

# Soybean\_DAS-68416-4

---

---

**Organisation: The European GMO-free Citizens (De Gentechvrije Burgers)**

**Country: The Netherlands**

**Type: Others...**

---

## **a. Assessment:**

### **Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)**

Study by Hoechst (Dr Arno Schulz) concerning the substrates of phosphinothricin acetyltransferase (PAT). In herbicide-resistant (i.e. PPT-resistant) crops, PAT is present.

Amsterdam, 7 November 1999.

Two studies that arrive at opposite conclusions, namely 1. Charles J. Thompson, 1987: Characterization of the herbicide-resistance gene bar from *Streptomyces hygroscopicus*; 2. Dr Arno Schulz, 1993: L-Phosphinothricin N-Acetyl-transferase -Biochemical Characterization - a report incorporated into Wehrmann 1996 (Schulz is co-author). The subject is the characterisation of the enzyme phosphinothricin acetyltransferase (PAT), and in particular the specificity of the substrates. The first study concerns the reaction of phosphinothricin with acetyl co-enzyme A under the influence of PAT and compares this with a number of structural analogues of phosphinothricin (PPT). One of the analogues is L-glutamate. The products of the reaction were identified via a mass spectrogram and the equilibrium constants (affinity) determined. In addition to phosphinothricin (PPT), a number of structural analogues were tested to determine whether there was an acetylation reaction. L-glutamic acid was one of the substances investigated. Compared with PPT the affinity of most of the substances was low: one substance did not react at all. In this test, where a numerically reportable reaction occurred to an identified product (the detection threshold is not an issue here) there does not appear to be any reason to doubt that glutamic acid is a substrate of PAT.

The second study concerns the reaction of a large number of amino acids, including L-glutamic acid, which was also involved in the first study, in a reaction mix together with a 100% excess of PPT in relation to the acetyl source acetyl co-enzyme A and PAT. Products of the reaction were identified via chromatography. Even with a very large excess of L-amino acid no products of reaction with the amino acids were found. Only acetyl phosphinothricin was found. The authors concluded that PAT very specifically has only PPT as a substrate. The following criticisms can be made of this conclusion, which conflicts with that produced in the first study. (Incidentally, the first study is cited in the bibliography to the second study): 1. No detection threshold was determined for acetylated L-glutamic acid. 2. The possibility of acetylated glutamic acid being a source of acetyl for the acetylation of PPT was ignored. This could have been tested in the study by adding acetylated glutamic acid to the reaction mix in a quantity above the detection threshold and examining whether this added quantity disappears during the reaction. Based on the results of the first study it could certainly be predicted to

disappear!! 3. The study was conducted using a reaction mix in which a large excess of a competing substrate, PPT, was present. Observations of the pure amino acids were not conducted. 4. There is no discussion whatsoever of the results of the first study, in particular as to why these were so different. 5. Essentially, the authors of the second study accuse the authors of the first study of fabrication, of fraud (the first study contains a wealth of numerical data; in the second there are no figures). In the second study this aspect is not fully explored. The background to the conclusion that PAT has only one substrate - PTT – is as follows: in herbicide-resistant (i.e. PPT-resistant) crops, PAT is present. In order to obtain approval for products to be placed on the market the toxicity of this gene-product must be examined. Could this gene-product react with the content of our GUT, e.g. with the – important – amino acid L-glutamic acid? It would cost a fortune in research to demonstrate that the dangers were minimal. For HOECHST, it would seem that total denial is a better strategy! We believe that the conclusion drawn in the second study is completely unfounded and that the so-called ‘study’ is unworthy of the name. It is an incompetent study and those persons who cite it need to be told about its incompetence. J. van der Meulen, L. Eijsten.  
<http://www.gentechvrij.nl/rvs9911.html>

EU to restrict herbicide glufosinate

Category: Crop Protection Products Tags: EU , restrict , herbicide , glufosinate The European Commission has announced the restrictions for the use of the herbicide glufosinate, which will be effective from Nov 13, 2013.

The decision is based on the additional information provided by the notifier, the Commission considered that the further confirmatory information required had not been provided and that a high risk for mammals and non- target arthropods could not be excluded except by imposing further restrictions.

The active ingredient will only be authorised for band or spot application at rates not exceeding 750 g ai/ha (treated surface) per application, with a maximum of two applications per year.

EU member states must amend or withdraw existing product authorizations in accordance with Regulation (EC) No 1107/2009 by Nov 13, 2013 .They may set a grace period of up to one year for use of existing stocks.New approvals should include the application of drift-reducing nozzles and spray shields, together with relevant labelling.

Glufosinate obtained EU approval for use in apple orchards in 2007. Source: EUR-Lex  
<http://news.agropages.com/News/NewsDetail---9598.htm>

---

## **b. Food Safety Assessment: Toxicology**

2,4-D Legislation Under European pesticides legislation, 2,4-dichlorophenoxyacetic acid was assessed and placed on the list of plant protection products that can be recognised by the Member States.[3] It was listed until 30 September 2012. Toxicology and safety 2,4-dichlorophenoxyacetic acid is a substance with moderate toxicity. It irritates the eyes, skin

and respiratory tract. It affects the nervous system. Long-term exposure can result in hypersensitivity and cause eczema. It can also affect the liver and kidneys. Acceptable daily intake (ADI) is 0.05 mg/kg body weight. <https://nl.wikipedia.org/wiki/2,4-dichloorfenoxiazijnzuur>

---

### **Statements by mothers in the USA, where GMOs are not labelled as such.**

Verklaringen van moeders in de USA waar GMO's niet gelabeld zijn.

"When my son was born he fussed a lot, the whole day, wouldn't nap. I breast fed until he was three months old. And because his gut was not right, he fussed and I could never console him. I tried all the gassy meds, not sure they are considered meds. Once on formula the fussing continued, we switched to different formulas, but not until we switched to Parents Choice organic, Walmart, his fussing stopped, he began taking naps. As a toddler, I fed him Cheerios, a main staple in our house. The tantrums began; two hours at a time couple times a day. This was with head banging or slamming his head into the wall repeatedly. He wouldn't let me hold him, not even touch him. Can you imagine not cuddling your baby? I cried everyday. I had watched the movie Food Inc. It touched on a subject I wasn't familiar with. After watching Genetic Roulette, I cleaned out the cupboards. After doing this, within two weeks my son's tantrums stopped completely, he started smiling, crawling into my lap for cuddles. I had no idea that was the issue. Even now when he gets something conventionally/ GMO poison, he'll have another tantrum like his past ones. So if there's a question as to where it's from-what kind of seed, I don't take it. So for me and my family, we bow out from being a guinea pig."- Stephanie Vanderyacht

"My husband was in the hospital 5 times last year. Doctors wanted to remove part of his intestine because it was so infected instead doctors pumped him full of antibiotics for a week when he got out of hospital I changed his diet and all our family food choices to NON- GMO foods WOW what a difference he's doing great and food never tasted so good! I will march, sign petitions, anything to reclaim our healthy labelled food choices. God Speed JUST SAY NO TO GMO'S ....MAAM! " Rhonda Bryne, MAA

My 7 year old son was diagnosed with asthma and needed glasses inside of two weeks. I started learning about asthma and natural ways to control it. Then I found out about GMO. I removed my family from GMO foods/drinks. My 7 year old went from needing a nebulizer 3x's a day to not at all. His asthma disappeared. He also no longer had the stigmatism that required glasses. The eye Dr. said he must have had 'some sort of inflammation' that is now gone for whatever reason. The reason was removing GMO from our diets. He was recommended for retention last year. This year, he is at the top of his class. Karen L.~Moms Across America The above testimonials are a sampling of the hundreds of testimonials which Moms have sent to us. More see:

[http://www.momsacrossamerica.com/zenhoneycutt/mom\\_s\\_testimonials](http://www.momsacrossamerica.com/zenhoneycutt/mom_s_testimonials)

---

## **Nutritional assessment**

No nutritional benefits!

---

## **Others**

Rising demand for organic and non-GMO grains outpaces U.S. production By Ken Roseboro  
Published: February 22, 2017 Issue: March Category: Organic/Sustainable Farming

Organic imports rise sharply as U.S. corn and soybean growers contemplate premiums, risk-reward scenarios Increasing consumer demand for organic and non-GMO foods led to a sharp rise in organic grain imports in 2016—prompting food manufacturers to explore new incentives for U.S. growers transitioning to organic production, according to a new report from CoBank. While U.S. production of non-GMO crops has risen, domestic production of organic corn and soybeans remains well short of demand. CUT <http://non-gmoreport.com/articles/rising-demand-organic-non-gmo-grains-outpaces-u-s-production/>

---

## **4. Conclusions and recommendations**

The European GMO-free Citizens (De Gentechvrije Burgers) do not want any GMOs on their plates, either as medicine, biological products or vaccine or as crops.

---

## **5. Others**

---

## **6. Labelling proposal**

Labelling in the Netherlands is a farce. If labelling is carried out, it should be effective and subject to strict supervision, especially in the case of GMOs obtained through parallel importing, which might contain prohibited GMOs, such as certain genetically modified sugars. Dairy products from genetically modified animals and all other applications that are not labelled at present, such as vitamins, enzymes, colorants, flavourings, etc should also be labelled. The European GMO-free Citizens (De Gentechvrije Burgers) of Lelystad have found out that:

all American (genetically modified) products at Jumbo are incorrectly labelled. Jumbo places the following warning as standard on all its products from the American range: “American products may contain genetically modified raw materials”, even on the ‘GMO Free’ products. Consumers are therefore unable to determine whether or not a product contains genetically modified organisms (GGO/GMO). This undermines the basic principles of compulsory

labelling of genetically modified food: • consumers have the right to know what they are eating • the freedom of consumers to choose whether or not to consume GMOs. Furthermore, such products may contain ingredients that are prohibited in the EU. This applies to all the American products from the Jumbo range (at least 36 products). Jumbo therefore infringes Dutch law and regulations concerning compulsory GMO labelling, in particular the Dutch Decision on new foodstuffs and EU Regulation No 1830/2003. GMOs must be labelled as such in the EU. The wording to be used is specified exactly and must not be deviated from. 5-7-2015 Request to the NVWA (Dutch Food and Consumer Product Safety Authority) to enforce the law at Jumbo. Because this behaviour by Jumbo undermines the principles of freedom of choice and the right of consumers to know what they are eating, we have asked the NVWA to intervene. (more info>>)

1-9-2015 Declaration by NVWA: Jumbo must change the labelling on all American products. The NVWA immediately started an investigation at the request of the European GMO-free Citizens (De Gentechvrije Burgers) . Quotation from the NVWA's letter dated 1 September 2015: “Appropriate measures have been taken by the NVWA and the sales organisation to stop the deviation. As at 14 August 2015, the incorrect information was removed from the website or amended. The said data were also amended on the labels”. (full text of the NVWA's letter >>)All's well that ends well? Unfortunately, Jumbo is still making a mess of things,

since in the meantime (2 September 2015), we have noticed the following with regard to the new labels: • products labelled as containing genetically modified wheat. GM wheat is prohibited in the EU; • products labelled as containing GMOs but stated as GMO-Free on the packaging; • products without any labelling (no Dutch declaration list); • we have no confidence in Jumbo actually checking whether the ingredients in these American products really are permitted. After two interventions by the NVWA, Jumbo has still not put its house in order!! . On 9 March 2015, the NVWA actually also took action at Jumbo at the request of the European GMO-free Citizens (De Gentechvrije Burgers). To date, around 30 completely unlabelled American GM products have been found. Quotation of NVWA declaration of 9 March 2015: A NVWA inspector took samples for analysis. Analysis revealed that the labels did not meet the legal requirements. The NVWA took appropriate action. (more info>>) 04-05-2016: The initial products have now finally been correctly labelled by Jumbo after repeated requests by the European GMO-free Citizens (De Gentechvrije Burgers), European Consumers' Platform to the NVWA. But what will happen if it starts using a different importer? And there are still articles that are incorrectly labelled on the shelves, with no Dutch text on the label stating that the product contains genetically modified (= manipulated) organisms. We are keeping an eye on things! 8 November 2016. Now Poptart labels have been found at Jumbo that are very difficult to read on more than one package.  
[http://www.gentechvrij.nl/DossierJumbo\\_2.html](http://www.gentechvrij.nl/DossierJumbo_2.html)

Via Facebook:

Miep Bos Jumbo Supermarkets 28 February: Dear Jumbo, we have now found yet another US product with incorrect labelling in one of your shops. Does it or does it not contain GMOs? "Bevat mogelijk GMO" [May contain GMOs] is not permitted under the EU directive.

Jumbo Supermarkets Hi Miep, that is a good point. That is not the intention. We will ask our colleagues how things stand. Can you perhaps also send us a photo of the barcode? · 28 February at 22:04

Jumbo Supermarkets Hi Miep, We have contacted the supplier and the product has been withdrawn from the range. Thank you for drawing this to our attention. 20 March at 10:14 [https://www.facebook.com/photo.php?fbid=324595777937632&set=o.156928557716372&type=3&theater-if\\_t=photo\\_comment-if\\_id=1488315874763235](https://www.facebook.com/photo.php?fbid=324595777937632&set=o.156928557716372&type=3&theater-if_t=photo_comment-if_id=1488315874763235) 24 March 2017 soft drinks Coca Cola Vanilla and A&W and Cheetos (cocktail biscuits, carton) at Jumbo do not bear any indication that they are produced using biotechnology. Cheetos don't even have a Dutch label. These products are manufactured in the USA and obtained through parallel importing. Jumbo should investigate this.

---

---

**Organisation: Testbiotech**  
**Country: Germany**  
**Type: Non Profit Organisation**

---

**a. Assessment:**  
**Molecular characterisation**

The expression of the newly introduced proteins was only measured in field conditions in the US. It is unclear to which extent specific environmental conditions can influence the overall concentration of the newly introduced proteins in the plants. The plants should have been subjected to a much broader range of environmental conditions to obtain reliable data on gene expression and functional genetic stability. Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see Trtikova et al., 2015).

Further, all parts of the plants should be taken into account for risk assessment. Expression data have to be considered as one of the starting points in the risk assessment of the plants and, therefore, assessment of the data cannot be reduced to those parts of the plants entering the food chain.

References:

Trtikova, M., Wikmark, O.G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions, PLoS ONE 10(4): e0123011.  
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011>

---

**Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)**

Field trials focussing on comparative analysis and analysis of agronomic traits were conducted at eight locations in the US in 2009. No field trials were conducted in other soybean producing regions such as Argentina and Brazil.

Several differences were found in the comparison of agronomical and phenotypical traits. The criteria 'days to 50% flowering' fell under equivalence category III (non-equivalence is more likely than equivalence) for untreated soybeans.

Several significant changes were also found in the composition analysis:

- DAS-68416-4/untreated Statistically significant differences from the conventional counterpart for 22 constituents (2 in forage and 20 in seeds). The level of 18 of the 22 constituents fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or IV.
- DAS-68416-4/2,4-D Statistically significant differences were identified for 23 constituents (2 in forage and 21 in seeds). The level of 19 of the 23 constituents<sup>29</sup> fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or I.
- DAS-68416-4/glufosinate Statistically significant differences were identified for 26 constituents (2 in forage and 24 in seeds). The level of 19 of the 26 constituents<sup>30</sup> fell under equivalence category I or II, while the level of seven seed constituents fell under equivalence category III or IV (Table 3).
- DAS-68416-4/2,4-D + glufosinate Statistically significant differences were identified for 20 constituents (1 in forage and 19 in seeds). The level of 16 of the 20 constituents<sup>31</sup> fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or IV.

EFSA's own guidance states that non-equivalence is more likely than equivalence for all significant findings that fall under equivalence category III or IV. Therefore, the genetically engineered soybean has to be considered to be different from its isogenic comparator in regard to several compounds: moisture, stearic acid, calcium, four amino acids, iron content, folic acid, raffinose and lectin activity.

Given this wide range of biologically relevant differences, it is not acceptable that EFSA failed to require further studies e.g.

omics studies (proteomics, transcriptomics, metabolomics) to assist the compositional analysis and the assessment of the phenotypical changes. Investigation into changes in the content of miRNA that can be taken up from the gut and render biological effects across borders of life domains. Exposure of the plants to a wide range of defined biotic or abiotic stressors to assess the true range of possible changes in the plants' composition Inclusion of more varieties inheriting the trait in order to investigate how the gene constructs interact with the genetic background of the plants. Feeding trials with the whole plants to assess potential health effects. The effects on the immune system were completely ignored in the assessment of potential health impacts from the increased levels of lectins.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

---

## **b. Food Safety Assessment: Toxicology**

The applicant conducted an acute toxicity study, a feeding study with chickens as well as three 28-day studies to confirm the safety of soybean DAS-68416-4. Two of the three 28-day studies were rejected by EFSA due to methodological flaws.

The third 28-day study on mice found changes in blood parameters and other significant changes. Only a small number of animals were examined.

Despite biologically relevant differences being found in the comparative assessment, no further testing of the whole plant was requested. Strikingly, no 90-day subchronic study was requested by EFSA. This gap in risk assessment was criticised by experts from several EU Member States (EFSA, 2017b). Implementation Regulation (503/2013) requests 90-day subchronic studies are undertaken as part of the risk assessment for all applications filed after 2014. In the light of this regulation, it is obvious that such data also have to be requested in cases where many biologically relevant differences between the event and its comparator are found, including compounds such as lectins. Further, multigenerational studies should have been performed to assess the impact on the reproductive system.

Beyond this, the residues from spraying were considered to be outside the remit of the GMO panel. However, without detailed assessment of these residues no conclusion can be drawn on the safety of the imported products: Due to the specific agricultural practices that go along with the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention.

Herbicide-resistant plants are meant to survive the application of the complementary herbicide while most other plants will die after short time. Thus, for example, residues of glufosinate and 2,4-D its metabolites and additives to the formulated product might accumulate and interact in the plants. As the publication by Kleter et al. (2011) shows, using herbicides to spray genetically engineered herbicide-resistant plants does indeed lead to patterns of residues and exposure that have to be assessed in detail.

While it is true that Pesticide Regulations 396/2005 and 1107/2009 are relevant in this context, in practice, they are not sufficient to generate the data needed to assess the residues from spraying with complementary herbicides. In addition, according to a reasoned legal opinion drawn up by Kraemer (2012), from a regulatory point of view, residues from spraying with complementary herbicides do, indeed, have to be taken into account in the risk assessment of genetically engineered plants.

There is a clear gap in the safety assessment of the genetically engineered soybeans that cannot be filled by adjustments to the MRLs applicable under the Pesticide Regulation. Consequently, the impact of spraying residues has to be assessed before the soybeans can be declared safe. The failure to do so poses real safety risks to humans, animals and the environment generally.

In conclusion, GMO risk assessment cannot avoid its obligation to make sure that the applicant provides all data necessary to assess the product derived from the soybean in all relevant health aspects.



There are good reasons to assess the residues from spraying with the complementary herbicides in detail: From scientific literature (not acknowledged by EFSA) it is known that metabolism in crops tolerant to 2,4-D may lead to the production of the compound 2,4-DCP (2,4-Dichlorophenol). According to a review by Lurquin (2016), 2,4-DCP may cause negative metabolic and genotoxic effects, and, like 2,4-D, is listed as “a possible carcinogen based on inadequate evidence in humans and limited evidence in experimental animals” by IARC. A new study has recently linked 2,4-D with Non-Hodgkin Lymphoma (Smith et al., 2017). Some of the complementary herbicides for use on DAS-68416-4 soybean will be phased out in Europe e.g. glufosinate, fluazifop and diclofop-Methyl. Combinatorial effects are likely to arise from the interaction of residues from spraying with glufosinate and 2,4-D together.

In any case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plant contains or produces other compounds of potential toxicity.

Consequently, the toxicological assessment as performed by EFSA is not acceptable.

#### References:

EFSA (2017) Application EFSA GMO-NL-2011-91 by Dow AgroSciences LLC, Comments and opinions submitted by Member States during the three-month consultation period, Register of Questions, <http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?0&panel=ALL>

Kleter, G.A., Unsworth, J.B., Harris, C.A. (2011) The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. *Pest Management Science*, 67(10): 1193-1210.

Kraemer, L. (2012) The consumption of genetically modified plants and the potential presence of herbicide residues, legal dossier compiled on behalf of Testbiotech, [http://www.testbiotech.de/sites/default/files/Legal\\_Dossier\\_Kraemer\\_Pesticide\\_RA\\_PMP.pdf](http://www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf)

Lurquin, P. F. (2016) Production of a toxic metabolite in 2, 4-D-resistant GM crop plants. *3 Biotech*, 6(1): 1-4. <https://link.springer.com/article/10.1007/s13205-016-0387-9#CR25>

Smith, A. M., Smith, M. T., La Merrill, M. A., Liaw, J., & Steinmaus, C. (2017) 2, 4-Dichlorophenoxyacetic Acid (2, 4-D) and Risk of Non-Hodgkin Lymphoma: A Meta-analysis Accounting for Exposure Levels. *Annals of Epidemiology*. <http://www.sciencedirect.com/science/article/pii/S1047279717302466>

---

## Allergenicity

There are several relevant issues that were left aside in risk assessment of EFSA regarding

allergenicity and the immune system. No non-IGE-mediated immune reactions were assessed although these effects must be considered relevant (Mills et al., 2013). This is especially relevant in this case since higher levels of lectins are present in comparison with the isogenic plants. The assessment did not take the risk for more vulnerable groups of the population, such as infants (EFSA, 2010), into account. The number of blood samples from patients with a known allergenicity to soybeans is too small to draw any conclusions. An analysis published by EFSA experts and other scientists recently found that in general open questions remain regarding the allergenicity assessment of genetically engineered plants, especially in the case of engineered soybeans (Selb et al., 2017).

Overall, the assessment is insufficient to exclude impacts on the immune system.

#### References:

EFSA Panel on Genetically Modified Organisms (GMO) (2010) Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010; 8(7):1700, 168 pp.

Mills, E.N.C., Marsh, J.T., Boyle, R., Hoffmann-Sommergruber, K, DuPont, D., Bartra, J., Bakalis, S., McLaughlin, J., Shewry, P.R. (2013) Literature review: ‘non-IgE-mediated immune adverse reactions to foods’, EFSA supporting publication 2013:EN-527.

Selb, R., Wal, J. M., Moreno, F. J., Lovik, M., Mills, C., Hoffmann-Sommergruber, K., & Fernandez, A. (2017). Assessment of endogenous allergenicity of genetically modified plants exemplified by soybean—Where do we stand?. Food and Chemical Toxicology. <http://www.sciencedirect.com/science/article/pii/S0278691517300212>

---

#### 4. Conclusions and recommendations

The risk assessment by EFSA is not acceptable in its present form. It does not identify knowledge gaps and uncertainties and fails to assess toxicity, impact on immune system and the reproductive system. The monitoring plan has to be rejected because it will not make essential data available.

---

#### 5. Others

Monitoring should be case specific. Exact data on the exposure to the soybean should be made available. Possible health impacts must be monitored in detail. Controls regarding residues from spraying with glufosinate and 2,4-D have to be established. Accumulated effects that might stem from mixtures with other genetically engineered plants have to be taken into account in the monitoring plan.

---