Comments on

Syngenta's Application for Commodity Clearance of Genetically Modified Maize, Event 3272

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A. First GM Industrial Crop, Unlimited Clearance

Syngenta's Event 3272 maize represents the very first genetically modified (GM) industrial crop for which commodity clearance is sought from the National Department of Agriculture. The intended industrial application is for fuel ethanol production:

> *"In those countries where Event 3272 maize will be cultivated (not in South Africa), the grain will be used in the dry-grind fuel ethanol process."* (NDA 2006)

In fact, it is the first such GM industrial crop for which any sort of commercial approval (whether for cultivation or import) has been sought anywhere in the world. This raises the question of how such a GM crop should be regulated.

Non-food GM crops are by definition not intended for food use, but can they reliably be kept out of the food and feed supply? In the case of maize, the answer is almost surely no. The astoundingly widespread contamination of the U.S. and world food supply with "animal-feed-only" GM StarLink corn in 2000 and 2001 abundantly demonstrates the virtual impossibility of segregating a commodity food crop like maize for any particular use (Freese 2001). StarLink is not an aberration. Syngenta mistakenly distributed large amounts of its unapproved Bt10 maize seed to farmers in the U.S. and elsewhere for nearly four years before the error was reported, resulting in 133 million kg of the untested, unreviewed, GM corn entering the food supply (Kleiner 2005). (There are many other examples.) This perhaps helps explain why the applicant, Syngenta, is seeking full clearance of Event 3272 for all food and feed products. According to the notice:

> *"It [Event 3272] is not intended to be used in other processing applications (e.g. wet milling and dry milling processes) or to be exported as a commodity crop. However,* ***it cannot be excluded*** *that the harvest originally intended to be used in the dry-grind fuel ethanol industry or the by-products of this processing* ***could enter international trade routes*** *at extremely low levels."* (NDA 2006)

This language makes it sound as if Event 3272 will enter the world's food and feed supply (i.e. "international trade routes") only by mistake, for instance when batches of Event 3272 are misdirected to wet or dry-milling plants. However, this is not the case.

Syngenta's application to the National Dept. of Agriculture (NDA) is presumably similar in scope to the company's application for Event 3272 to the European Union, which provides the following additional information:

> ***"By-products of the dry-grind ethanol process produced from Event 3272 will be commingled with by-products from conventional maize****… Derived products from Event 3272 maize can be used in a manner similar to those products derived from conventional maize."* (EU Application 2006)

This additional information (again, assuming that Syngenta's application to the NDA has the same scope as its EU application) makes it abundantly clear that Event 3272, if it is commercialized, will enter the world's animal feed and food supply ***as a matter of course,*** not merely due to occasional mishaps.

The EU also states that:

> *"The application also covers the import and industrial processing of Event 3272* ***for all potential uses, as any other maize****. … the scope of the application includes* ***all feed and food products*** *containing,* ***consisting or produced from*** ***the genetically modified Event 3272*** *including products from inbreds and hybrids obtained by conventional breeding of Event 3272."* (EU Application 2006)

Use of the words "consisting or produced from" clearly indicates that granting the application would permit import of maize-based food and feed products containing unlimited amounts of Event 3272, up to and including 100%. In other words, Syngenta is asking the EU and (presumably) South African authorities to allow unlimited use of the world's first GM industrial crop in food and feed. The extremely broad scope of the application contrasts sharply with the contention that Event 3272 could enter international trade routes only "at extremely low levels." This huge disparity between a presumed "extremely low level" of Event 3272 in food or feed and import clearance permitting food and feed "***consisting or produced from***" 100% Event 3272 is puzzling, and raises several questions.

Upon what basis has Syngenta and/or NDP concluded that only extremely low levels of Event 3272 will enter the world's food and feed? Presumably, the conclusion is based on Syngenta's ***current plans*** to limit its marketing of Event 3272 to fuel ethanol production. If the commodity clearance is granted, however, nothing in South African law or regulation would prevent Syngenta from expanding its marketing of Event 3272 to food and animal feed applications. There is even reason to believe Syngenta might have such plans. The company is reportedly developing "self-processing" GM corn varieties that contain starch-degrading enzymes similar or identical to the one in Event 3272 specifically for use as animal feed to increase digestibility of the feed corn (Patricio 2006).

But even assuming that Syngenta continues to limit its marketing of Event 3272 for industrial ethanol use, where is the analysis to demonstrate that it will be present at extremely low levels in food and feed? The proportion of U.S. maize (the one country where, at present, Event 3272 might be grown) devoted to industrial ethanol production has risen sharply the past few years, and now constitutes at least 13% (more than $1/8^{th}$) of U.S. corn. If Event 3272 is approved, commercialized and proves to be popular, and industrial ethanol use of U.S. corn continues to rise as predicted, millions of acres in the U.S. could be planted to Event 3272 in the near future. And since the U.S. is by far the world's largest exporter of corn and corn by-products, this would mean a substantial amount of Event 3272 and its by-products in the world's food and feed supply.

In addition, the huge potential for misdirection of Event 3272 at all stages of the maize supply chain must not be underestimated. Recall that Syngenta is the company that sold enough unapproved Bt10 corn to farmers in the U.S. and elsewhere to generate 133 million kg of maize for nearly 4 years before detecting, or at least reporting the mishap to the U.S. Environmental Protection Agency. Can we depend on this company and its innumerable seed dealers to do any better job with Event 3272?

Finally, the potential for shipments of Event 3272 to South Africa to be misdirected to food or feed processing plants, or to be mistakenly planted and contaminate South African maize and maize seed supply must not be discounted.

Thus, we urge NDA to ***completely disregard any representations by Syngenta concerning the level of Event 3272 to be expected in food and feed imported into South Africa, and to disregard as well any representations by Syngenta regarding its current marketing plans (i.e.***

only for ethanol production), because such representations are not enforceable by NDA through either regulation or law.

Event 3272 should therefore be subjected to the strictest review, and to the highest standards, on the assumption that it may constitute 100% of some food items and feed lots consumed by people and animals in South Africa. The need for strict review is much more pressing for South Africa, where maize is a staple food crop, than in the U.S. or Europe, where it is consumed very little. For instance, only 10% of U.S. maize is processed into food products (e.g. high fructose corn syrup and other sweeteners, some breakfast cereals, etc.).

B. Description of Event 3272 and Alpha-Amylase in Ethanol Production

Event 3272 has been genetically modified to produce two novel proteins: a thermostable alpha-amylase enzyme and phosphomannose isomerase, a selectable marker. The thermostable alpha-amylase enzyme is expressed from a genetic construct consisting of segments of DNA that presumably[1] come from microorganisms of the domain *Archaea*, order *Thermococcales*, isolated from hydrothermal vents deep below the surface of the ocean (Richardson et al 2002; Anon. 2005). This genetic construct was introduced into Event 3272 via Agrobacterium-mediated transformation (EU Application 2006).

Other alpha-amylase enzymes are already employed to process corn to produce ethanol and high fructose corn syrup. Since 1980, the most widely used alpha-amylase for these applications has been isolated from the common soil bacterium *Bacillus licheniformis* (Richardson et al 2002). The alpha-amylase enzyme in Event 3272 is said to be superior to currently used enzymes by virtue of its higher activity at the pH of the ethanol production process (pH = 4.5) and its greater thermostability (Ibid). Currently used enzymes are not incorporated into maize. Instead, they are produced using GM bacteria in contained fermentation vats, then extracted and introduced into the ethanol production process. It should also be noted that a version of the alpha-amylase enzyme incorporated into Event 3272 has recently become available as a "stand-alone" product ("Ultra-Thin" enzyme) for use in ethanol plants in the traditional manner. It is being offered for sale by Diversa Corporation in partnership with Valley Enzymes (Diversa 2005). Syngenta holds a major stake in Diversa. The commercial availability of this enzyme means whatever advantages it might offer can be had without use of Event 3272.

In short, ethanol production with Event 3272 would have several unique and radically new features distinguishing it from traditional ethanol production that NDA should carefully consider:

1) The enzyme is incorporated in maize kernels rather than generated in contained fermentation systems. Incorporation into maize means that Event 3272 and its enzyme can spread to other maize plants through cross-pollination, or enter the food and feed supply by seed dispersal or human error. Promises of containment simply cannot be taken seriously given the horrendous record of the biotechnology industry in this regard.

[1] It is interesting to note that Richardson et al (2002) seem uncertain as to the origin of the three separate alpha-amylase genes upon which the genetic construct is based: "Phylogenetic analysis revealed that all three enzymes…are likely to be from organisms closely related to the order of Thermococcales."

2) The enzyme in Event 3272 is derived from novel deep-sea organisms that have never been a part of the human food supply.

3) The deep-sea organisms are said to be from the domain *Archaea*, one of three domains into which all living organisms are classified (the others being *Bacteria* and *Eucarya*). Very little is presently known about organisms of the Archaea domain, as they were only recently discovered, and are ubiquitous mainly in inaccessible regions such as the deep sea.

4) The enzyme is not only from an unfamiliar source, but its corresponding gene sequence is an artificial construct made by randomly mixing alpha-amylase gene segments from three *Thermococcales* microorganisms (Richardson et al 2002).

C. Evaluation of Event 3272 for Potential Human Health Impacts

Given the fact that maize is a staple food crop of South Africa, we urge NDA to conduct a rigorous safety review of Event 3272. NDA should demand safety tests that meet the highest international standards for safety review of GM crops, even if such standards are more rigorous than those commonly employed for GM maize in the U.S. and European countries, where maize is a minor component of the food supply.

Event 3272 presents at least two human health concerns that deserve extremely careful consideration: allergenicity and unintended amplification of toxic compounds through the genetic modification process.

Allergenicity

Allergies are one of the most commonly cited health risks presented by GM crops, particularly when they express novel proteins that have never been a part of the human food supply. Allergies are often taken less seriously than they deserve to be. First, they affect a great many people, for instance an estimated 2-2.5% of adults and 6-8% of children in the U.S. (or roughly 8 million Americans). While many allergic reactions are mild or moderate, a significant number of people experience allergic reactions known as anaphylactic shock, which can be life-threatening. It can be difficult to determine which food component has caused an allergy sufferer's reaction. Normal maize rarely causes allergies. Thus, allergists often discount maize as a potential cause of allergies, and sometimes even prescribe corn-based diets to allergy-prone infants and young children. Thus, the hidden presence of an allergenic protein in GM maize would give rise to particular concern, as it would likely go undetected as the cause of an allergic reaction. For more on GM crops and allergies, see Appendix 1.

The alpha-amylase in Event 3272 (AA3272) may pose an allergenic risk based on three considerations: the demonstrated allergenicity of alpha-amylase enzymes derived from fungi; the extreme thermostability of AA3272; and the likely failure of Syngenta to subject AA3272 to an allergenicity assessment that meets authoritative international standards.

1. *Allergenicity of fungus-derived alpha-amylases*

Appendix 2 contains the abstracts of three peer-reviewed scientific articles documenting the known allergenicity of alpha-amylase enzymes used in the baking industry.

> *"Several studies have reported sensitization and respiratory disorders among bakery workers caused by enzymes in dough improvers. Fungal alpha-amylase is the most frequently reported cause of allergy."* (J. Allergy Clin. Immunol. 1997 Mar; 99(3): 289-92)
>
> *"Fungal alpha-amylase is an important occupational allergen in the bakery industry."* (Am. J. Respir. Crit. Care Med. 1996 Jul; 154(1): 130-136)

It is not known whether AA3272 shares the allergy-causing properties of its fungal relatives, but the structure of a particular type of protein in one organism is often quite similar to those of the same type of protein in other organisms. Fungal alpha-amylases have been demonstrated to cause respiratory rather than food allergies (it is not clear whether they have been tested as potential food allergens). But inhalational allergens can in some cases also cause food allergies. In addition, South African workers who process Event 3272 would presumably be exposed to AA3272-containing corn dust by the inhalational route, and so could be at risk of developing allergies to it, just as bakery workers have become allergic to fungal alpha-amylase. The allergenicity of fungal alpha-amylases at least argues for a rigorous allergenicity assessment of AA3272.

2. *AA3272 exhibits great thermostability, a characteristic property of food allergens*

Thermostability is resistance to breakdown and inactivation by heat. AA3272 is substantially more thermostable, and operates best at a lower pH (4.5), than currently used ethanol production enzymes. The most widely used alpha-amylase at present, from *B. licheniformis*, operates optimally at pH = 6.0 and 90 degrees C, and is virtually inactive at 100 degrees C and pH = 4.5. AA3272, on the other hand, exhibits great activity at 100 degrees C and even at 115 degrees C., substantially above the boiling point of water (Richardson et al 2002). This activity indicates that it survives intact at this high temperature.

According to one of the leading food allergists in the United States, Dr. Hugh Sampson, thermostability is a characteristic of proteins that cause allergies. "The allergenic fraction of food is generally comprised of heat-stable, water-soluble glycoproteins…" (Sampson 1999). Another leading U.S. food allergist, Steven Taylor, also notes that food allergens are often stable to heat (Taylor & Hefle 2001, p. 768). The US EPA concurs, citing stability to heat as a characteristic of allergenic proteins (EPA Cry1Ab [MON 810] Fact Sheet 2000). In fact, the EPA has formally adopted heat stability as a criterion of potential allergenicity of novel proteins produced in GM crops (EPA BRAD 2001, p. IIB2). It should be noted that the EPA will ***not*** review Syngenta's application for Event 3272 in the U.S., as the Agency is only responsible for GM crops that produce pesticidal proteins (i.e. Bt crops).

In the year 2000, a GM corn variety known as StarLink that was not approved for human food use due to concerns that it could cause food allergies, was found to have massively contaminated the U.S. and world's food supply. As a result, numerous food companies, including Kraft Foods, were forced to conduct massive recalls of maize-containing food products such as taco shells and tortillas (Freese 2001). StarLink was genetically modified to produce an insecticidal protein, Cry9C, in its grain and all its tissues (e.g. leaves, pollen, etc.). Cry9C was suspected of being an allergen for several reasons, among which was its resistance to breakdown by heat (i.e. thermostability). Dr. Hubert Noteborn, an expert on this type of GM crop, conducted heat stability tests on the Cry9C protein at 90 degrees C.

> *"The protein was stable for 120 min independent of whether Cry9C was assayed as a purified protein or as a component of TM [tomato matrix]…"* (Noteborn 1998, p. 22).

Whether or not Cry9C is in fact an allergen has never been determined. Leading food allergists advising the U.S. EPA strongly criticized an inconclusive US government analysis of people suspected of having experienced allergic reactions to StarLink, and recommended against approving ***any*** level of Cry9C, even the extremely low level of 20 parts per billion, in the food supply.

> *"The test, as conducted, does not eliminate StarLink Cry9C protein as a potential cause of allergic symptoms."* (EPA SAP 2001, p. 29)

> *"… the Panel concluded that based on reasonable scientific certainty, there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public."* (EPA SAP 2001, p. 35)

As noted above, AA3272 is at least somewhat stable at 115 degrees C, a substantially higher temperature than the 90 degree C at which Cry9C exhibited stability. AA3272's stability to extreme heat is another reason to demand the strictest possible allergenicity assessment.

3. Allergenicity assessment of AA3272 should meet authoritative international standard

The authoritative international standard for assessing the potential allergenicity of GM crops was drafted by an expert consultation of the UN's Food and Agriculture and World Health Organizations (FAO-WHO 2001). The group that drafted this report comprised leading allergists from around the world.

It is beyond the scope of these comments to discuss FAO-WHO's allergenicity assessment protocol. We will only say that neither U.S. nor EU food safety authorities adhere to this standard. Numerous GM crops have been approved in both the U.S. and EU on the basis of demonstrably substandard tests that do NOT meet FAO-WHO standards (see Freese & Schubert 2004; Freese 2001). Therefore, we urge NDA to carefully examine this report (especially its detailed protocols for tests of digestive stability and amino acid homology to known allergens), and demand that Syngenta submit tests that meet FAO-WHO standards before NDA considers issuing a commodity clearance for import of Event 3272.

Potentially hazardous unintended effects caused by the genetic modification process

The methods used to genetically modify plants involve random introduction of foreign genetic constructs into the plant's genome. Because genetic modification is a random process, foreign genes may be inserted within the plant's natural genes or in other genomic locations where they cause disruptions in cellular metabolism. Scrambling of native plant DNA, often but not always adjacent to the site(s) of insertion, is another common occurrence (Wilson et al 2004). Such scrambling occurred with Monsanto's Roundup Ready soybeans, and was only discovered after years of commercial cultivation of the crop on tens of millions of acres (Windels et al 2001). Disruptions caused by the genetic modification process are sometimes evident in the form of non-viable or debilitated plants, in which case development can be terminated. Other

disruptions may have subtler effects that go undetected in the development process, in which case potentially hazardous GM crops may end up being approved and commercialized.

One potential unintended effect that should be tested for is reduced levels of plant nutrients. For instance, Roundup Ready soybeans have been reported to have lower phytoestrogen levels (Lappe et al 1999) and lower levels of important aromatic amino acids. Other unintended effects observed in Roundup Ready soybeans include depressed root development, nodulation and nitrogen fixation; lower average yields than their conventional counterparts (Benbrook 2001); as well as increased lignin content (Coghlan 1999).

Another potential unintended effect is the unintended amplification of native plant toxins that are normally present at low, unobjectionable, levels.

Current assessment procedures examine a very limited array of key nutrients and selected anti-nutrients and toxicants for potential changes in levels of expression relative to a non-engineered control plant; such changes may signal unintended effects that require further analysis. With this "targeted approach:"

> *"…unexpected changes are merely identified by chance. The targeted approach has severe limitations with respect to unknown anti-nutrients and natural toxins…"* (Kuiper et al, 2001, p. 516).

This is because the number of compounds evaluated is a small fraction of the cell's full complement of compounds, and their selection is somewhat arbitrary due to limited knowledge concerning which are most likely to be affected. This has led to calls for a "non-targeted" approach utilizing profiling methods (Ibid, p. 516).

Profiling methods currently available or under development include DNA expression analysis, proteomics, two-dimensional gel electrophoresis, and chemical fingerprinting. These techniques – used singly or in combination – permit simultaneous, small-scale, quantitative analysis of a large array of plant components, including messenger RNA, proteins and metabolites. The virtue of this "non-targeted" approach is that it casts a wide net, implicitly acknowledging what genetic engineers often prefer to ignore: that genetic engineering often causes completely unintended effects, making the crude "targeted" analysis of a few cellular components ineffective as a means for detecting them. Kuiper et al urge rapid refinement and application of these profiling techniques to ensure the most complete assessment possible of unintended effects caused by any application of genetic engineering.

In part because profiling techniques have not been perfected, long-term animal feeding studies with the whole GM plant are also needed to ensure that any subtle, long-term effects (such as reproductive disorders, cancers, or endocrine disruption) do not go undetected.

It should be noted that neither US nor EU regulatory authorities demand either comprehensive profiling assessments or long-term animal feeding studies with the whole GM plant. For one recommended GM crop safety testing scheme, see Freese and Schubert (2004).

Recommended tests for Event 3272

We urge NDA to demand comprehensive testing of the sort described above before it considers whether to grant commodity clearance for import of Event 3272. In particular, we urge NDA to demand testing for the levels of two toxic compounds that have been recently discovered in corn – and which are present in some varieties of GM corn as well – tetrahydrofuran diols and leukotoxins (Markaveritch et al 2005, 2002). These compounds have been shown to cause severe reproductive disorders in mice and rats at extremely low

concentrations, roughly 200 times below the level at which some plant phytoestrogens have been found to affect reproductive behavior.

Conclusion

Event 3272 is not just another GM corn variety. It is the first GM crop to be proposed for commercial approval that has been specifically modified for an industrial use. It contains a novel enzyme derived from little-known deep-sea organisms. It belongs to a class of enzymes known to cause allergies, and exhibits extreme thermostability, a characteristic of food allergens. The hazardous unintended effects that top scientists (such as Harry Kuiper, a leading EU GM food safety expert) believe can be caused by the genetic modification process deserve much more intensive analysis than has been accorded to GM crops in the past.

Because maize is a staple food of South Africa, it is incumbent on regulatory authorities to assess this novel crop rigorously – certainly more rigorously than the often *pro forma* assessments conducted by U.S. (and increasingly EU) regulatory authorities, where maize in particular is a minor part of the food supply. Accordingly, we urge NDA to demand an allergenicity assessment in strict accordance with the internationally recognized standard, FAO-WHO (2001). For other safety issues, we recommend the GM crop safety testing scheme outlined in Freese & Schubert (2004). In particular, long-term (lifetime) rodent feeding trials with the whole crop are absolutely essentially to detect more subtle, but potentially quite serious, health impacts such as endocrine disruption, reproductive disorders and cancers. With respect to reproductive disorders, it is absolutely essential that Event 3272 be tested for the presence of tetrahydrofuran-diol and leukotoxin-diol derivatives of linoleic acid (Markaverich 2002, 2005).

It is also unclear why the National Dept. of Agriculture is being asked to give ***complete food and feed import approval*** to a crop that its developer says will be limited to use in ethanol production. Granting commodity clearance would remove Event 3272 from any further oversight by NDA, no matter how Syngenta decides to market this crop in the future. It is also worth noting that Event 3272 has not been approved anywhere in the world, even in the U.S., which at present is the only country where approval for cultivation is being sought. What is the rush to approve import of a crop that may never even be approved for cultivation or commercialized? Surely there is no need to rush this.

Finally, the existence of a "stand-alone" version of Event 3272's incorporated alpha-amylase argues strongly that there is no pressing need for this GM maize variety at all.

We respectfully urge you to protect South African citizens by conducting a rigorous assessment of Event 3272, and to defer any decision until such time as all relevant data has been collected and considered.

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Appendix 1
GM Crops and Allergies[2]

The potential of genetically engineered (GE) crops to induce food allergies has been the focus of intense concern ever since they were first introduced over a decade ago, as evidenced by the many studies, conferences and workshops devoted to the issue (FDA 1994; Metcalfe et al, 1996; Wal 1998; Consumer & Biotechnology Foundation 1999; Lehrer 1999; SAP II, 2000; EC Scientific Steering Committee 2000; FAO-WHO 2001; to name just a few). ….

Food allergies afflict an estimated 2-2.5% of the adult population and 6-8% of children (SAP III, p. 11; Sampson 1999), or about 8 million people in the US alone (based on US Census data). Symptoms range from rashes, hives and swelling to life-threatening anaphylactic shock. An estimated 29,000 episodes of anaphylaxis occur each year in the US, killing an estimated 150 people (Bock et al. 2001). Allergy sufferers can often avoid foods they are allergic to by checking the list of ingredients (e.g. peanut labeling). This is why serious reactions often occur at restaurants and other food service establishments, where meals can be contaminated with allergenic ingredients unbeknownst to the allergy suffer. Genetically engineered (GE) foods pose a similar problem. Without labeling of these novel foods, which requires traceability from field to table, no one can know whether an allergic reaction they have suffered is due to the genetic modification of that food, especially if it comes from a crop like corn that is rarely allergenic. This casts great doubt on the frequent claim that GE foods have not harmed anyone, especially when one recalls the similar claims once made for numerous chemicals now known to be toxic, such as asbestos, PCBs and dioxins. Then as now, "don't look, don't find" is not a very convincing scientific protocol. In part from concern over the rising incidence of food allergy (Wal 1998, p. 413; SAP I, p. 12), the causes of which are still obscure, an increasing number of scientists have recommended consideration of post-marketing surveillance of GE foods (Wal 1998 & 2001; SAP I, p. 11; SAP II, p. 11; Consumer & Biotechnology Foundation 1999, section 5.2; FAO-WHO 2001, p. 9).

Supporters of labeling and post-market surveillance of GE foods believe that the huge gaps in our knowledge of food allergy, coupled with the immunologic uncertainties of novel, genetically engineered proteins in the food supply, warrant such a precautionary approach. As allergists readily admit, it is extremely difficult to make valid generalizations in this field. Some prior exposure to the allergen is necessary for "sensitization" (acquiring the allergy), but how much exposure and for how long varies greatly depending on the allergen, the person, age and frequency of exposure, and many other factors (SAP I, p. 10). In some cases, exposure to billionths of a gram is sufficient to induce an allergy or allergic reaction (Businco et al, 1999). Even a single exposure could possibly sensitize the immune system (Dr. Ricki Helm, SAP Transcript, p. 446). There are reports of infants becoming sensitized through breast milk and fetuses acquiring allergies *in utero* (SAP III, p. 16). Some people may even become allergic to a food through inhalation of trace quantities (FDA 1994, pp. 219-20; Urisu 2001, p. 7). Many allergens are common components of the foods in which they are found; yet proteins present in "infinitesimally small quantities" (e.g. in soybeans) can also be allergens (FDA 1994, p. 145). The only thing that everyone seems to agree upon is that children, especially infants, are at the greatest risk of allergic sensitization to novel, genetically engineered proteins (see Section 8).

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[2] Excerpted from Freese, B. "The StarLink Affair," Friends of the Earth US, July 2001. Full report complete with references available from http://www.foe.org/camps/comm/safefood/gefood/starlink.pdf

Appendix 2
Allergenicity of Fungus-Derived Alpha-Amylases in the Baking Industry

Ann Occup Hyg 1997 Jun;41(3):287-96
Grouping strategies for exposure to inhalable dust, wheat allergens and alpha-amylase allergens in bakeries.
Houba R, Heederik D, Kromhout H. Department of Epidemiology and Public Health, Wageningen Agricultural University, The Netherlands, Dick.Heederik@Medew.hegl.wau.nl

This paper describes repeated measurements of inhalable flour dust, wheat allergens and alpha-amylase allergens in the bakery industry. A total of 571 full-shift personal dust samples was collected. Wheat allergens and alpha-amylase allergens were measured in 449 and 507 samples, respectively, by the use of recently developed immunoassays. For all three measures of exposure, the main components of exposure variability were determined. Different grouping strategies for studying exposure-response relationships were compared. The specific job of a bakery worker was identified as the most important source of variability in inhalable flour dust concentrations. For exposure to wheat allergens, the job performed was also the most important source of variation, but type of bakery also explained some of the variability. For alpha-amylase allergen exposure, information on type of bakery was more important then job information. For exposure to inhalable dust and wheat allergens, a classification by job title would lead to sufficient contrast in average exposure levels. By contrast, a grouping strategy based on a combination of job and type of bakery appeared to be essential to obtain a useful classification of exposure to alpha-amylase allergens. 1997 British Occupational Hygiene Society. PMID: 9204756 [PubMed - indexed for MEDLINE]

2: J Allergy Clin Immunol 1997 Mar;99(3):286-92
Airborne levels of alpha-amylase allergens in bakeries
Houba R, van Run P, Doekes G, Heederik D, Spithoven J. Department of Epidemiology and Public Health, Agricultural University Wageningen, The Netherlands.

BACKGROUND: In the baking industry the use of enzymes has increased throughout the 1980s. Several studies have reported sensitization and respiratory disorders among bakery workers caused by enzymes in dough improvers. **Fungal alpha-amylase is the most frequently reported cause of allergy.** alpha-Amylase allergen exposure levels in the bakery industry, however, have not yet been reported.
OBJECTIVE: The main objective of this study was to quantify personal alpha-amylase exposure levels of bakery workers.
METHODS: alpha-Amylase allergens were measured in 507 personal samples of airborne dust taken in bakeries by using a newly developed sandwich enzyme immunoassay with affinity-purified polyclonal rabbit IgG antibodies. A cascade impactor was used to estimate the size of dust particles carrying alpha-amylase allergens.
RESULTS: The rabbit IgG antibodies used in the assay showed, in immunoblotting with commercially available alpha-amylase, a reaction profile very similar to that of IgE from sensitized bakers. **The enzyme immunoassay appeared to be highly specific for fungal amylase**. Allergen exposure levels varied considerably among bakery workers, depending on the

type of bakery and job category (range, 0 to 40 ng/m3). In confectioneries no alpha-amylase allergens were detected. In other bakeries alpha-amylase exposure was only found for workers directly involved in dough making. Measurements of the particle size distribution in these bakeries showed that alpha-amylase allergens are most likely to be deposited in the nose and ciliated airways.
CONCLUSION: **This study shows that personal monitoring of fungal amylase allergen exposure in bakeries is possible. This permits the identification of high-risk tasks and allergen sources, as well as the study of exposure-response relationships.** PMID: 9058682 [PubMed - indexed for MEDLINE]

3: Am J Respir Crit Care Med 1996 Jul;154(1):130-6
Exposure-sensitization relationship for alpha-amylase allergens in the baking industry
Houba R, Heederik DJ, Doekes G, van Run PE. Department of Epidemiology and Public Health, Agricultural University, Wageningen, the Netherlands.

Fungal alpha-amylase is an important occupational allergen in the bakery industry. Epidemiologic studies focusing on the relationship between alpha-amylase allergen exposure and work-related respiratory allergy, however, have not been reported yet. In this cross-sectional study, sensitization to occupational allergens and work-related symptoms were studied in 178 bakery workers and related to allergen exposure. Alpha-amylase allergen concentrations were measured in personal dust samples, using a sandwich enzyme immunoassay. All workers were categorized into groups on the basis of their job histories and the alpha-amylase exposure levels of their job titles. Of all workers 25% had one or more work-related symptoms. **As much as 9% of the bakery workers showed a positive skin prick test reaction to fungal amylase, and in 8% amylase-specific IgE was demonstrated**. Alpha-amylase exposure and atopy appeared to be the most important determinants of skin sensitization, with prevalence ratios for atopy of 20.8 (95% CI, 2.74 to 158) and for medium and high alpha-amylase exposure groups of 8.6 (95% CI, 1.01 to 74) and 15.9 (95% CI, 1.95 to 129), respectively. Furthermore, a positive association was found between positive skin prick tests to alpha-amylase and work-related respiratory symptoms. In conclusion, this study has shown that there is a strong and positive relationship between alpha-amylase allergen exposure levels in bakeries and specific sensitization in bakery workers. PMID: 8680668 [PubMed - indexed for MEDLINE]