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Comments to FSANZ on A1081

Application for approval of
Food derived from Herbicide-tolerant
Soybean Line SYHT0H2

Prepared by Bob Phelps

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Our comments on the assessment

FSANZ says: "This safety assessment report addresses only food safety and nutritional issues." So it is unacceptable that this assessment relies almost exclusively on unpublished and non-peer-reviewed Syngenta and Bayer documents which we critically review below. Most of the documents submitted do not include:

- Assessments of risks, hazards and safety among their goals
- Quantification or justification for treatments or sample sizes
- Statistical analyses or data suitable for such analysis
- Tests of the significance of differences between groups or treatments
- Control groups or control treatments
- Openness over rejected data and the reasons for such rejection
- And other deficiencies as detailed

In our submission, FSANZ would be unable to conclude, on the basis of the papers that the applicants provided, that: **"**No potential public health and safety concerns have been identified in the assessment of soybean line SYHT0H2. **On the basis of the data provided in the present Application,** and other available information, food derived from soybean line SYHT0H2 is considered to be as safe for human consumption as food derived from conventional soybean cultivars."

We therefore ask FSANZ to reject the application.

Our comments on the applicants' documents

Appendix 1: Event SYHT0H2 Soybean - Insert Sequence Analysis

Assessments of risks, hazards and safety were not among the goals of this study.

This was not a scientific paper as: "No statistical analysis was conducted during this study."

Tests of statistical significance were also not used which renders the study useless as the basis for a safety assessment of Event SYHT0H2 Soybean.

There is also no quantification or justification for selecting three samples of each colony. The secrecy of any rejected data is also unacceptable.

"PCR products were cloned, and for each product three colonies were randomly chosen. Any rejected data and the documented reasons for the rejection of those data were retained in the study file."

The paper concludes that the: "SYHT0H2 insert consists of two inverted and truncated copies of the pSYN15954 T-DNA …."

This is a mere description, without a conclusion or discussion that would inform regulators or the public. As there is no complete and intact copy of the inserted DNA, we question whether it is possible to accurately predict the allergenicity and toxicity of the foreign genetic fragments identified.

Appendix 2: Functional Element Copy Number Southern Blot Analysis

Assessments of risks, hazards and safety were not among the goals of this study.

This can in no sense be regarded as a scientific study as the report says: "No statistical analysis was conducted during this study."

It was helpful from a scientific perspective that a control sequence was included with each sample but:

"A positive control representing one copy of a fragment of known size in the soybean genome was included in each Southern blot to demonstrate the sensitivity of the experiment."

However, it is unacceptable that secrecy surrounds rejected data and reasons as this makes any objective regulatory assessment subject to error.

"Any rejected data, and the documented reasons for the rejection of those data, were retained in the study file."

The study also arrived at a conclusion that is a mere description of what was observed and not a meaningful finding.

“SYHT0H2 soybean contains a single copy of avhppd-03, four copies of pat, a single copy of the avhppd-03 enhancer complex sequence, two copies of the 35S promoter, two copies of the CMP promoter, two copies of the TMV enhancer (contained in the pat-03-02 cassette), and five copies of the NOS terminator, as expected for a single insertion site consisting two truncated copies of the pSYN15954 TDNA (de Framond 2012).”

Also, the supposed de Framond reference is self-referential: de Framond A. 2012. Event SYHT0H2 Soybean: Insert Sequence Analysis. Report No. TK0059645 (unpublished). Research Triangle Park, NC: Syngenta Crop Protection, LLC.as it is another unpublished and non-peer-reviewed study that adds nothing to the credibility of the so-called conclusion.

Appendix 3: Flanking Sequence Determination

Assessments of risks, hazards and safety were not among the goals of this study.

“No statistical analysis” or tests of significance were conducted in this study. The inclusion of controls adds rigour but alone is insufficient to have this study qualify as scientific.

Secrecy over any rejected data and the reasons for rejection is unacceptable. No complete regulatory assessment could be conducted without access to all data.

“PCR fragments were cloned, and one colony from each cloning reaction was randomly chosen. **Any rejected data and the documented reasons for the rejection** of those data were retained in the study file.”

The study conclusion is merely descriptive, saying:

"The soybean genomic sequences flanking the SYHT0H2 insert were determined.”

Appendix 4: Event SYHT0H2 Soybean: Allergenicity and Toxicity Assessment of Start to Stop T-DNA ORFs with a Minimum Size of 30 Amino Acids

Assessments of risks, hazards and safety were not among the goals of this study.

No controls were used in this study, the rationale for a minimum of 30 amino acids is unclear, and although there is reference to a test of significance we cannot see how it was calculated or applied.

We therefore question the conclusion that:

“Evaluation of these sequences using an allergen and toxin database supports the conclusion that these sequences show no biologically relevant similarity to any known or putative allergens or toxins.”

This aanlysis is also only as good as the database used for comparison and it is

unclear how exhaustive its entries may be.

Appendix 5: "Event SYHT0H2 Soybean: Mendelian Inheritance Analysis"

Assessments of risks, hazards and safety were not among the goals of this study.

Control samples were used and the report claims to have done statistical analysis of its data. However, it appears no tests of statistical significance were performed, as usual in scientific papers. We therefore question the study's conclusions.

"**Statistical analysis of segregation data** from three generations of SYHT0H2 soybean confirmed that the observed segregation ratios for avhppd-03 and pat were as expected for a gene inherited according to Mendelian principles. The data indicate that the insert is inherited according to Mendelian principles and, thus, integrated into a chromosome within the nuclear genome of SYHT0H2 soybean."

Appendix 6: Event SYHT0H2 Soybean: Genetic Stability Analysis

Assessments of risks, hazards and safety were not among the goals of this study.

The report says: "No statistical analysis was conducted during this study." So its credibility as science is open to serious question.

It also appears that some relevant data and the reasons for its exclusion from the report are retained by the applicant and are not in the public domain, without explanation.

"The test and control substances were analyzed on the same Southern blot. One or more positive controls representing one copy of a fragment of known size in the soybean genome was included in each Southern blot to demonstrate the sensitivity of the experiment. **Any rejected data, and the documented reasons** for the rejection of those data, were retained in the study file."

For the foregoing reasons, we do not accept the reliability of the studies conclusions.

"Southern blot analyses of SYHT0H2 soybean demonstrated that (1) SYHT0H2 soybean carries a single insert consisting of two partial copies of the pSYN15954 T-DNA with no extraneous T-DNA fragments of plasmid pSYN15954 inserted elsewhere in the soybean genome, (2) the transgenic locus is stable across all the SYHT0H2 generations analyzed, and (3) every generation SYHT0H2 soybean examined is free of backbone sequence from the transformation plasmid pSYN15954."

Appendix 7: to determine the concentrations of the proteins p-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) and phosphinothricin acetyltransferase (PAT) in food and feed fractions processed from soybean seed derived from transformation Event SYHT0H2

Like most of the other Appendices, no tests of statistical significance were applied to the data produced in this study so no valid conclusions can be drawn.

"The paper merely states that: "All calculations, including means and standard deviations (SD), were performed with Microsoft Excel® 2007 spreadsheet software. All decimal places associated with the concentrations determined for each replicate sample were used in calculation of the means, and were then rounded to two decimal places for reporting consistency."

This study is not scientific so we cannot accept its conclusions that:

"Southern blot analyses of SYHT0H2 soybean demonstrated that (1) SYHT0H2 soybean carries a single insert consisting of two partial copies of the pSYN15954 T-DNA with no extraneous T-DNA fragments of plasmid pSYN15954 inserted elsewhere in the soybean genome, (2) the transgenic locus is stable across all the SYHT0H2 generations analyzed, and (3) every generation SYHT0H2 soybean examined is free of backbone sequence from the transformation plasmid pSYN15954."

It is not acceptable either because exempt data is not available for public scrutiny.

"Protein extractions were performed on representative aliquots of homogeneous samples, and each extract was analyzed in triplicate. **Any rejected data, and the documented reasons for the rejection of those data, are retained in the study file."**

Appendix 8: "Event SYHT0H2 Soybean Gel-based, Event-specific Polymerase Chain Reaction Method Assessment**"**

Assessments of risks, hazards and safety were not among the goals of this study. Like most of the other Appendices, no tests of statistical significance were employed either so no valid conclusions can be drawn.

Nonetheless, the report claims:

"A gel-based, event-specific polymerase chain reaction method was developed to detect SYHT0H2 soybean deoxyribonucleic acid (DNA). This method uses two oligonucleotide primers to amplify a 140 base pair DNA fragment that spans one of the junctions between the soybean genome and the SYHT0H2 insert."

But the test is not foolproof, does not provide reliable results, and the proposed techno-fix does not resolve its problems. The applicant has failed to produce a test of satisfactory standard of reliability, replicability and robustness.

"The absence of the expected PCR product **may indicate** that the DNA extract is inhibiting the PCR analysis. In this case, re-extraction of DNA and further purification **may be necessary**. To determine if a DNA extract is inhibiting the PCR analysis, add 1 µl of 0.04 ng/µl 100% SYHT0H2 DNA into a well containing the reaction mixture and the DNA extract that did not show the expected PCR product. Perform PCR analysis (as described in this method). **If the expected PCR product is still absent, the DNA extract is inhibiting the PCR."** So, where to from here?

Appendix 9: "Event SYHT0H2 Soybean Validation of a Gel-based, Event-specific Polymerase Chain Reaction Method Assessment"

Assessments of risks, hazards and safety were not among the goals of this study. And the report says: "No statistical analysis was required for any parameter evaluated in this study."

There is no explanation for the absence from the study of a: "Negative control containing no DNA"

Nor is there any explanation for the decision that: "Any rejected data, and the documented reasons for the rejection of those data, will be retained in the study file." Is it commercial in confidence? Is it accessible to the FSANZ assessors?

For these reasons we should be skeptical of the conclusions:

"A gel-based, event-specific PCR method was developed to detect SYHT0H2 DNA. This method uses two oligonucleotide primers to amplify a 140 bp DNA fragment that spans one of the specific junctions between the soybean genome and the SYHT0H2 insert. Syngenta tested the sensitivity, repeatability, and reproducibility of this method using SYHT0H2 soybean DNA and nontransgenic soybean DNA mixed to various final concentrations of SYHT0H2 DNA: 1%, 0.5%, 0.1%, 0.05%, and 0.01%. The method demonstrated sensitivity, repeatability, and reproducibility at all concentrations of SYHT0H2 DNA tested, and the limit of detection of the method was determined to be at least 0.01% SYHT0H2 DNA. The repeatability and reproducibility of this method were confirmed by an inter-laboratory validation. The method was specific to SYHT0H2 soybean DNA. The method described by Carlin (2012)"

The Carlin reference is to Syngenta's own work: "Carlin, R. 2012. Event SYHT0H2 soybean Gel-based, Event-specific Polymerase Chain Reaction Method. Report No. TK0059653. Research Triangle Park, NC: Syngenta Biotechnology, Inc."

This document has not been peer-reviewed or published, which is another reason to reject the conclusions.

Appendix 10: "Quantification of p-Hydroxyphenylpyruvate Dioxygenase and Phosphinothricin Acetyltransferase in Event SYHT0H2 Soybean Tissues

Assessments of risks, hazards and safety were not among the goals of this study.

"The concentrations of AvHPPD-03 and PAT measured in this study represent the levels of these proteins in SYHT0H2 soybean in various tissue types and developmental stages across four different field environments. Concentrations of AvHPPD-03 and PAT were quantifiable in all SYHT0H2 soybean tissues types analyzed." So, what? Does that make it safe?

Like most of the other Appendices, no tests of statistical significance were employed so no valid conclusions can be drawn.

"All calculations, including means and standard deviations (SD), were performed with Microsoft Excel® 2007 spreadsheet software. All decimal places associated with the concentrations determined for each replicate sample were used in calculation of the means, which were then rounded to two decimal places for reporting consistency."

Appendix 11: Comparison of p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant Escherichia coli and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants Final Report

The rationale for this study is substantial equivalence. Though safety gets three anecdotal mentions, it appears that strict assessments of risks, hazards and safety were not among the goals of the study.

It compares p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein from Recombinant E coli with AvHPPD-03 Protein from Soybean Plants Event SYHT0H2, claiming that this establishes the safety of the Protein Produced in Event SYHT0H2. But the study is short on statistical testing and merely states:

"The results of this study demonstrate that the microbially produced AvHPPD-03 is biochemically and functionally equivalent to AvHPPD-03 produced in SYHT0H2 soybean and supports the conclusion that the microbially produced AvHPPD-03 is a suitable surrogate to evaluate the safety of AvHPPD-03 produced in SYHT0H2 soybean."

We cannot rely on this conclusion as it is not fully supported by rigorous evidence.

As in several other appendices supplied by the companies to justify approval, deviations from the norm are unexplained and are forgiven. By whom, on what grounds, and using what methods, is never explained. The term 'significance' is used here just as window-dressing.

"The lack of the first four amino acids from the N-terminus of the plant-produced AvHPPD-03 **is not considered to have a significant impact** on the overall integrity of the protein, based on the observation of comparable specific enzymatic activity with the fully intact microbially produced AvHPPD-03."

Appendix 12: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein Produced in Recombinant Escherichia coli and PAT Protein Produced in Event SYHT0H2 Derived Soybean Plants

Safety gets four anecdotal mentions in this paper but it appears that stringent assessments of risks, hazards and safety were not among the goals of this study.

This study compares the protein produced in the transgenic soy plant and in E coli claiming that this establishes the safety of the PAT Protein Produced in Event SYHT0H2. But the study is short on statistical testing and merely states:

"Means and relative standard deviations were calculated using Microsoft Office Excel® 2007 software."

There is no satisfactory explanation of why the surrogate E coli protein should be preferred for testing rather than the PAT Protein Produced in Event SYHT0H2.

"The results of this study demonstrate that the microbially produced PAT is biochemically and functionally equivalent to PAT produced in SYHT0H2 soybean and supports the conclusion that the microbially produced PAT is a suitable surrogate to evaluate the safety of PAT produced in SYHT0H2 soybean."

Appendix 13: AvHPPD-03: Assessment of Amino Acid Sequence Similarity to Known or Putative Toxins

Assessments of risks, hazards and safety were not among the goals of this study.

The word 'significant' is constantly used throughout this report but there is no reference to, or calculation of, tests for statistically significant differences. The following summary therefore has minimal credibility:

"The threshold value from the Basic Local Alignment Search Tool for determining potential significance of matches was based on searches conducted with randomly shuffled sequences of the amino acids comprising AvHPPD-03. Of 916 protein sequences identified as having potential significance to AvHPPD-03, inspection of these alignments showed that there were no biologically relevant sequence homologies with any proteins known to be toxins. AvHPPD-03 is unlikely to share toxicity or other biological activity with known toxins that are harmful to human or animal health."

Appendix 14: PAT/pat PROTEIN AMINO ACID SEQUENCE HOMOLOGY SEARCH WITH KNOWN TOXINS (BAYER)

Safety is referred to just once in passing in this uncontrolled study. It is a classic case of finding what you are seeking and expecting to find, and also forgiving the exceptions and outliers without adequate explanation or rationale.

"**As expected** based on the good safety profile of the PAT protein, none of the 30 sequences from the toxin database matching with the PAT/bar protein sequence were **true toxins.** Several matched sequences were derived from complete genomes of various organisms, or from complete sequences of chromosomes, and did not correspond to an identified protein. In addition, several matched sequences were inaccurately included in the Bayer Toxin database because of the presence of specific keywords (see section 1.4) in their phylogeny. Therefore, these homologies are not relevant. Similarly, several matched sequences from non toxic proteins (e.g., sortase, Accession number GQ352402_11) were not true toxins and were unaccurately (sic) included in the toxin database because of the presence of specific keywords in their description."

Such anecdotes do not support the conclusion that:

"Therefore, no significant similarities were found between the PAT/pat protein and any

toxic proteins from the Toxin database."

Data was also systematically excluded from the report.

"If the statistical significance ascribed to a match was greater than the E-value threshold, the match was not reported." Why not?

The Bayer toxins database appears to be incomplete and unreliable, as is the following conclusion.

"The PAT/pat protein shows a high degree of homology with other proteins of its respective family. No records were found on potential hazard associated with this protein family. In addition, **no biologically significant similarities** were found with any toxic protein from the Bayer Toxin database. Therefore, the PAT/pat protein does not show any evidence of potential toxic properties."

Appendix 15: In vitro Digestibility of p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein under Simulated Mammalian Gastric Conditions

Assessments of risks, hazards and safety were not among the goals of this study.

The report says: "No statistical analysis was required for any parameter evaluated in this study." In the absence of such analysis and any rigorous measurement, the following conclusion must be discounted and the study replicated.

"The AvHPPD-03 protein degraded rapidly upon exposure to SGF. No intact AvHPPD-03 or AvHPPD-03 derived fragments were detected following its incubation in SGF for 1 minute. The results of this study support the conclusion that AvHPPD-03 will be readily digested under typical mammalian gastric conditions."

Appendix 16: In vitro Digestibility of p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein under Simulated Mammalian Intestinal Conditions

Assessments of risks, hazards and safety were not among the goals of this study.

There is no reference to, or calculation of, test for statistically significant differences between the treatment and control samples in the study. Anecdotal opinions about the significance of some observed similarities and differences do not justify the conclusion.

"The AvHPPD-03 protein degraded rapidly upon exposure to SIF. No intact AvHPPD-03 was detected by SDS-PAGE or Western blot analyses after digestion in SIF for 1 minute. Furthermore; no AvHPPD-03 derived fragments were detected after incubation for five minutes. The results of this study support the conclusion that AvHPPD-03 will be readily digested under typical mammalian intestinal conditions."

Appendix 17: Effect of Temperature on the Enzymatic Activity of p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein

Assessments of risks, hazards and safety were not among the goals of this study.

The relevance of this conclusion to the safety of the protein is unclear and is unexplained in the report.

"The data presented in this report supports the conclusion that the enzymatic activity of AvHPPD-03 is greatly decreased after incubation for 30 minutes at 37ºC. Enzymatic activity is no longer detected after incubation for 30 minutes at 65ºC and above, indicating the loss of its dioxygenase enzymatic function."

There is minimal analysis of the statistical significance of the measurements in the study. The production of information for the assessment of risks, hazards and safety was not among the goals of this study.

Appendix 18: PAT/pat PROTEIN IN VITRO DIGESTIBILITY STUDY IN HUMAN SIMULATED GASTRIC FLUID (BAYER)

This was a simulation study of the digestion of foreign protein in gastric fluids.
It was not a scientific study as no controls were used.
There is no reference or calculation of statistical significance in the study.
Assessments of risks, hazards and safety were not among the goals of this study.

Appendix 19: PAT/pat PROTEIN IN VITRO DIGESTIBILITY STUDY IN HUMAN SIMULATED INTESTINAL FLUID

This was a simulation study of the digestion of foreign protein in gastric fluids.
It was not a scientific study as no controls were used.
There is no reference or calculation of statistical significance in the study.
Assessments of risks, hazards and safety were not among the goals of this study.

Appendix 20: AvHPPD-03 AvHPPD-03: Single-Dose Oral (Gavage) Toxicity Study in Mice with a 2-Day or 14-Day Observation Period

This study assessed only acute toxicity, chronic or long-term toxicity. Thus, the evidence of safety is incomplete.

Moreover, when statistically significant differences between experimental and control groups were observed, they were discounted for insufficient reasons and without justification:

"Statistically significantly lower mean body weight gains were noted for the 1500 mg/kg group males from study day 1 to 2 and for the 2000 mg/kg group females from study day 7 to 8 when compared to the control group. **These statistically significant differences were sporadic and did not occur in a dose-related manner; therefore, they were considered to be incidental in nature and unrelated to AvHPPD-03 administration.** For the 1500 mg/kg group females, statistically significantly higher mean cumulative body weight gains were noted from study days 0 to 13 and 0 to 14. These differences were not seen in the 2000 mg/kg group females, and therefore, were not considered to be dose-related."

This was not a feeding study as only one dose of soy was administered.

"The test substance, AvHPPD-03-0209 containing the active ingredient AvHPPD-03 protein (72.2% purity w/w), was **administered as a single oral dose via gavage** to 3 groups of Crl:CD1(ICR) mice (Groups 2, 3, and 4). Dose levels were 500, 1500, and 2000 mg active ingredient/kg body weight, respectively. The vehicle, deionized water, was administered to a concurrent control group (Group 1) on a comparable regimen. The dose volume was 20 mL/kg for all groups.

There is no explanation for the sample size of 10 animals yet FSANZ criticized independent scientist Seralini for using groups of ten animals too.

"Each group consisted of 10 animals/sex. **Five animals/sex/group were euthanized a minimum of 48 hours following dose administration (study day 2). The remaining 5 animals/sex/group were euthanized following a 14 day observation period."**

Appendix 21: AvHPPD-03: Assessment of Amino Acid Sequence Similarity to Known or Putative Allergens

There is no reference to statistical significance in the study.
Assessments of risks, hazards and safety were not among the goals of this study.

Appendix 22: PAT/pat PROTEIN AMINO ACID SEQUENCE HOMOLOGY SEARCH WITH KNOWN ALLERGENS (BAYER)

There is no reference to statistical significance in the study, only this anecdotal opinion.
"The lack of any **significant** amino acid sequence homology with known allergens supports that it is unlikely that the PAT/pat protein possesses allergenic properties."

Assessments of risks, hazards and safety were not among the goals of this study.

Appendix 23: Compositional Analysis of Forage and Seed from Soybean Event SYTH0H2 Grown During 2010 in the USA

Assessments of risks, hazards and safety were not among the goals of this study.

"In the across-location comparisons between untreated SYHT0H2 soybean (test) and the control soybean, or between SYHT0H2 soybean treated with the trait-specific herbicides mesotrione and glufosinate (test + TSH) and the control soybean,

statistically significant differences were observed. However, the magnitudes of most differences were less than 10% and some differences were observed in only one of the comparisons."

This is just an opinion, with no statistical validity, so the following conclusions are not justified.

"Based on these data, it is concluded that forage and seed from SYHT0H2 soybean differs in composition when compared to the near-isogenic control soybean. Based on comparisons with ranges of component levels published in the ILSI database, and with ranges of component levels in reference varieties of soybean grown simultaneously at the same locations, it is concluded that the nutrient composition of SYHT0H2 soybean is not materially different from that of conventional soybean varieties."

Appendix 24: Endogenous Allergen Assessment of Event SYHT0H2 Soybean: 2-Dimensional Western Blotting with Human Sera

Assessments of risks, hazards and safety were not among the goals of this study. There are only anecdotal references to significance not statistical analysis as should be required, and no mention of safety.

Appendix 25: Event SYHT0H2 Soybean The Effect of Diets Containing Soybean Meal from SYHT0H2, Nontransgenic Control and a Commercial Variety on Broiler Growth Performance and Carcass Parameters

Assessments of risks, hazards and safety were not among the goals of this study. This is a commercial, not safety study. It sought to determine if there were differences in carcass weight between the GM, conventional and control groups.

Much is hidden from view in this study report. For instance, 12 birds were placed in each of 3 treatment groups – GM, non-transgenic and control. Seven birds died in each of the two treatment groups, leaving only 5 at Day 43.

They say: "The apparent cause of death identified at necropsy for most birds that died was bacterial infection, ascites and sudden death syndrome; these occur commonly in chickens."

This is insufficiently robust to provide any confidence in a safety assessment that accepts this as scientific reporting.

What does **biologically relevant** mean in the following?

"A 42-day broiler feeding study evaluated whether standard poultry diets prepared with SYHT0H2 transgenic soybean meal had an effect on male and female broiler chickens. There were **no biologically relevant differences in broiler performance or carcass yield** between broilers fed diets containing soybean meal produced from SYHT0H2 soybean and those fed diets containing meal from a nontransgenic, near-isogenic control soybean variety.

And the following is also prevarication.

"Although there were three parameters that differed for the commercial reference variety there were no statistical differences specific for SYHT0H2 soybean meal-fed birds compared with the nontransgenic, near isogenic control meal-fed birds."

The following is not a credible report of results and appears unrelated to the data.

“8.0 STATISTICAL ANALYSIS
Statistical analyses were conducted on growth performance (body weight), carcass weight and portions, feed consumption, and feed conversion ratio (unadjusted for mortality). The pen was used as the experimental unit. Statistical significance was defined as alpha = 0.05. ANOVA was used to assess treatment-related differences. The statistical model included block, all treatments, sex, and the treatment-by-sex interaction as fixed effects. If the treatment-by-sex interaction was not significant, the main effect of treatment was evaluated. If the treatment-by-sex interaction was significant ($P \leq 0.05$), this was judged to undermine the validity of comparing treatments across genders, in which case within-sex treatment effects were assessed. Within the model framework stated above, the significance of the specific comparison between test and the nontransgenic control treatments were determined in all cases, regardless of the significance of the overall treatment effect (two-sided test).”

Appendix 26: Event SYHT0H2 Soybean

No significant findings or safety references.

Appendix 27: Characterisation of the PAT protein batch # 995 produced in E coli

No conclusions, significant findings or safety references.

Appendix 28: Event SYHT0H2 Soybean: Allergenicity and Toxicity Assessment of Start to Stop, Genome to Insert Junction ORFs with a Minimum Size of 30 Amino Acids

Assessments of risks, hazards and safety were not among the goals of this study.

No rationale is given for testing only ORFs with a Minimum Size of 30 Amino Acids.

The provenance of the database NCBI Entrez® Protein Database used for comparison is not given.

“An evaluation of this sequence using a toxin database created from the NCBI Entrez® Protein Database (2012) supports the conclusion that these sequences show no relevant similarity to any known or putative toxins.”

Similar reports to the following should have characterized all of the Appendices.

“No significant sequence similarity (E-value less than 1 × 10-5), nor under an E-value of 10 was observed between the translated junction sequence and any entry in the toxin database.”