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Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 19.06.2013

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringen **EFSA/GMO/NL2012/106** for genmodifisert soya DAS-444Ø6-6 fra DOW AgroSciences LLC.

Vennligst ta kontakt hvis du har noe spørsmål.

Med vennlig hilsen,

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# Assessment of the technical dossier submitted under EFSA/GMO/NL2012/106 for approval of DAS-444Ø6-6 soy from DOW AgroSciences LLC

**Submitted to**

**Direktoratet for Naturforvaltning**

**by**

**Idun Grønsberg, Lise Nordgård, Vinicius Vilperte**

**Centre for Biosafety – GenØk**
**June 2013**

## KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av soya DAS-444Ø6-6.

Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til DAS-444Ø6-6 som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

**Hovedkonklusjon og anbefalinger**
Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring som omfatter DAS-444Ø6-6 for bruksområdet mat, fór, import og prosessering. DAS-444Ø6-6, er en stablet hybrid med ulike herbicid-kodende gener innebygd. Planten blir i følge søknaden resistent mot glyfosat og tolerant mot plantevernmiddlene 2,4D og glufosat ammonium.

CP4 EPSPS-proteinet gjør maisplantene tolerante overfor ugrasmidler med virkestoffet glyfosat. I den senere tid har laboratorie forsøk vist at glyfosat kan føre til celleskader, blant annet i humane embryoceller. Undersøkelser har også vist en skadelig effekt på vassdrag og vannorganismer. I tillegg forstyrrer glyfosat næringsstoffomsetninga i jord. Søker bør utføre analyser av viktige kjemiske prosesser som erfaringsmessig vites å være aktuelle problemstillinger for denne type genmodifiserte planter (herbicid toleranse medfører akkumulering av pågjeldende stoffer). I tillegg er plantevernmidlene 2,4D og glyfosat-ammonium ikke lovlig i Norge eller EU. Vi mener en godkjennelse av DAS-444Ø6-6 vil skade grunnleggende etiske og sosiale kriterier for bruk, som omtalt i den norske Bioteknologiloven.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke vedlagt søknaden om omsetting av mat produsert fra DAS-444Ø6-6 eller inneholdende ingredienser produsert fra DAS-444Ø6-6

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av DAS-444Ø6-6 for bruksområdene mat, fór, import og prosessering som det søkes om.

**SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL2012/106**

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of DAS-444Ø6-6, setting out the risk of adverse effects on the environment, including other consequences of proposed release under the pertinent Norwegian regulations.

**Specific recommendations**

Based on our findings, we propose a few specific recommendations, summarized here and detailed in the critique below.

- The regulator is encouraged to ask the Applicant to extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- The regulator is encouraged to ask the Applicant to re-design the probes in order to have a set of smaller ones and re-design the strategy for the restriction enzymes.
- The regulator is encouraged to ask the Applicant to conduct generational sequencing studies.
- The regulator is encouraged to ask the Applicant to specify whether it is the plant or the microbially derived protein that is used in the analysis.
- The regulator is encouraged to ask the Applicant to use newly expressed proteins from real field studies and clarify whether the soy was sprayed or not.
- The regulator is encouraged to ask the Applicant to provide western blots with visible standard so that it is possible to interpret size data. Also, some of the blots should have been exposed more to visualize additional bands better.
- The regulator is encouraged to ask the Applicant to include herbicide treated soya in the animal experiments, and analyze the residue level of the herbicides and their metabolites.
- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.
- The regulator is encouraged to ask the Applicant to submit required information on the social utility of DAS-444Ø6-6 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

**Overall recommendation**

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of **DAS-444Ø6-6**. **Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway.** Hence at minimum, the dossier is deficient in information required under Norwegian law. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of DAS-444Ø6-6, we conclude that based on the available data supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

**ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL2012/106**

**About the event**

The genetically modified DAS-444Ø6-6 soy was generated through *Agrobacterium* mediated transformation using a modified procedure to incorporate the *aad-12* gene from *Delftia acidovorans*, the *mepsps* from Zea mays and the *pat* gene from *Streptomyces viridochromogenes* into soyabean. The Applicant is requesting the authorization for GM plants for food, feed, and import and processing.

## Assessment

***Herbicides***

**Glyphosate**

The genetically modified DAS-444Ø6-6 expresses *2mEPSPS* that confers tolerance to herbicides products containing glyphosate. In recent years glyphosate has received more risk-related attention due to negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003), and also because of constantly increasing number of glyphosate herbicide applications since the introduction of this chemicals in 1971 (Dill et al. 2010, Cuhra et al 2012).

Studies in animals and cell cultures indicate possible health effects in rodents, fish and humans. Glyphosate given in the feed to pregnant female rats resulted in higher embryonic mortality and aberrations in the skeleton (Dallegrave et al. 2003). Nile-tilapia (Oreochromis niloticus) fed sublethal concentration of Roundup (active ingredient: glyphosate) resulted in a number of different histopathological changes in organs (Jiraungkoorskul et al. 2003). Experiments with sea urchins exposed to Roundup influenced early cell divisions (Marc et al 2002), effects that have relevance to potential health effects in many eukaryotic organisms, including domestic animals and humans. Exposure to Roundup affected the CDK1/CyclinB regulator which is nearly identical in sea urchins and humans.

Glyphosate has also been shown to negatively affect the differentiation of nerve cells (Axelrad et al. 2003). In human placenta cells, Roundup is more toxic than the active ingredient glyphosate (Richard et al. 2005). The authors concluded that additional components of Roundup increase the biological availability and accumulation in organisms.

In a recently published study by Seralini et al (Seralini et al. 2012) the authors concludes that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats.

**Glufosinate-ammonium**

The *pat* gene derived from *Streptomyces viridochromogenes* confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Studies have shown that glufosinat ammonium is harmful by inhalation,

swallowing and by skin contact and serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

**2,4-D**

DAS-444Ø6-6 has been genetically modified for tolerance to pesticides containing 2,4-D and AOPP ("fop"). This trait is likely to influence agricultural practices. In order to evaluate the effects of DAS-444Ø6-6 on sustainability in a global context, data on the nature of these changes and the effects of the changes is important. The evaluation of co-products, that is, secondary products that are specifically designed and intended to be used in conjunction with the GMO, is also considered important in the risk assessment of a GMO (Dolezel et al, 2009). Therefore, considerations of the co-products also warrant an evaluation of safer use, particularly when there is precedence in policy concerning its use.

**Recommendation**:
The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.

**Molecular characterization**

2.2 Information relating to the GM plant

- The size of some probes used in the Southern Blot analysis is considered too long (RB7 probe – 1010bp; 2mEPSPS probe – 1712bp; Histone promoter probe – 1516bp; AtUbi10 Promoter probe – 1313bp; aad-12 probe- 882bp; AtuORF1 UTR probe – 799bp; Ori-Rep probe – 1087; Backbone 2 probe – 1714bp; Backbone 1 probe- 1254bp; SpecR probe – 795bp; and also all the probes used for the southern blot studies covering the small gaps (Poorbaugh, 2011)). That can lead to false negative results since the strength of the interaction between probe and target is based on the number of bonds that form between the single strand od DNA (probe) and the matching recombinant DNA (target). A long probe that binds perfectly to a short fragment will not bind strongly and might be washed of depending on the stringency of the wash.
- Most of the Southern blot results showed really clear results and contained the molecular weight marker. However, some of them showed very weak bands, which could be explained by the use of long probes. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length.
- For southern blot studies, the probes were designed to bind in only a single fragment generated by the restriction enzymes. The probes could have been designed to bind also in the restriction site, allowing it to bind in two different fragments. Thus, this strategy would be able to confirm the strength of interaction

between the probe and the target. A set of different restriction enzymes could have been used.

- The promoter used for the *pat* gene expression cassete is the viral sequence from the Cassava vein Mosaic Virus (CsVMV), a virus from the genus Caulimovirus, the same genus as the Cauliflower Mosaic Virus (CaMV). Scientists recently reported the overlap between CaMV 35S promoter regions (P35S) and the viral gene VI (Podevin and du Jardin, 2012). The authors state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes. In light of these new findings, the present viral sequence should be examined carefully to exclude possible overlaps with other viral genes.
- The sequencing studies were conducted only with plants from one generation. Since Southern blot analyses for five generation were conduct, and this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.
- The electropherograms for the sequencing studies are not available therefore is not possible to check the quality of the sequences.

**Recommendations:**

- The regulator is encouraged to ask the Applicant to extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- The regulator is encouraged to ask the Applicant to re-design the probes in order to have a set of smaller ones and re-design the strategy for the restriction enzymes.
- The regulator is encouraged to ask the Applicant to conduct generational sequencing studies.

**4. Toxicology Assessment.**

Biochemical characterization was performed by analysis of mode of action, heat lability and equivalence between microbially and plant derived proteins.
All safety assessments were performed with microbially derived transgenic proteins. From our point of view, the plant version should be used for such purposes even though the concept of equivalence is proven by structure analysis (sequencing). Plants and bacteria do differ in their post-translational processing of proteins, and this is not considered.

**Enzymatic activity of newly expressed proteins.**

Only AAD-12 was assessed when it comes to enzymatic activity. The applicant considers it unnecessary to assess 2mEPSPS and PAT considering their "history of safe use". However, their level of expression and activity should have been analyzed in the context of being expressed together in the stacked soy. Also, the applicant does not specify whether it is the plant or the bacterial version of the AAD-12 protein that is used for the enzyme activity studies.

**Equivalence studies of microbial and DAS-444Ø6-6 soybean expressed protein.**
All three proteins, PAT, 2mEPSPS and AAD-12 were found biochemically equivalent to the soybean expressed protein. The protein isolated from soybean was from greenhouse and not field studies. Also, the applicant do not state whether the soy had been sprayed with the herbicides or not. This was also not found in the referenced document (Schafer et al 2011)

- The 2mEPSPS protein was isolated from the soy leaf tissue and not the bean, which is used in food and feed.
- Figure 54 representing SDS-PAGE (with total protein stain) of plant and microbially derived 2mEPSPS shows additional bands in lanes 1 and 2 with plant derived protein (at around 80 kDa). These bands are not further analyzed.
- Figure 55 with western blots of plant and microbially derived 2mEPSPSproteins lacks molecular weight standard. Thus it is impossible to interpret size of bands. Also, there is a double band in lane 3 (plant derived protein) which is not commented. The weak band of 2mEPSPS in non-transgenic soy is commented as native EPSPS with 76% homology to the microbially derived one.
- Figure 62 representing SDS-PAGE of microbially and plant derived AAD-12 for glycoprotein analysis should have been exposed longer. The glycoprotein membrane has very weak bands/signals, even for the positive control.
- The PAT protein western blot analysis (Figure 67) reveals additional bands in all lanes reacting with the monoclonal antibody used. These bands are very weak. They are not mentioned in the explanation of the figure which says that" no immune reactive proteins, consistent with the PAT protein, were observed in the control…" However, there are no analyses provided for the bands visible on the membrane in the control or any of the other lanes. Also, more exposure time would have revealed these bands better.

**Detection of glycosylation.**
Glycoprotein staining of gels after SDS-PAGE did not reveal any glycosylated proteins. However, the signals on the membranes are very weak, even for the positive control. Thus a longer exposure time would be recommended in the analysis of potentially glycosylated proteins.

**Stability of proteins and resistance to proteolytic proteins.**
Heat stability studies indicate that industrial processing of 2mEPSPS and AAD-12 degrades the tertiary structure of the proteins as they lose their immunoreactivity. PAT is not analyzed here because of its "history of safe use". A mixture of the three transgenic proteins was not analyzed together for heat stability; or at least the application does not say anything about it. This should have been tested as proteins in a mixture seem to tolerate more external stress (heat, digestion, etc) than single proteins.
Also, simulated gastric fluid studies show that these three proteins are rapidly degraded. However, several major food allergens have previously been found to be labile in SGF studies (Kenna and Evans, 2000). Resistance to SGF can therefore not be major characteristics of food allergens as there is not a clear correlation between the digestibility of an allergen and its allergenic potential (Fu, 2002).

The SGF studies seem to have been performed with the microbial version of the transgenic proteins. However, in the text the applicant write that the plant version also is tested, but this is from another event of soy (DAS-68416-4). The plant version of the transgenic proteins from the event in question should have been tested, as this has a different combination of genes and thus, expressed transgenic proteins.

**Assessment of the whole food and/or feed derived GM plants.**
Toxicity Assessment was performed with background in history of safe use, amino acid sequence comparison to known toxins, bioinformatics analysis and assessment of toxicity to animals.
The transgenic proteins were used separately in acute oral (OECD 423) studies and AAD-12 was subjected to a 28-day repeated dose (OECD 407) study. These studies did not reveal any toxic effects on the animals. No acute oral of repeated dose study was performed with a mixture of the transgenic proteins. A 90-day feeding study was performed with 20% DAS-444Ø6-6 in the diet. No fully transgenic diet was analyzed. Also, there was no mentioning of whether sprayed and unsprayed transgenic soy had been used. Herbicide treated soya should have been included in the animal experiments, and the residue level of the herbicides and their metabolites should have been analyzed. The applicant found no treatment related effects.

**5. Allergenicity assessment**
Safety of donor organism, homology with known allergens and *in vitro* simulated gastric fluid (SGF) studies were performed in order to analyze the potential allergenicity of the transgenic proteins. Donor organisms were analyzed and found safe. The proteins were found to not have any sequence homology to known allergens. We have not analysed these data further. SGF studies are mentioned in the above part under toxicology.
Allergenicity assessment of the whole GM plant was performed using ELISA and IgE immunoblot. No change in the level of endogenous allergens was detected by the immunoblot method used, and no change in IgE binding patterns were detected with these two methods.
Serum screenings were not performed in order to look for immune-related reactions because of the assumed non-allergenicity of the proteins.

**Adjuvanicity**
Only the AAD-12 protein was evaluated for adjuvanicity. Due to the “history of commercial use “(and assessments by EFSA), the two other proteins were not analysed.
It was however not considered or discussed whether the three transgenic proteins <u>together</u> could increase the intrinsic immunogenicity of the antigen(s) to elicit strong responses.

**Recommendations:**

- The regulator is encouraged to ask the Applicant to specify whether it is the plant or the microbially derived protein that is used in the analysis. This is not always the case.
- The regulator is encouraged to ask the Applicant to use newly expressed proteins from real field studies and mention whether the soy was sprayed or not.
- The regulator is encouraged to ask the Applicant to provide western blots with visible standard so that it is possible to interpret size data. Also, some of the blots should have been exposed more to visualize additional bands better.
- The regulator is encouraged to ask the Applicant to include herbicide treated soya in the animal experiments, and analyze the residue level of the herbicides and their metabolites.

***Missing information in relation to requirements under the Norwegian Gene Technology Act***

**Social utility and sustainability aspects**

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act. In accordance with the aim of the Norwegian Gene Technology Act, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of sustainable development. This is further elaborated in section 10 of the Act (approval), where it is stated that

> *"significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development"*.

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of DAS-444Ø6-6. In fact, there are important doubts regarding the sustainability of the product as the potential transfer of herbicide tolerant genes to wild relatives might create weed problems and thereby increase herbicide use (other than glufosinate-ammonium and glyphosate-based, as recognized by applicant in section 11.4)..

Further, the Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental

effects in other countries, such as where GMOs are grown. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. Hence it cannot be expected that the same effects will apply between different environments and across continents.

**Recommendation**:
The regulator is encouraged to ask the Applicant to submit required information on the social utility of DAS-444Ø6-6 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

**Ethical considerations**

DAS-444Ø6-6 contains a *aad-12* gene (from *Delftia acidovorans*) and a phosphinothricin acetyl transferase gene (PAT) (from *Streptomyces viridochromogenes)* that confers tolerance to herbicides containing glufosinate-ammonium and 2,4-D, herbicides that are banned in Norway. While it is understood that the Applicant has not applied for deliberate release of DAS-444Ø6-6 in Norway, the acceptance of a product in which the intended use includes the use of a product banned in Norway would violate basic ethical and social utility criteria, as laid out in the Act. That is, we find that it would be ethically incongruous to support a double standard of safety for Norway on one hand, and safety for countries from which Norway may import its food and feed on the other. This line of reasoning is consistent with the provisions under the Act to assess ethical, social utility and sustainable development criteria not only for Norway, but for countries from which Norway imports food and feed.

Therefore, we find it difficult to arrive at justified use of this event without engaging in such an ethical double standard. Specifically, this issue is relevant particularly in revised regulations of 2005 Section 17 “Other consequences of the production and use of genetically modified organisms” points 2 and 3 “ethical considerations that may arise in connection with the use of the genetically modified organism(s), and “any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)”, respectively.

## Conclusion

### Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature provided in the application is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

### Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use of DAS-444Ø6-6 that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of DAS-444Ø6-6 we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

**References**

Axelrad JC, Howard CV, Mclean WG, (2003). The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicology 185:67-78.

Blackburn LG, Boutin C, (2003). Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup (R)). Ecotoxicology 12:271-285.

CBD (2003). Cartagena Protocol on Biosafety. http://www.cbd.int/biosafety/

Codex (2003) Principles For The Risk Analysis Of Foods Derived From Modern Biotechnology; Codex Alimentarius Commission, CAC/GL 44-2003

Codex (2003a) Codex Work on Foods Derived from Biotechnology. In CAC/GL 45-2003. Codex. http://www.who.int/foodsafety/biotech/codex_taskforce/en/.

Codex (2003b) Codex Alimentarius Commission, Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June- 5 July, 2003. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity, pp. 47-60

Cuhra M, Traavik T and Bøhn T (2012) Colone- and age-dependent toxicity of a glyphosate commercial formulation and ist active ingredient in Daphnia magna. Ecotoxicology, DOI 10.1007/s10646-012-1021-1

Dallegrave E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, Langeloh A (2003). The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats. Toxicology Letters 142:45-52.

De Schrijver A, Devos Y, Van den Blucke M, Cadot P, De Loose M, Reheul D and Sneyer M (2007) Risk assessment of GM stacked events obtained from crosses between GM Events. Trends in Food and Sci Technol 18:101-109

Dolezel M, Miklau M, Eckerstorfer M, Hilbeck A, Heissenberger A, Gaugitsch H, (2009). Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU / Standardisierung der Umweltrisikoabschätzung gentechnisch veränderter Pflanzen in der EU. BfN – pp.259.

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A, Bleeke M, Honegger JL, Farmer D, Wright D, Haupfear EA (2010) Glyphosate: discovery, development, applications, and properties. In: Nandula VK (ed) Glyphosate resistance in crops and weeds: history, development, and management.Wiley, New York, pp 1–33.

Fu, JT (2002) Digestion Stability as a Criterion for Protein Allergenicity Assessment Ann. N.Y. Acad. Sci. 964: 99–110.

Halpin C (2005) Gene stacking in transgenic plants- the challenge for 21st century plant biotechnology. Plant Biotechnol, 3:141-155.

Hung D (2007) Diffused brain injury in glufosinate herbicide poisoning. Clinical Toxicol 45:617

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P, (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus). Environmental Toxicology 18:260-267.

Kenna, J.G and EVANS, RM ( 2000). Digestibility of proteins in simulated gastric fluid. Toxicologist 54: 141.

Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, Belle R (2002). Pesticide roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chemical Research in Toxicology 15:326-331.

Matsumura N, Takeuchi C, Hishikawa K, Fujii T and Nakaki T (2001) Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice. Neuroscience Letters 304:123-125

Ono, MA, Itano EN, Mizuno LT, Mizuno EHF, Camargo ZP (2002). Inhibition of Paracoccidioides brasiliensis by pesticides: Is this a partial explanation for the difficulty in isolating this fungus from the soil? Medical Mycology 40:493-499

Podevin N and du Jardin P (2012) Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants. GM Crops and Food 3: 1-5

Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1-23, Available from http://eur-lex.europa.eu/JOHtml.do?uri¼OJ:L:2003:268:som:en:html

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives 113:716-720.

Schafer B.W., Juba A. N., Harpham N.V. J., and Mayes M.R. (2011) Characterization of the Aryloxyalkanoate Dioxygenase-12 (AAD-12) and double mutant 5-Enol Pyruvylshikimate-3-Phosphate Synthase (2mEPSPS) proteins derived from transgenic soybean event DAS-444Ø6-6 Regulatory Sciences and Government Affairs—Indianapolis Lab Dow AgroSciences LLC

Schulte-Hermann R, Wogan GN, Berry C, Brown NA, Czeizel A, Giavini E, Holmes LB, Kroes R, Nau H, Neubert D, Oesch F, Ott T, Pelkonen O, Robert-Gnansia E and Sullivan FM, (2006) Analysis of reproductive toxicity and classification of glufosinate-ammonium. Regulatory Toxicology and Pharmacology 44:S1-S76

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois JS (2012) Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol. 2012 Nov;50(11):4221-31. doi: 10.1016/j.fct.2012.08.005.

Solomon KR, Thompson DG (2003). Ecological risk assessment for aquatic organisms from over water uses of glyphosate. Journal of Toxicology and Environmental Health-Part B Critical Reviews 6:289-324.

Watanabe T, Sano T (1998) Neurological effects of glufosinate poisoning with a brief review. Human & Experimental Toxicology 17:35-39