SUBMISSION TO THE IRISH ENVIRONMENTAL PROTECTION AGENCY

In connection with the notification for the release into the environment of genetically modified (GM) potatoes with improved resistance to *Phytophthora infestans* (2006-2010); notification number: B/IE/06/01.

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Although the permission sought only for the field trial of these GM potatoes, if the field trial is deemed successful, they are obviously intended for human (and animal) consumption. In view of this, it is regrettable that although the purpose of the release is "to compile data on agronomical performance and environmental effects and evaluate resistance against *Phytophthora infestans* and collect material for further (and unspecified) analyses…", none of the scope of these studies, nor the methods to be employed are detailed. This serious omission is further compounded by the fact that the only reference to possible toxic or allergenic effects of the GM potatoes is described in a scientifically totally unacceptable sentence that "no toxic or allergenic are expected on the basis of…or the expressed AHAS protein". Indeed, under subsection F: "Summary of planned field trials designed to gain new data on the environment and human health impact of the release" is dismissed as ***not applicable***.

This sort of cavalier treatment of the regulatory authorities and consumers is totally unacceptable particularly in view of the accumulating worrying evidence for the potential negative health effects observed with many other GM potato lines. Every part of the potato plant is toxic for human and animals and even the tuber may become toxic when exposed to sunlight, etc. Thus because all cells of the potatoes carry the same genetic information, the importance of the exclusion of possible unintended alterations resulting from gene-splicing ought to have featured prominently in the submission. It is not even mentioned. This is further aggravated by the fact that most experimental work reported in science journals is full of negative findings and most GM potato lines do not even satisfy the conditions of substantial equivalence with their isogenic parent lines. Some examples:

GM-potatoes

The gene of soybean glycinin was transferred into potatoes with the aim to increase their protein content (Hashimoto et al., 1999a). However, as the expression level of glycinin in potatoes was only between 12-31 mg/g total soluble protein, the improvements in protein content or amino acid profile were minimal. In fact, the total protein content of the GM potatoes after the gene transfer became significantly less than that of the control line. Even more unfortunately, the contents of some vitamins were reduced while the amounts of both solanine and chaconine increased in the GM lines. In this light the claimed substantial equivalence of the GM and parent lines was not supported by the published results.

Because of finding significant differences in a number of tuber components, the results of compositional analyses of some macro- and micro-nutrients of insect- and virus-resistant potatoes and those of untransformed lines (Rogan et al., 2000) did not *appear to support their substantial equivalence. However, in the absence of animal studies it is difficult to ascertain whether these differences could have any biological consequences for humans/animals, particularly as known antinutrients, such as lectins or enzyme inhibitors were not analysed.*

It has been shown by modulating the adenylate pool by genetic manipulation of the plastidial adenylate kinase in transgenic potato plants that it was possible to increase the level of starch in the tuber by 60%, the concentrations of several amino acids and, at the same, time increase tuber yield as well (Regierer et al., 2002). Unfortunately, no feeding studies have been reported on this GM potato. No modern analytical methods for establishing substantial equivalence between GM- and non-GM potatoes have been used in either of these two studies.

The composition of two GM lines G2 and G3 expressing Cry V gene from Bacillus thuringiensis was compared by conventional analytical methods with that of the Egyptian parent line called Spunta in a recently published paper (El Sanhoty et al., 2004).. A general comment: it would have been helpful if the authors had made clear whether the potatoes used for the analyses were from the Egyptian markets as stated in the introduction or from Michigan field plots in the USA as indicated under materials and methods. According to the authors, none of the 14 main chemical components in the two GM lines and the parent potatoes was significantly different.

However, a closer examination of the results in the Tables 2-5 showed that the contents of some components of the control and either or both of the GM-lines may have differed significantly. Thus, the ascorbic acid, phosphorus and calcium contents in Table 2 appeared to be different. The contents of some amino acids in the three lines (Table 3) also showed major differences, chief amongst them were methionine, cystine, tryptophan, histidine. Curiously, phenylalanine was not listed in Table 3 and each, the methionine and tryptophan contents exceeded that of aspartic acid. Both genetically modified potato lines contained significantly higher protease inhibitor activity than the parent line (Table 5). The general state of the presentation of the results was rather poor and left the reader confused whether to believe the text or the Tables. In fact, the results appeared to contradict the conclusions of the paper that the parent- and GM potatoes were substantially equivalent.

There is also plenty of evidence to show that most of the GM potatoes when tested in animal experiments were not nutritionally equivalent to the parent line potatoes either.

Here are some examples:

Glycinin-expressing potatoes

In a 4 week rat feeding study both the experimental and control groups were fed the same commercial diet but daily they were also given by gavage 2 g of respective potato lines/kg body weight. These were the parental control line and two transformed GM lines, one with the glycinin gene and another one with a designed glycinin gene (coding for four additional methionines in the gene product), respectively. Commendably, the authors (Hashimoto et al., 1999b) measured the growth, feed intake, blood cell count and blood composition and internal organ weights of the rats. However, it is unclear whether the animals were fed with raw or boiled/baked potatoes and this makes the interpretation of the results difficult.

Bt toxin potatoes

In a mainly histology study of the ileum of mice fed with potatoes transformed with a Bacillus thuringiensis var. kurstaki CryI toxin gene and as control, the effect of the toxin itself (Fares and El-Sayed, 1998) it was shown that both the delta-endotoxin and, to a lesser extent the Bt-potato, caused villus epithelial cell hypertrophy and multinucleation, disrupted microvilli, mitochondrial degeneration and increased numbers of lysosomes and autophagic vacuoles and the activation of crypt Paneth cells. Unfortunately there were some flaws in the experimental design such as the lack of proper description of the Bt potatoes and their gene expression level or the uncertainty whether the potatoes in the diet were cooked/baked or raw or the failure to describe the amount of the Bt toxin used for the supplementation of the control potato diet, which makes it difficult to quantitatively compare the effects on the ileum of the Bt potato with the spiked control potato diets. All the same, this was an important study because it once and for all established that, in contrast to general belief, exposure of the mouse gut (ileum) to the CryI gene product has caused profound hypertrophic and hyperplastic changes in the cells of the gut absorptive epithelium and these could lead to mucosal sensitization as it was later demonstrated (Vazquez Padron et al., 1999; 2000). These changes could only have occurred because, in contrast to the artificial stability shown in the in vitro simulated gut proteolysis tests, the Bt toxin did in fact survive in a biologically active form the passage through the digestive tract in vivo. Clearly, concerns about the possible biological consequences of exposure to GM food such as those expressing the Bt toxin should be addressed under in vivo conditions. As a result it was recommended that "thorough tests of these new types of genetically engineered crops must be made to avoid the risks before marketing".

GNA-GM potatoes

The work concerning the effect on the histology of the different gut compartments of feeding rats on diets based on GM potatoes expressing the snowdrop (Galanthus nivalis) bulb lectin (GNA) gene (Ewen and Pusztai, 1999b) revealed some major changes in gut structure and function. The significance of these results was further expanded in the authors' reply (Ewen and Pusztai, 1999a) to the invited comments by the Lancet (Kuiper et al., 1999) and also in a recent review (Pusztai et al., 2003). Some other selected results of the nutritional/metabolic studies published on the website of the Rowett Research Institute (http://www.rri.sari.ac.uk) where most of the work was done (Bucksburn, Aberdeen, Scotland UK) will only be briefly mentioned. Young, rapidly growing rats (starting weight of 84 ± 1 g) were strictly pair-fed on iso-proteinic (60 g total protein/kg diet; most of which was from potatoes) and iso-caloric diets supplemented with vitamins and minerals for 10 days. The test diets contained GM potatoes either raw or boiled. The control diets contained the same amount of parental line potatoes (raw or boiled) alone or supplemented with GNA at the same concentration as expressed in the GM potatoes. As a system control a lactalbumin control group of rats was also included. Samples of stomach, jejunum, ileum, caecum and colon were, after fixation and staining with haematoxylin and eosin, subjected to full quantitative histological evaluation which revealead that the mucosal thickness of the stomach was increased a part of which was caused by GNA, the gene product (Ewen and Pusztai, 1999b). However, the proliferative hyperplastic growth of the rat small intestine leading to crypt enlargement and a part of the stomach enlargement was not a GNA lectin effect but was probably either due to some other component of *the gene vector used for the genetic modification and/or the disruption caused by the incorporation of the vector in the plant genome. Indeed, unlike the strongly mitotic lectins such as the kidney bean phytohaemagglutinin, GNA from snowdrops is a non-mitotic lectin whose binding to and growth-promoting activity for the small intestinal epithelium is slight and not significant (Pusztai et al., 1990) and as measured by staining with anti-GNA antibody + PAP (peroxidase - antiperoxidase), it remains unchanged after the expression of its gene in GM potatoes. Hyperplasia was also confirmed by measuring the increase in crypt cell numbers and crypt mitotic figures in the jejunum of GM potato-fed rats (Pusztai et al., 2003). However, as the solanine glycoalkaloid content of the GM potatoes was significantly less than that of the parent lines (Birch et al., 2002) the suggestion that the jejunal growth was caused by potato glycoalkaloids could be ruled out. Overall the results suggested that crypt hyperplasia and the observed epithelial T lymphocyte infiltration caused by GM potatoes might also occur with other GM plants which had been developed using the same or similar genetic vectors and method of insertion. It is therefore imperative that the effects on the gut structure and metabolism of all GM crops should be thoroughly examined as part of the regulatory process before their release into the human food chain.*

Potatoes expressing cationic peptide chimeras

Desiree and Russet Burbank potatoes expressing N-terminus modified cecropin-melittin cationic peptide chimeras and control line potatoes fed to mice caused severe weight loss (Osusky et al., 2000). The animals did not grow even after supplementing these potatoes with Rodent Laboratory Chow. Apparently, mice fed with tubers from transgenic potatoes were as healthy and vital (sic) as those from the control group and their faecal pellets were comparable. The severe weight loss seriously questioned the value of the results of this poorly designed feeding experiment.

GM-potatoes expressing a cysteine-proteinase inhibitor, rice cystatin, are partially (or in some case fully) resistant to nematodes without apparently harming non-target anthropods or perturbing soil microbial communities (Urwin et al., 2003). As it occurs naturally in rice, maize and even in potatoes, cystatin is not new for the mammalian digestive system. Moreover, the expression of cystatin can be limited to the roots, further reducing the already potentially low concern of toxicity or allergenicity with these GM potatoes. According to recent claims by the Atkinson group, their nutritional studies presented prima facie evidence that cystatin-expressing GM-potatoes do not present toxic risks to mammals (Atkinson et al., 2004). However, in reality these authors did not test the safety of the GM-potatoes. Neither did they carry out a proper nutritional evaluation of them. What they actually tested was the in vitro stability to digestion with simulated gastric fluid at pH 1.2 and at abnormally high concentrations of pepsin, of a surrogate recombinant E. coli protein and not that isolated from GM-potatoes or, even more importantly, not of the GM-potatoes themselves. Thus, this in vitro simulation test was flawed both in principle and execution. Instead of a proper nutritional evaluation of the GM-potatoes the authors used this recombinant surrogate protein in a LD_{50}-type oral toxicity study, the design of which was seriously flawed. Rats of unknown starting weight were kept in groups of five, and not individually, making it impossible to follow their individual feed intakes and growth and to relate therefore their body tissue weights and other measured parameters to final body weights. Although some body organs were weighed and some, such as the liver showed differences, the only *part of the gastrointestinal tract that was measured was the caecum but not the small intestine or the colon, the very tissues that are the most sensitive indicators of dietary effects. This omission is the more serious because the weight of the caecum which is a relatively small part of the large intestine whose weight (unrelated to the final body weights!) was found to be significantly increased. For all these flaws the authors' conclusion that these cystatin-expressing GM potatoes do not present toxicity risks to mammals may need to be re-assessed by further studies..*

Raw Punta G2 and G3 GM- and non-GM-potatoes whose analytical composition was given above were also subjected to a 30-day feeding study with rats (El Sanhoty et al., 2004). Some of the annoying discrepancies between data in the text and the Tables should have been removed during the peer-review and editing. As the potato inclusion in the diet was only 30%, less than 15% of the protein in the diet was provided by the GM potatoes. More seriously, the composition of control group 1 was substantially different from the potato-containing diets as its protein content was about 15% less. It was not made clear why all the diets were supplemented with 0.3% methionine when the potatoes were not deficient in this amino acid. Differences in rat starting weights (± 10%) were too high although they improved after pre-feeding.

However, despite assurances by the authors that feed efficiency values were not significantly different between the test and the control groups this cannot be accepted on the basis of the data in Table 6. Similar considerations apply to testes weights in Table 7. Apart from the specific criticisms above, the design of the feeding study was rather simplistic and static without looking at more dynamic novel features in the GM-fed rats, such as possible changes in immune-, hormonal- and metabolic functions, and no attempt was made to use histopathology methods to detect and/or exclude potential alterations in the cells or tissues of the rats on different diets. A particularly serious omission in the work is the absence of a critical look at the structure and function of the small intestinal epithelium.

In view of all the accumulating data showing that GM potatoes of all kinds investigated to date have shown unacceptable compositional, metabolic, immunological effects and potentially toxic behaviour, it is imperative for the Irish EPA to reject this request by BASF for field trial of their GM potatoes until and unless they are first subjected to independent environmental and health risk assessments using a scientific protocol openly agreed and approved by independent scientists and representatives of the public and consumer groups.

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