Zealand white rabbits. A 25% solution in water with a MW 350 was applied for 24 h to 2.5 cm2 clipped, abraded and occluded skin (Leberco Testing Inc 1994-a, cited in CIR 2013). No further details are available.

Industry has submitted reports on an *in vitro* skin irritation test with HWP and with hydrolyzed wheat gluten (DS_B_Kelyamin_E1 2008, DS_C_Glusol_E1 2008). The model used is a human epidermis model for skin irritation potential cytotoxicity (EPISKIN-SM tm), later described in OECD guideline 439: after exposure of the test substance, cell viability (% viable cells) and histopathological parameters (expressed as a sum of morphological scores) are assessed as degree of irritancy.
For the HWP product (Kelyamin liquid, details not given), exposure of the test system was with 10 microliter of an undiluted sample, with a positive control of 5% sodium dodecyl sulphate (SDS) in water and saline as negative control substance.
Results: Only summary data were given. In terms of % viability there was no difference between the HWP product and saline (both 100% viability), but much lower viability (about 10%) was noted when SDS was applied. Also the histopathology scores for the product and the saline were similar (23 and 24 respectively, versus 12 for SDS).
The conclusion was that the HWP product was non-irritant according to this *in vitro* test method.

For the hydrolysed gluten product (Glusol powder, details not given) exposure of the test system was with 10 mg of the powder, with a positive control of 5% SDS in water and saline as negative control substance.
Results: Only summary data were given. In terms of % viability there was no difference between the hydrolysed gluten product and saline (both 100% viability), but much lower viability (about 10%) was noted when SDS was applied. Also the histopathology scores for the product and the saline were similar (20 and 24 respectively, versus 12 for SDS).
The conclusion was that the hydrolysed gluten powder product was non-irritant according to this *in vitro* test method.

In the same *in vitro* models, irritancy was also evaluated by transepithelial electrical resistance; according to the interpretation of the data (only summary data presented in a bar-chart) the tests confirmed the tolerability data of the above mentioned EPISKIN *in vitro* tests (DS_C_Kelyamin_E1 TEER 2008, DS_D_Glusol_E1 TEER 2008).

**SCCS comment**
Based on the submitted information, hydrolysed wheat protein is not an irritant to the skin.
It is not clear whether the MW 350 in the study in rabbits denotes Da or kDa.
The TER (transepithelial electric resistance) method is validated for rat skin, not for use on human culture system.
Furthermore the TER test can only detect corrosive substances.

### 3.3.2.2. Mucous membrane irritation

According to data submitted for a review (CIR 2013), HWP (25% aq solution, MW 350) was not an eye irritant in 6 albino rabbits (Leberco Testing Inc 1994-b, cited in CIR 2013).

Industry submitted a report on an *in vitro* eye irritation test (Eytex) with 15% hydrolyzed wheat gluten. Different amounts were applied: 20, 30, 50 and 100 microliter. The results are presented as scores in a summary table. According to the interpretation of the data it was not irritating.

Ref.: DS_B_Glusol_E10 (2002)

**SCCS comment**
Based on the submitted information, HWP does not appear to be an irritant to the eyes.
This is not a guideline method. The report includes one page only.

### 3.3.3. Skin sensitisation

| | |
|---|---|
| Guideline: | OECD 406 |
| Species/strain: | Guinea Pigs – Dunkin Hartley Crl:(HA)BR, females |
| Group size: | 20 (treatment) + 10 (controls) + 8 (preliminary study) |
| Test substance: | Gluadin AGP (wheat protein hydrolysate) 50% (w/w) in water |
| Negative control: | Water |
| Batch: | BG00814259 |
| Purity: | / |
| Vehicle: | water |
| Concentration: | 50% |
| Dose volume: | 0.5 ml |
| Observation: | grading of skin reaction from 0 to 3 |
| GLP: | yes |
| Study period: | Dec 1994 – Jan 1995 |

Industry submitted the results of a non-adjuvant occluded patch-test assay (Buehler Test) in guinea pigs (Henkel 1995).
A specific brand of HWP in a test concentration of 50% in water was used for induction and challenge. (Induction with HWP was performed in 20 animals, 10 animals were controls). Preliminary irritation tests and dose-finding to establish the appropriate concentration of the test substance to induce sensitization were performed in 8 animals.
Results: At 24 hours after challenge one animal in the control and none of the treated animals group showed a slight patchy erythema. At 48 hrs after challenge one control animal and one treated animal showed a slight patchy erythema.
Conclusion: The report concluded that this brand of HWP does not have to be labeled as 'sensitizing to the skin' choosing a non-adjuvant method.

| | |
|---|---|
| Guideline: | / |
| Species/strain: | Mouse, Balb-C |
| Group size: | 10 (treatment) + 5 (controls) + 8 (preliminary study to determine the highest non-irritant concentration) |
| Test substance: | Protein derivate L 21625 (proceeded by one injection with adjuvant) |
| Batch: | 01258 |
| Purity: | / |
| Vehicle: | none |
| Concentration: | 100% for induction and for elicitation |
| Dose volume: | 100 mg for induction, 20 mg for elicitation |
| Adjuvant: | FCA 25 microliter by intradermal injection |
| Observation: | ear swelling measured by micrometer |
| GLP: | yes |
| Study period: | April – June 1996 |

The sensitising potential of a specific brand of HWP was evaluated in a report about a mouse-ear swelling test (Scantox 1996).
On day 0, FCA was injected intradermally on the margin of the application site. Induction was performed on day 0, 1, 2 and 3 on the shaved skin of the thorax and abdomen. Induction with HWP was performed in 10 animals, in 5 animals there was induction with water (controls). Challenge/elicitation with same concentration was performed on day 9 on the left ear. The right ear was exposed to water as control substance. Swelling of the ears was measured with a micrometer.

Results: There were no differences in ear-swelling between the exposed and control ears in both animal groups.
Conclusion: The report concluded that the results show no indication of delayed-type hypersensitivity.

Results of a LLNA are presented at the ECHA website (ECHA 2014). According to the registrants there was no indication of sensitisation by hydrolysed wheat proteins. Because of legal restrictions no further details can be cited and discussed here.

**SCCS comment**
More data would be needed to assess the potential to induce delayed-type (IV) sensitization. However, given the chemical characteristics of HWP, this potential seems unlikely. The method is not suitable to assess type-I sensitization. In view of this, it is interesting to note the remark in the guinea pig study that one hour after the 3rd induction, 14 out of the 20 treated animals showed erythema around the application area. This might suggest a type I sensitization.
The report on the mouse-ear swelling test did, besides the code name "Proteinderivat L 21625", not have any description of the nature of the test substance. From another document in the submission it can be assumed that this was HWP (EFFCI 2009-2)

To date, only one animal study on immediate-type (type I) sensitization could be identified (Adachi 2012)

| | |
|---|---|
| Guideline: | / |
| Species/strain: | Mouse, BALB/c |
| Group size: | 40 (5 groups of 8 animals) |
| Test substances: | hydrolysed wheat proteins and Gluten (both as suspension in Tris solution), with and without SDS |
| Batch: | / |
| Purity: | / |
| Vehicle: | phosphate-buffered saline |
| Concentration: | 500 microgram per mouse |
| Positive control: | none |
| Negative control: | SDS 0.5% w/v in phosphate-buffered saline |
| GLP: | / |
| Study period: | / |

The shaved and tape-stripped skin of 5 groups (8 in each group) of BALB/c mice were exposed to vehicle (phosphate buffered saline with 0.5% SDS), HWP (one group with and one group without 0.5% SDS) and gluten (one group with and one group without 0.5% SDS). The HWP had been produced by acid hydrolysis, and contained mainly proteins of molecular weight 10 – 60 kDa. Skin application in order to induce sensitisation was done after tape-stripping on day 1, 8 15 and 24 (i.e. 8 applications) for a period of 3 days each under occlusion.
On day 23 HWP-specific IgE in the sera of the five groups was measured (as well as IgG1).
In addition, an immediate hypersensitivity reaction on day 18 or 25 was elicited by intraperitoneal injection of the antigens HWP or Gluten. Moreover, plasma histamine and cytokine release from cultured splenocytes was measured after elicitation of the immediate hypersensitivity reaction.
Results: All animals that were exposed to HWP showed (compared to the vehicle group) a dose-dependent production of HWP-specific IgE and IgG1, and a systemic reaction upon intraperitoneal challenge.
Conclusion
HWP, at least in the 10-60 kDa range, has (type-I) sensitizing potential through the animal skin.

**SCCS Comment**
The HWP formulation was applied on shaved and tape-stripped (damaged) skin. Sensitization was more easily achieved in the presence of SDS, which may have implications for its use in combination with surfactants in cosmetics.
The dosing and the elicitation schedules in the different groups of animals were not clear.

### 3.3.4. Dermal / percutaneous absorption

No data were submitted.

### 3.3.5. Repeated dose toxicity

/

### 3.3.6. Mutagenicity / Genotoxicity

/

### 3.3.7. Carcinogenicity

/

### 3.3.8. Reproductive toxicity

/

### 3.3.9. Toxicokinetics

/

### 3.3.10. Photo-induced toxicity

#### 3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

#### 3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

### 3.3.11. Human data

*Type I sensitisation*
From different parts of the world, there are reports of immediate-type contact allergic reactions, even with anaphylaxis, to cosmetic products that contain hydrolysed wheat proteins (HWP): for a summary, see table 1 below. From Japan it is reported that more than 1600 individuals who had used a HWP-containing soap developed allergic symptoms after ingesting natural wheat proteins (Chinuki 2013, Japanese Society Allergology). Also other brands of soap, containing the same HWP product, have been implicated (Matsunaga 2014). In most reported cases, the time sequence of events supports an induction of sensitisation via the skin. Immunological aspects are further discussed in paragraph 3.3.14.

Table 1
Overview of reports on patients with immediate-type hydrolysed wheat proteins (HWP) allergy (publications in Japanese language excluded).

| | nr of cases | contact urticaria | other reactions* |
|---|---|---|---|
| Varjonen 2000 | 1 | 1 | - |
| Pecquet 2002 | 1 | 1 | 1 |
| Pecquet 2004 | 7 | 7 | 6 |
| Codreanu 2006 | 1 | 1 | 1 |
| Laurière 2006 | 9 | 9 | 6 |
| Olaiwan 2010 | 2 | 2 | 1 |
| Bouchez-Mahiout 2010 | 4 | 2 | 2 |
| Fukutomi 2011 | 5 | 5 | 5 |
| Barrientos 2012 | 1 | 1 | - |
| Ishii 2012 | 1 | - | 1 |
| Iwamoto 2012 | 1 | - | 1 |
| Chinuki 2012 | 1 | 1 | 1 |
| Leheron 2013 | 1 | 1 | 1 |
| Chinuki 2013 | 7 | 6 | 7 |
| Japanese Soc Allerg 2012 | 1617 | | |
| Yokooij 2013 | 22 | ? | 22 |
| Hiragun 2013 | 30 | ? | 30 |
| Mimura 2014 | 1 | - | 1 |

* other reactions: conjunctivitis, generalised urticaria, angio-oedema, exercise-induced anaphylaxis, reactions to HWP in food

In a study among 9 French patients with contact urticaria to HWP containing cosmetics, immunoblotting analysis with HWP showed that IgE from all subjects reacted to a range (upwards from 20kDa) of peptide aggregates (Lauriere 2006). None of these patients had allergic reactions after eating bread, traditionally baked products or pasta, while some did react to food products containing HWP's. The authors state that their findings suggest that hydrolysis of wheat proteins did not destroy the pre-existing epitopes and may create new epitopes. This may be supported by the fact that the patients had no reactions to skin prick tests with flour extracts, and that only 3 had flour-specific IgE detected by commercial test-kits.
In another French study of 5 patients, 2 had contact urticarial reactions to HWP-containing cosmetics, and 2 had urticarial reactions to food containing HWP (Bouchez-Mahiout 2010).

Serum IgE reactivity was tested against 4 commercial HWP preparations. In the immunoblots of the sera of these patients there was no IgE reactivity towards the HWP preparation that was treated to limit the size of the peptides. From the immunoblots with the other HWP preparations the authors conclude that all IgE reacting components of HWP had molecular weights higher than 31 kDa. They also conclude that hydrolysis does not destroy all pre-existing epitopes and that multi-epitopic entities can be created.
In all patients, the time sequence favoured induction of sensitisation by means of HWP exposure of the skin.

The IgE reactivity towards different HWP's was studied in Japanese patients (Fukutomi 2011, Chinuki 2012, 2013, Yokooji 2013, Hiragun 2013). Contrary to the European patients, the Japanese are also reacting to food intake of normal wheat products. In a series of 7 patients, assumed to be sensitized by exposure to HWP in a soap, the hydrolysed wheat protein allergy was associated wheat dependent exercise induced anaphylaxis (HWP-WDEIA: explained in 3.3.14); in the serum of all patients there was IgE reactivity to a range of polypeptide aggregates (Chinuki 2013). In addition, a basophil activation test was performed. Although the authors claim that HWP's composed of large polypeptide aggregates possibly induce sensitization to a greater degree than lower-MW HWP's, the immunoblot analyses of three patients showed also IgE reacting with hydrolysates below MW 30 kDa, down to MW 15 kDa. None reacted with omega-5-gliadin.
In a series of 22 patients with HWP-WDEIA and 10 patients with conventional WDEIA, immunoblotting and histamine release tests showed a difference in sensitization pattern (Yokooji 2013): most patients with conventional WDEIA reacted to omega-5-gliadin and high-molecular weight glutenin. The HWP-WDEIA patients reacted to different gliadins. The blot showed a wide range of molecular weights of HWP binding to the patients' serum IgE. The study by Hiragun et al (2013), performed in patients who were sensitized by a HWP-containing soap, confirmed the low level of IgE reactivity and histamine release to omega-5 gliadin in these HWP-WDEIA patients.
The sera of at least three of the five patients in the study by Fukutomi (2010) showed serum IgE reactivity to HWP fractions below molecular weight 20 kDa.

**SCCS comment**
Although the blots from the sera indicate higher reactivity to molecular weights above 30 kDa, reactivity to HWP with molecular weight below 30 kDa has been demonstrated. It is as yet unknown whether the in vitro IgE reactivity to HWP fractions of lower molecular weight have clinical relevance in these individuals.

*Type IV (delayed type) sensitisation*
Delayed-type contact allergy with positive patch-tests to HWP or HWP derivates has been reported, but appears to be rare (Hann 2007, Livideanu 2007, Sanchez-Perez 2000). Interestingly, the contact urticaria case described in Spain also showed a positive patch-test reaction to HWP (Barrientos 2012)

Industry submitted HRIPT data (EFFCI 2009-a): a human repeated insult patch test (HRIPT) was conducted to confirm the lack of a sensitizing potential of a low molecular weight hydrolyzed wheat protein (Gluadin WLM; approx. 500 D). The undiluted substance (22% active matter) was repeatedly applied (9 applications within 3 weeks) under occlusive conditions to the backs of 113 volunteers for 24 h. Challenge took place two weeks after the last application by applying the same concentration to the same site in addition to a naive site of the back. During induction and elicitation, the reactions were graded 24 h after removal of the patches. In addition reactions were assessed 72 h after removal of the challenge patches. Among the 107 volunteers completing the study, the test substance showed no evidence of primary irritation or allergic sensitization during the induction and the challenge phase (report C0500323-2). According to the study authors the results from this HRIPT further confirm the low sensitizing potential of the tested hydrolyzed wheat protein.

In the same report, two other HRIPT studies in, respectively, 54 and 49 human subjects are summarized in a table; no skin responses were seen.

**SCCS comment**
The HRIPT is not suitable to evaluate IgE-mediated sensitization. The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

*Skin irritation*
According to data submitted for a review (CIR 2013), HWP was non-irritating in a patch test performed in 42 subjects. It was tested as 25% in water, MW 350, as a single dose under occlusion for 48 hrs (Japan Hair Science Assoc 1991, cited in CIR 2013). Occlusive human repeated insult patch testing (HRIPT) with HWP (25% aqueous solution, MW 350 in 52 subjects showed no skin irritation or sensitization (AMA Laboratories 2006, cited in CIR 2013).

Industry has submitted reports on clinical scoring and TEWL measurements after single-dose occlusive patch testing (DS_A_Kelyamin_E10 2002, DS_A_Glusol_E10 2002).
A HWP product (Kelyamin powder, diluted with water to 15%, no further product specifications) and hydrolysed wheat gluten (Glusol powder, diluted with water to 15%, no further product specifications) were tested on 20 human volunteers.
Both products were tested by a single-dose occlusive patch test randomised on the upper forearm or the upper back on either side. Patch-test removal was 48 hours after application, followed by local clinical assessment after 15 min or one day later.
According to the data, presented in a table, there was only slight erythema in 3 subjects.
The report concluded that according to the test and the scoring scale used, the test products can be considered to be non-irritant when applied to the human skin.
In the same experiment, the skin reactions were also instrumentally evaluated by transepidermal water loss (TEWL) measurements at 24, 48 and 72 hours after patch removal. The results are presented in a table as relative % change in TEWL.
The reports state that according to the evaluation test used and the statistical method adopted, the test product can be said to be unable to cause significant alterations in the stratum corneum barrier function.

### 3.3.12. Special investigations

**/**

### 3.3.13. Safety evaluation

**/**

### 3.3.14. Discussion

The proteins or fractions thereof in HWP are also present in food consumed as wheat. Therefore a safety evaluation of HWP cannot be based on classic dose-response scenarios derived from systemic exposure doses. Many individuals who are sensitized to HWP's can consume wheat-based food (non-hydrolysed wheat protein), although a number of these individuals react to food-products that have HWP's as additive. In Japan the proportion of HWP-sensitive individuals that react on the ingestion of wheat-based food is higher.
The safety issue of concern is the ability of HWP to induce sensitization and to elicit reactions in sensitized individuals; evaluation of the allergenic risk is at present based on observations in sensitized humans.
This can be further translated into the questions:

- whether there is evidence that HWP can *elicit* an allergic (IgE mediated) reaction after exposure of the human skin
- whether there is evidence that HWP can *induce* sensitization by exposure of the skin
- whether anything is known about the molecular weight and/or other characteristics of the proteins and/or polypeptides that are responsible for sensitization

*Physico-chemical properties*

Hydrolysed Wheat Protein (HWP) is obtained from the grains of wheat (*Triticum aestivum* and other *Triticum* species).
The enzymatic method is the most widely used technique in the cosmetics industry, obtaining hydrolysates of molecular weights between 25 and 90 kd (Autorités françaises / Note French authorities 2009). These hydrolysates can be further separated by, for example, centrifugation and ultrafiltration in order to obtain hydrolysates that are considerably smaller. There are no data on the enzymatic fractioning of the potentially allergenic structures of the proteins during the hydrolysis; retention of the original epitopes has been demonstrated. Also rearrangement of the HWP fractions to larger polypeptides is assumed (Lauriere 2006, Bouchez-Mahiout 2010, Yokooji 2013).
Industry has supplied information that some brands have an average MW of 350 Da and 2300 Da (CIR 2014).
The use of HWP's in cosmetics is based on their function as surfactant, film-former, foaming agent, hydrating agent, antistatic and softener. HWP is used in a wide variety of products such as facial care products, make-up, manicure products, soaps & shampoos, shower gels and bath oils (CTFA 2008). In addition, HWP's can be present in food, for example in liver pâté and ham. According to information provided to the CIR Expert Panel the use concentration of HWP in cosmetic product categories varies from $2x10^{-5}$% to 1.7% (CIR 2014).

*Irritation*

The submitted studies in humans and animals do not indicate that HWP is an irritant to the skin.

*Sensitisation*

A. Wheat.

Allergenic wheat proteins are present in different classes of wheat proteins. Wheat proteins can be divided into water soluble proteins (albumins and globulins, of which the amylase and trypsin inhibitors have been identified as allergens) and water insoluble proteins (belonging to the prolamin superfamily). Glutenins and gliadins found in wheat are a subgroup of prolamins, called cereal prolamins. They are the major storage proteins found in the endosperm of cereals (Breiteneder 2004, Radauer 2007).
A number of different wheat allergens associated with different manifestations of clinical symptoms in humans have been identified and labelled (WHO/IUIS).

Table 2. Wheat allergens. Taken from WHO/IUIS (www.allergen.org)

| Allergen | Biochemical name | MW | Food allergen |
|---|---|---|---|
| Tri a 12 | Profilin | 14 | Yes |
| Tri a 14 | Non-specific lipid transfer protein 1 | 9 | Yes |
| Tri a 15 | Monomeric alpha-amylase inhibitor 0.28 | | No |
| Tri a 18 | Agglutinin isolectin 1 | | Yes |
| Tri a 19 | Omega-5 gliadin, seed storage protein | 65 | Yes |
| Tri a 21 | Alpha-beta-gliadin | | No |
| Tri a 25 | Thioredoxin | | Yes |
| Tri a 26 | High molecular weight glutenin | 88 | Yes |
| Tri a 27 | Thiol reductase homologue | 27 | No |
| Tri a 28 | Dimeric alpha-amylase inhibitor 0.19 | 13 | No |
| Tri a 29 | Tetrameric alpha-amylase inhibitor CM1/CM2 | 13 | No |
| Tri a 30 | Tetrameric alpha-amylase inhibitor CM3 | 16 | No |
| Tri a 31 | Triosephosphate-isomerase | | No |
| Tri a 32 | 1-cys-peroxiredoxin | | No |
| Tri a 33 | Serpin | | No |
| Tri a 34 | Glyceraldehyde-3-phosphate-dehydrogenase | | No |
| Tri a 35 | Dehydrin | | No |
| Tri a 36 | Low molecular weight glutenin GluB3-23 | 40 kDa | Yes |
| Tri a 37 | Alpha purothionin | 12 kDa | Yes |
| Tri a 39 | Serine protease inhibitor-like protein | | No |

In view of the high number of humans who consume wheat-based food products, immediate-type allergies with clinical reactions to wheat proteins are not common. Wheat allergy should be distinguished from celiac disease, which is an abnormal cellular response to gluten. Serum reactivity (allergen-specific IgE) to wheat is regularly seen in medical practice, but often without clinical relevance; often it is a matter of cross-reactivity with grass pollen. A review by Inomata (2009) reported that prevalences are in the order of 0.5%, with higher prevalences of serum-IgE reactivity to wheat proteins (which does not necessarily cause clinical symptoms). Estimates of prevalences may be inflated by selection bias. In children, prevalences of food-based wheat allergy with positive clinical provocation tests are reported to vary between 0 and 0.5% (Zuidmeer 2008).
There is a clear overlap between the spectra of proteins responsible for diverse clinical conditions of wheat allergy. Based on the allergens involved in wheat food allergy, allergic

responses to the ingestion of wheat can be divided into clinical subtypes: for example, wheat-dependent exercise induced anaphylaxis (WDEIA) is mainly associated with gluten, particularly omega-5 gliadins and high-molecular-weight (HMW) glutenin subunits. Other responses that include classic food allergy relate to a wide range of water/salt-soluble and insoluble wheat proteins (Inomata 2009). In children with allergic reactions after eating wheat products, omega-5 gliadin is the most important allergen (Palosuo 2001)

Baker's asthma has been extensively documented. Although a wide range of wheat allergens (with molecular weights between 12 – 90 kDa) may be involved in allergic sensitization by the inhalation route, especially the α-amylase / trypsin inhibitors (10 -16 kDa) are considered as major allergens in Baker's asthma (Quirce 2013). Most patients with Baker's asthma can eat wheat and pasta without problems.

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is an allergic reaction (varying from generalized urticaria, dyspnoe to collapse and shock) induced by the ingestion of wheat and subsequent physical exercise. Acetylsalicylic acid ("aspirin") can also be a trigger. WDEIA is associated with [omega]-5 gliadins and high-molecular-weight (HMW) glutenin subunits. Of interest is that in Japan a number of patients with WDEIA seem to be sensitized by HWP in soap, whereby they show no or low levels of IgE against omega-5 gliadin (Chinuki 2012a, Yokooji 2013). Based on these observations, a distinction between conventional WDEIA and HWP-WDEIA is proposed.

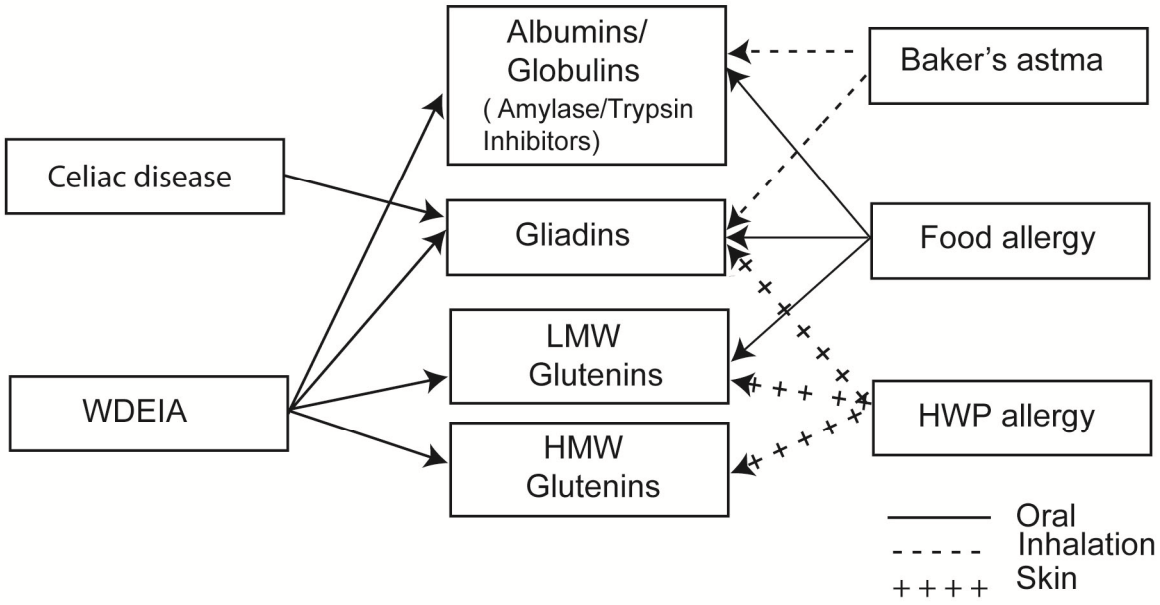


*Figure 1. Immunologically overlapping reactivity (arrows are based on the presence of allergen-specific serum IgE) to wheat proteins according to clinical reaction patterns. The presence of a specific type of IgE in the serum may, but not necessarily, explain or predict clinical symptoms. Note that most individuals with HWP allergy can eat wheat products (bread, pasta), but a number of them react after eating food to which HWP's have been added (e.g. ham, pâté). Note that Celiac (or Coeliac) Disease is based on a different mechanism: intolerance to gliadins due to an auto-immune reaction to the gut enzyme Tissue Transglutaminase.*
*WDEIA: wheat-dependent exercise-induced anaphylaxis. LMW: low molecular weight HMW: high molecular weight. Figure taken and modified from Uvackova (2013).*

B. Hydrolysed wheat proteins (HWP).

Skin exposure to proteins or fractions thereof can give immune responses; this principle is also under further investigation for new immunization technologies (Mitragotri 2005). And there is ample evidence that in sensitized individuals, skin exposure to proteinacious allergens can provoke topical and even severe systemic reactions (Smith Pease et al. 2002). An important question is whether topically applied proteins, and hydrolyzed fractions of proteins such as wheat proteins, can induce sensitisation by gaining access to the immune system through intact or minimally damaged skin. In their review, Kimber et al. conclude that epicutaneous exposure is an effective route for the initiation of immune responses to protein antigens and favours the acquisition of allergic sensitization (Kimber 2013).
Impaired skin barrier is regarded as a sensitization-promoting factor Smith Pease 2002). An impaired barrier is present in the (many) individuals with overt or subclinical atopic eczema. However, in many reported cases of HWP allergy, there was no history or serologic evidence of atopy. Barrier impairment may also be achieved by skin exposure to surface-active chemicals ("surfactants"), which can be present in soaps or detergents. In a large number of cases due to exposure to a specific HWP reported from Japan, the exposure was apparently in combination with a facial soap.
In Japan the abovementioned 'epidemic' of WDEIA attributable to a particular brand of HWP in soap has been labelled HWP-WDEIA, distinct from the conventional WDEIA that also occurs in Japan.

There is discussion whether exposure to low molecular weight HWP (i.e. below 30 kDa) would be 'safe' because it does not carry a risk of sensitization (CIR 2013). Although the study by Chinuki et al (Chinuki 2013) shows that HWP's composed of large polypeptide aggregates possibly induce sensitization to a greater degree than lower-MW HWP's, the immunoblot analyses of three patients also showed IgE reacting in vitro with hydrolysates below MW 30 kDa, down to MW 15 kDa. The sera of at least three of the five patients in the study by Fukutomi (2010) showed in vitro serum IgE reactivity to HWP fractions below molecular weight 20 kDa. It is not known whether the in vitro IgE reactivity to HWP below 30 kDa was responsible for the clinical reactions in the patients who were studied. There is at present insufficient evidence to support the claim that HWP's with lower molecular weights (e.g. 10 - 30 kDa) are safe in terms of capacity to sensitize.
The raising evidence pointing to the skin as an important route of induction of sensitisation to food proteins is of concern. At present there are insufficient data to set a safe level of skin exposure (total dose or dose per cm2) to HWP. In order to cross-link IgE in sensitised individuals, a protein needs to have a minimum size which is assumed to be in the order of 3 kDa (Huby 2000). It can be argued that proteins of this size are also unlikely to induce clinically relevant (type I) sensitisation. The CIR Expert Panel concluded that HWP is safe for use in cosmetics when formulated to restrict peptides to a weight average MW of 3.5 kDa or less (CIR (2014).

## 4. CONCLUSION

The SCCS is of the opinion that,

In view of the numbers of reported cases of immediate-type contact urticarial and systemic allergic reactions, the overall risk of sensitization to Hydrolysed Wheat Proteins (HWP) appears to be low, with the exception of an 'epidemic' in Japan associated with one particular HWP product used in some brands of soap.

Scientific concerns with regard to the use of HWP in cosmetic products include that

- there is evidence that sensitisation to HWP is via exposure to cosmetics, not via food

- there are indications that the risk of sensitisation is higher when HWP's of higher molecular weight are used on the skin, in particular as an ingredient of products that have strong surfactant properties such as soaps and liquid soaps.

The SCCS considers the use of hydrolysed wheat proteins safe for consumers in cosmetic products, provided that the maximum molecular weight average of the peptides in hydrolysates is 3,5 kDa.

## 5. MINORITY OPINION

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