

**Chardon LL Hearing**

**Analysis of key documents relevant to the safety of  
Chardon LL for animal feed purposes**

**Proof of Evidence**

**of**

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**on behalf of Friends of the Earth**

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This evidence was submitted at the National Seed List Hearings which ran from October to November 2000 in Manchester and London, England. In April 2000 the UK Government proposed to add the genetically modified maize seed, Chardon LL, to the UK National Seed List. Chardon LL is a variety of T25 maize developed by Aventis. The hearings considered public objections to this government proposal. On 15 November 2000 the National Seed List Hearings were indefinitely suspended by the UK Government.

This is part of a series of evidence submitted. For the rest of the evidence and for Friends of the Earth's case against Chardon LL maize see:

[www.foe.co.uk/campaigns/food\\_and\\_biotechnology/information/gm\\_food/](http://www.foe.co.uk/campaigns/food_and_biotechnology/information/gm_food/)

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## **1. Introduction and summary**

- 1.1 I have been asked by Friends of the Earth to analyse documents which were produced by Aventis and relevant to the safety of Chardon LL. I present the results of my analysis in this Proof of Evidence.

I qualified in Medicine in 1970 at the University of Liverpool and after registration with the General Medical Council started a career in research at that institution. My research has always been centred on the development of the fetus and neonate. I am currently a Senior Lecturer and Head of the Fetal and Infant Toxicology-Pathology Group at the University of Liverpool. I am a Fellow of the Royal College of Pathologists.

In 1983, I obtained the degree of Doctor of Philosophy at the University of Liverpool for research into the development of the immature brain, using 3-D microscopy techniques. At this time I was appointed an Editor of the Journal of Microscopy, which is an internationally recognised publication and the official journal of the Royal Microscopical Society. In 1985 I was appointed General Editor in overall charge, a post that I held until 1992.

- 1.2 I have analysed the relevant parts, or all, of certain documents which we are informed have been submitted by the company to the European Commission, The United States Food and Drug Administration and to the UK Government. The documents are:

- (1) the Food and Feed Safety Assessment of T25 maize voluntarily submitted to the US Food and Drug Administration (CHD 10b, page 4 onwards; FOE File 3, Tab6; original document reference GSM/S2);
- (2) the Submission to the European Union for Placing on the Market of T25 maize (CHD 12, page 10 onwards; FOE File 4, Tab 2); and

- (3) a report of a feeding study in rats ( CHD 10b, page 300 onwards; FOE File 3, Tab 15).

1.3 The principal conclusions of my analysis can be summarised as follows:

(1) It is stated that T25 maize is not “*materially different*” from current commercial varieties. However the measurements made suggest a different conclusion. Statistically significant differences were found in the composition of fat, protein and fibre between the GM maize silage and silage from the non GM counterparts, as well as statistically significant differences in fat and carbohydrate values of the GM and non GM grain samples. Increases were observed in the levels of the amino acids arginine, histidine and lysine, and analysis of fatty acid composition found the levels of steric, linolenic and aracidic fatty acids to be statistically significantly different from the non-GM counterparts. The levels of linolenic and aracidic fatty acids had values outside the range recorded in the literature.

(2) It is stated that “*AgrEvo GmbH has confirmed experimentally that PAT protein and pat DNA in glufosinate resistant canola [oilseed rape] is degraded in vitro by the gastric juices from swine, chicken, and cattle (bovine rennet bag fluid and paunch)*”. However, I do not consider that this statement is supported by the experiments outlined in the document. The experiments did not accurately model the conditions likely to be found in animals eating the GM maize as part of their diet, and consequently do not represent a realistic assessment of the likely degradation of PAT protein in real animals. In addition, I am not convinced that experiments conducted using PAT protein extracted from canola can be applied to the PAT protein in T25 maize.

(3) In the case of Chardon LL, the ultimate intention is to feed it to cattle, probably representing >50% of the total diet. The only toxicological study

conducted was a two-week feeding trial with rats using a refined extract from GM oilseed rape. I do not consider that this study using rats can be used as a basis for making judgements about the safety of Chardon LL maize with respect to cattle. The studies presented do not address the main question with respect to Chardon LL, namely "What is the effect of feeding glufosinate resistant maize to ruminants at >50% of the diet?" All the data that has been presented is just a surrogate for a well-designed feeding trial, which would be both relevant and informative.

(4) Safety can only be proven by a properly designed feeding trial in which the whole plant is fed to the intended target species in a relevant proportion of the diet.

I now consider each of the documents: firstly, documents (1) and (2) together, and thereafter document (3).

**2. The Food and Feed Safety Assessment of T25 maize voluntarily submitted to the US Food and Drug Administration in 1995 (CHD 10b, page 4 onwards; FOE File 3, Tab6; document reference GSM/S2); and the Submission to the European Union for Placing on the Market of T25 maize made in 1996 (CHD 12, page 10 onwards; FOE File 4, Tab 2)**

2.0 The first document was submitted by AgrEvo (now Aventis) to the US FDA. It was then largely recycled for submission to the European Union. The document for the US FDA includes references to another GM maize (T14) which was not submitted for approval in the European Union but in many cases the text of the two documents is the same.

The summary of AgrEvo's submission to the US FDA states that

*"The food and feed safety assessment summarised below establishes the safety of transformation events T14 and T25 and all progeny derived from crosses with*

*traditional corn varieties*” (CHD 10b, page 10, last sentence of the first paragraph on page 7 of the original document; FOE File 3, Tab 6).

and that

*“AgrEvo’s data and findings in every case lead to the conclusion of ‘no concern’ for the safety and nutrition of GRC [Glufosinate Resistant Corn] events T14 and T25 and their progeny”* (CHD 10b, page 10, last sentence of second paragraph on page 7 of the original document; FOE File 3, Tab 6).

In their submission to the EU Aventis state that

*“Analyses showed that GTC [glufosinate tolerant corn] silage and grain are not materially different from current commercial varieties in essential nutrients or antinutrients”* (CHD 12, page 49, being in section A/Compositional and Nutritional Analyses on page 40 (bottom numbering) of the original document; FOE File 4, Tab 2).

## **2.1 Establishment of Equivalence**

2.1.1 Statistically significant differences were found in the composition of fat, protein and fibre between the GM maize silage and silage from the non-GM counterparts. Although the GM and non-GM maize were clearly not equivalent, there was no further investigation of this obvious area of difference between the non-GM and GM maize (CHD 12, pages 49/50, including Figure C1, being pages 40/41 (bottom numbering) of the original document; FOE File 4, Tab 2).

2.1.2 The analyses of the GM grain and non-GM counterparts also showed statistically significant differences in fat and carbohydrate values. Once again,

this difference was not investigated further because, according to the authors, “*the values do fall within the range of nutrient values reported*” . (CHD 10b, page 32, at the end of the penultimate sentence in the top paragraph on page 30 (bottom numbering) of the original document; FOE File 3, Tab 6).

2.1.3 In the case of silage, Aventis state that the above-mentioned “*differences are attributable to two of the pairs*” of cultivars (CHD 10b, page 30, first sentence on top of page 28 of the original document; FOE File 3, Tab 6). If this is offered as a reason to dismiss the differences, the reason is unclear. If a statistically significant difference is detected in the presence of high variability, then it should be further investigated, not ignored.

2.1.4 In any event, the fact is that this experiment is not optimally designed, with too few specimens analysed to allow for a suitably powerful statistical analysis. Where differences are observed between the GM maize and non-GM counterparts, these are dismissed without adequate explanation. This means that the experiment is not sufficiently rigorous to support any conclusion about the equivalence or not of T25 maize to its non-GM counterparts.

### **Pre-ensiling analysis**

2.1.5 An additional problem is the fact that silage analyses were performed on material taken prior to ensiling. However there are only literature values for ensiled material, which, the authors state, is highly variable and not directly comparable to their data. They then proceed to do precisely that comparison.

2.1.6 I do not consider that compositional analyses performed on pre-ensiled material are a reasonable or adequate base upon which to examine the composition of ensiled material. The authors should have been aware before they conducted the analyses that the results would not have been directly comparable with any

literature values. This means that the analyses cannot be used to support any judgement about the equivalence or not of T25 silage to non-GM silage.

## **2.2 Compositional and nutritional analysis**

- 2.2.1 The amino acid assay for substantial equivalence showed statistically significant increases in the levels of the amino acids arginine, histidine and lysine (CHD 10b, page 32, being page 30 of the original document; FOE File 3, Tab 6). This is interesting in itself because these three amino acids are similar in chemical composition, possessing an additional  $\text{NH}_2$  group. No further investigation was made into this difference, despite the fact that the introduced PAT-protein is an N-acetyl transferase, an enzyme that might be expected to react with such molecules. It is possible that the increased levels of these three amino acids are connected to the introduction of the PAT protein.
- 2.2.2 Strangely, no analysis was made for the levels of the amino acids asparagine and glutamine or, if this was done, no data are presented. These amino acids also have an additional  $\text{NH}_2$  group. The levels of the three other amino acids with an additional  $\text{NH}_2$  were found to be increased, and therefore one might expect that asparagine and glutamine might similarly be raised.
- 2.2.3 Enzyme kinetics and specificity of the novel protein PAT were tested against all amino acids (CHD 12, pages 37/38, being pages 28-29 (bottom numbering) of the original document; FOE File 4, Tab 2), in contrast to the investigations for substantial equivalence, where only 18 were tested. No activity of the enzyme towards any amino acid was demonstrated under test conditions. To me, this raises the question as to what the possible mechanism could be that led to the raised levels of three amino acids. There is no discussion by the authors of mechanisms which could lead to the observed changes in amino acid composition, for example interference between the naturally present

acetyltransferases and PAT n-acetyltransferase. I am concerned that this has not been addressed.

2.2.4 The differences in the three amino acids measured were not investigated further because, according to the authors, *“both[ the] GRC [Glufosinate Resistant Corn] and its counterparts was qualitatively and quantitatively similar to that reported by USDA”* and *“because of the varied diet of both humans and animals, and the degradation of excess amino acids in the body, any additional protein contributed by PAT or the GRC would be nutritionally inconsequential”*(CHD10b, around (owing to photocopying error) page 32/33, being page 31 of the original document; FOE File 3, Tab 6).

2.2.5 This statement does not concur with that made in 1992 by Dr Gerald B. Guest, DVM, Director of the FDA Centre for Veterinary Medicine, with which I agree. In commenting on the FDA consultation paper “Statement of Policy: Foods from Genetically Modified Plants”, Dr Guest said (CHD 15, pages 283/4 (at point 1); FOE File 5, Tab 25):

*“Unlike the human diet, a single plant product may constitute a significant proportion of the animal diet. For instance 50 – 75 percent of the diet of most domestic animals consists of field corn. Therefore, a change in nutrient or toxicant composition that is considered insignificant for human consumption may be a very significant change in the animal diet.”*

I note that this statement is repeated, almost verbatim, in the US Federal Register dated 29<sup>th</sup> May 1992 (CHD 15, page 263, middle column under section H ‘Issues Specific to Animal Feeds’; FOE File 5, Tab 24).

He also pointed out that *“ Residues of plant constituents or toxicants in meat and milk products may pose human food safety problems. For example,*

*increased levels of glucosinolates or erusic acid in rapeseed may produce a residue problem in edible products”* (CHD 15, page 284, (point 5); FOE File 5, Tab 25).

- 2.2.6 An analysis of fatty acid composition found that the levels of steric, linolenic and aracidic fatty acids were statistically significantly different from the non-GM counterparts. The levels of linolenic and aracidic fatty acids in fact had values outside the literature value range. This difference was not investigated further because, according to the authors, *“the qualitative and quantitative profiles of total lipids and specifically the fatty acids are similar to those reported for grain by USDA”* (CHD 12, pages 53/4, being pages 44/5 (bottom numbering) of the original document; FOE File 4, Tab 2).
- 2.2.7 The importance of the measurement of anti-nutritional factors was noted by the authors, who state that *“The possible presence of anti-nutritive substances which occur naturally needs to be taken into consideration with respect to animal feed”*. They then use the example of phytates *“which bind phosphorus and other minerals making them unavailable to monogastric animals”* (CHD 12, pages 50/1, being pages 41/2 (bottom numbering) of the original document; FOE File 4, Tab 2). They then proceed to examine the levels of phytic acid in silage, a feed which is fed almost exclusively to ruminants, such as cattle, not to monogastric animals.
- 2.2.8 Even laying the appropriateness of the analysis to one side, the variability of the data is extreme, with a Coefficient of Variation in excess of 100%.
- 2.2.9 The summary of the section dealing with variation in nutritional and compositional parameters between GM T25 maize and non-transgenic counterparts in the document submitted to the US FDA, acknowledges that *“there is some variation”* but that *“none of these differences is significant*

*nutritionally*” (CHD10b, around (owing to photocopying error) page 32/33, being page 31 of the original document; FOE File 3, Tab 6). No rationale or methodology for arriving at this opinion is offered. The final conclusion (CHD 10b, page 49, final paragraph on page 47 of the original; FOE File 3, Tab 6) is that T25 maize is not “*materially different*” from current commercial varieties. However the measurements made suggest a different conclusion.

## **2.3 The Introduced PAT Protein**

2.3.1 In the voluntary submission to the US FDA, it is stated that “*both the ensiling process and heat treatments used for the processing of grain should eliminate most PAT activity*” (CHD 10b, page 36, being the first sentence of the final paragraph on page 34 of the original; FOE File 3, Tab 6). However, whilst statements are made about the relationship and sequence of changes in pH and temperature in the making of silage, these are not supported by experiments using the GM maize. Therefore it is not possible to interpret the information given on page 34 of the original document with respect to the length of time the enzyme is active during the process of ensiling. It is possible that if the PAT protein has considerable activity during the ensiling process this could affect the nutritional value of the resulting silage. No examination of this was presented.

2.3.2 On the same page 34 of the original document, in the middle of the final paragraph, it is stated that “*AgrEvo GmbH has confirmed experimentally that PAT protein and pat DNA in glufosinate resistant canola [oilseed rape] is degraded in vitro by the gastric juices from swine, chicken, and cattle (bovine rennet bag fluid and paunch)*”. However, I do not consider that this statement is supported by the experiments outlined in the document. With respect to the gastric juices of monogastric animals, the pH of 1.5 quoted on page 35 will only be present in the fasting condition. It is well understood by nutritional physiologists that the pH of the stomach rises after ingestion of food, definitely

higher than pH 5.5, the highest pH tested in these experiments. It remains elevated above pH 4 in the contents for a considerable time. The experiments do not represent a realistic assessment of the likely degradation of PAT protein in real animals.

- 2.3.3 I am concerned that these tests were carried out using PAT protein from GM canola (oilseed rape). The authors state that *‘these results can be extended to corn, as the PAT enzyme is the same size when expressed in canola, corn and bacteria’* (CHD 10b, page 37, from the penultimate sentence of the top paragraph on the original page 35; FOE File 3, Tab 6). However there is no further explanation as to why this should mean that tests conducted using transgenic canola can be translated to transgenic maize. This does not seem to be a justifiable comparison.
- 2.3.4 With respect to DNA, this is not as readily degraded as the protein and indeed, at all pH >1.5 the gene was still detectable after 1 hour. The possible impacts of this are not discussed.
- 2.3.5 There is no evidence that the influence of high protein diets in animals on the deactivation of PAT protein been investigated. It appears to us that the available level of pepsin, an enzyme required for digesting protein, seems to be crucial.

## **2.4 Allergic and Toxic Potential**

- 2.4.1 The allergenic potential of the introduced PAT protein is examined by examining its mass profile, heat and acid stability, glycosylation and homology of its amino acid sequence to known allergens (CHD 10b, pages 41/2; FOE File 3, Tab 6).
- 2.4.2 Searching for sequence homologies such as the amino acid sequence for potential glycosylation sites is no substitute for human volunteer trials, which are the only reliable way of detecting human allergy. Although Chardon LL is proposed for

animal consumption, T25 maize varieties have, as far as I am aware, also been produced for human consumption.

2.4.3 The final statement in section 2 on page 40 of the original document (CHD 10b, page 42; FOE File 3, Tab 6) that “*there is no evidence that PAT protein should pose any significant toxic or allergenic risk*” is based on little relevant data.

2.4.4 The variability of PAT expression is impressive - the authors admit on page 41 of the original document that PAT expression is susceptible to effects from location, part of the plant, time of harvesting and year of harvest. In my opinion, it seems likely that inadequate testing has been done.

2.4.5 The statement that allergenic proteins “*are usually present in high concentrations*” (CHD 10b, page 41, in the penultimate sentence of the first paragraph of section C on page 39 of the original document; FOE File 3, Tab 6) is not relevant. Some food components which are very hazardous to a susceptible minority can have an effect at trace levels.

2.5 The statement is made at the beginning of the voluntary submission to the US FDA that “*AgrEvo’s data and findings in every case lead to the conclusion of ‘no concern’ for the safety and nutrition of GRC [Glufosinate Resistant Corn] events T14 and T25 and their progeny*” (CHD 10b, page 10; from the final sentence of the second paragraph on page 7 of the original document; FOE File 3, Tab 6). I do not consider that this conclusion is justified by the data presented. The tests to give realistic answers on the question of toxicity simply have not been performed.

## **2.6 Gene transfer**

2.6.1 One of the major concerns about antibiotic resistance marker genes in GM crop plants is horizontal gene transfer between the plant and bacteria in the

SK/TK/HMI

environment. The question of whether or not bacteria can take up the disrupted *ampR* gene and reactivate it has not been addressed – even to the extent of stating that it is not relevant.

**3. RCC Project 616307, AgrEvo Document Number: A57935. PAT-PROTEIN – Repeated dose oral toxicity (14-day feeding) study in rats. Authors: Pfister Th, Schmid H, Luetkemeier H, Biedermann K & Weber K. Dated 29<sup>th</sup> April 1996 (CHD 10b, page 300 onwards; FOE File 3, Tab 15)**

3.1 This document was submitted by Aventis to the European Union in support of its application for GM marketing consent for T25 maize. I have examined a full copy of the final version of this document.

Detailed comments about this experiment are included in the Appendix, our general comments are as follows:

3.2 We note that it is stated on page 15 of the report, under section entitled ‘PURPOSE/RATIONALE’, that:

*“This study should provide a rational basis for toxicological risk assessment in man.”* (CHD 10b, page 315; FOE File 3, Tab 15).

Therefore it cannot, and does not, purport to be a rational basis for toxicological risk assessment in cattle.

3.3 The study was performed on immature rats which underwent a doubling of weight during the study. This, in combination with a low protein diet, represents good toxicological practice, in that it is more sensitive to detecting anti-nutritional effects than studies performed on fully mature animals on high protein diets.

3.4 There is no specific statement as to the provenance of the PAT protein made in the Report. However, by inference it appears that it came from purified Canola meal (page 18 of the report, or CHD 10b, page 318). An implicit model assumption is made, that the toxicology of PAT-Protein from Canola is identical to that from the fodder maize, which is the subject of the current listing application. There is no

evidence presented to support this assumption. The folding of the protein in the two species, which will not necessarily have the same chaperone proteins, may not be identical. It would have been a more logical experimental design to use PAT-Protein from the fodder maize in an application to licence that particular crop.

- 3.5 By feeding the purified PAT-protein, rather than the whole plant, this experiment is specifically designed to NOT detect the pleiotropic effects which should be anticipated. In addition, the relatively short-term exposure of 14 days cannot be expected to realistically model the type of toxicity that might occur from lifelong exposure to GM maize derived forage at > 50% of the diet. Typically, sub-chronic toxicity testing in laboratory rodents is usually performed for 28 or 90 days.
- 3.6 In the case of Chardon LL, the ultimate intention is to feed the GM fodder maize in question to cattle, probably representing >50% of the total diet. Under these circumstances it would seem logical, for the purposes of registration of such a crop variety, to perform feeding studies of whole plant diets to immature cattle under conditions of protein restriction. Then the target species will have been tested against the actual food in conditions likely to uncover toxic effects if they exist. I do not consider that this study using rats can be used as a basis for making judgements about the safety of Chardon LL maize with respect to cattle.
- 3.7 Small laboratory animals such as rats are known to be able to detoxify toxic substances at much higher rates than larger animals. Therefore the approach adopted is clearly not adequate to determine the toxicological status of this maize if used as a fodder crop for cattle.
- 3.8 I have set out in the Appendix of this Proof of Evidence more comments on this study.

## **4 Overall Opinion**

- 4.1 The studies seem to avoid posing the main toxicological question, namely "What is the effect of feeding glufosinate resistant corn to ruminants at >50% of the diet?" The data from the three documents that have been presented are collectively a surrogate for a well designed feeding trial, which would be both relevant and informative. The transformed line is clearly not substantially equivalent to the parent strain and therefore proper hazard assessment needs to be performed in the form of an *in vivo* feeding trial, to be sure that pleiotropic effects can be detected.
- 4.2 In my opinion, the transformed line does not appear to be substantially equivalent with the parent line. We do not consider that feeding the PAT protein produced by another plant species (Canola) to a non-relevant species (rat) is sufficient to prove the safety of this product.
- 4.3 If, despite the problems with substantial equivalence, licensing is still sought, then safety can only be proven by a properly designed feeding trial in which the whole plant should be fed to the intended target species in a relevant proportion of the diet.

## Appendix

### **Detailed comments on RCC Project 616307, AgrEvo Document Number:**

### **A57935. PAT-PROTEIN – Repeated dose oral toxicity (14-day feeding) study in rats. Authors: Pfister Th, Schmid H, Luetkemeier H, Biedermann K & Weber**

### **K. Dated 29<sup>th</sup> April 1996 (CHD 10b, page 300 onwards; FOE File 3, Tab 15)**

- A.1 This is a Good Laboratory Practise (GLP) toxicology study which is presented in a detailed report. The study design involves feeding immature rats for 14 days on either a standard protein diet to a control group or low protein diets to three experimental groups, consisting of either 100% soyamin, 100% PAT-Protein or 10% PAT-Protein/90% soyamin respectively.
- A.2 The PAT-Protein was apparently extracted from Canola seeds. No significant differences between any of the groups for any parameter measured was reported.
- A.3 The mean weight of the animals at the commencement of the experiment was very variable. The range of weights for males was 53 to 82 grams and for females 50 to 74 grams. The standard deviation (SD) of mean animal weights in different groups varied by up to 50%. Furthermore in the male animals, while the SD of the mean weights in the protein deprived animals increased during the experimental period, that for the control group decreased.
- A.4 These observations indicate potential causes of confounding in an experiment with group sizes of only 5 animals, which is generally regarded as a working minimum. The power of the experiment to discriminate minimal change would have been higher if the weights of animals had been more closely matched and/or the numbers of animals in each group had been increased at the commencement of the investigation.

## A.5 Protein deprived diet

A.5.1 The nominal protein contents of the diets provided were (page 156A of the original report, or CHD 10b, page 447):

Group 1	193 g/kg	(Standard diet)
Groups 2 –4	155 g/kg	(Low protein)

Thus the low protein diet contained 80% by weight of the protein found in the standard diet.

A.5.2 It is interesting to note that for part of the time the experiment was running, the food consumption of some of the protein-deprived animals was considerably higher than in the control group. For example, in the female Group 3 at day 7, mean food consumption was 38% higher than in controls. Also in the female group, the food consumption of Group 4 was consistently about 20% higher than in the control group (page 38 of the original report or CHD 10b, page 338). This means that for much of the experiment, many of the female animals in the ‘low protein group’ would have been on iso-protein intakes with the controls. For males, any such trends were not obvious. However, at the end of the experimental period in the male group, food consumption was increasing in Group 3 animals. A longer period of experimental feeding may have shown whether this was a significant change.