

Expression of Bt toxins in 'SmartStax'

**Analyses of Stilwell & Silvanovich, 2007 and Phillips, 2008
Report number MSL0021070 and Sub-Report ID: 61026.05**

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Summary:

It is important to know the rate of expression of the additional proteins in the plant in order to assess their genetic stability as well as environmental and food chain exposure.

Further, it is especially relevant to determine just how much insecticidal toxin is produced in the different parts of the plant, and whether this content is dependent on certain environmental conditions. The plants should, therefore, be exposed to defined environmental conditions to identify factors impacting the rate of expression and the actual range of variation.

It is also important to investigate the persistence of the toxins. The Bt toxins are introduced into the environment via manure and parts of the plants (such as roots, pollen and parts remaining on the field after harvest). It is important to know if they can accumulate and/or persist over longer periods of time in order to assess the exposure of soils and water.

It is absolutely necessary in this context, to define the protocol for measuring the toxins, since different methods for measuring can result in highly varying results. The technical protocols should be fully published, and evaluated by independent laboratories to allow other institutions to conduct further measurements to control the exact level of toxins.

The investigations were commissioned and conducted by Monsanto and Dow AgroSciences. No independent laboratories were involved. The results were not published in peer-reviewed magazines.

The data as provided by the applicants show a large range of variations in the Bt content of the maize plants. In several cases, the data show a tenfold variation in the Bt content. However, twentyfold and even higher ranges of variation do occur. The exact range of variation under changing environmental conditions and the specific impact factors has not been determined. Thus, it is not known if the range of variations in the Bt toxins under specific environmental conditions might be even greater, or whether genetic stability can be expected under stress conditions.

The protocols used by Dow AgroSciences have never been published. The company only ever refers to its own unpublished reports, which are even classified as "*draft method*". Moreover, the protocols used by Monsanto for conducting the measurements have not been fully published. None of the protocols that are specific to each of the different Bt toxins were evaluated by independent laboratories. As a result, no independent institution can make comparable measurements to monitor

the actual range of Bt concentration in the plants during cultivation and in food and feed products. This in turn excludes adequate monitoring after market authorisation.

There was, in addition, no investigation into the persistence of the Bt toxins. Therefore, the actual exposure of the environment via manure or parts of the plants and the potential accumulation of the toxins in the soil cannot be assessed.

The investigations of industry show some outcomes that give cause for concern: The overall content of the Bt toxins can amount to more than 1600mg/ kg in leaves (dry weight) which is a much higher content of Bt toxins than described in other genetically engineered maize plants.

EFSA did not ask for additional investigations but simply declared the data to be “comparable” with the data from parental events. It did not discuss the huge variations. EFSA also failed to act upon requests from several Member States for more data and a much more detailed investigation.

In conclusion, the investigations of Stilwell & Silvanovich (2007) Phillips (2008) do not render the necessary scientific evidence. The quality of the data is not reliable. Because the expression rate of the foreign protein is a very basic element in risk assessment of genetically plants, the overall risk assessment of SmartStax suffers from unacceptable deficiencies.

1. Background:

Smartstax produces a combination of six Bt toxins and two enzymes (EPSPS and PAT) that confer herbicide tolerance (glyphosate and glufosinate). The gene constructs for PAT are actually doubled because they are present in two of the parental events used to produce SmartStax.

As mentioned by experts from the Austrian government, the plants also inherit “among others sequences from the 35S-promoter in all inserts, and sequences from the ubiquitin promoter, the rac intron, the nos-terminator, the 35S-terminator.” (page 2 of EFSA 2010 b). These various gene constructs and their elements are not meant to be controlled by the plants’ gene regulation. They are, in fact, designed to evade biological mechanisms such as silencing and down regulation by the plant’s overall gene regulation (see for example Diehn et al., 1996). The expression rate in single events and stacked events can be influenced by various factors and interactions with external factors and plant metabolism.

It is known that due to the combination of gene constructs in stacked events, the expression rate of the foreign proteins can be higher than in the single events, but the reason for this is not known (EFSA 2010a). It is also known that the content of Bt toxins in genetically engineered plants is influenced by environmental factors and can show a wide range of variation (Ngyuen & Jehle, 2007, Then & Lorch, 2008). Furthermore, genetically engineered plants can show unexpected reactions to environmental conditions and stress factors (see for example Zeller et al, 2010), that can also impact the content of its foreign proteins.

Therefore, it is necessary to explore the actual range of variation, especially of the Bt toxins. Their expression rate does not only impact their efficacy on pest insects, but also concerns the exposure of the food chain and the environment to insecticidal proteins.

In general, the range of concentration of the foreign proteins is relevant to the exploration of genetic stability in genetically engineered plants. It is also relevant to the assessment of potential health

impacts such as toxicological and immunological hazards as well as combinatorial effects. Further, it is relevant for the assessment of environmental impacts such as risks for non-target organisms, pest resistance and exposure of soils and other areas of the environment to Bt toxins.

Methods and protocols for measurements and their quality control are decisive in acquiring reliable data and carrying out risk assessment. As for the application of pesticides, fully publishing technical protocols and evaluation by independent institutions are indispensable prerequisites for determining exposure rates. In the case of Bt toxins, so-called ELISA systems are used to determine the content, but their outcome is highly dependent on details of the protocol (see for example Then & Lorch, 2008).

Further, it is necessary to determine environmental impact factors that can influence the rate of gene expression. It is known that the environment can impact Bt content in genetically engineered plants (Then & Lorch, 2008). To determine the most relevant impact factors and the true range of possible variations it is necessary to expose the plants to defined environmental conditions. Then & Potthof (2009) propose a 'stress test' (or 'crash test') for this purpose.

2. Overview of investigations of Stilwell & Silvanovich and Phillips

Stilwell & Silvanovich (2007) measured the expression rate of Cry1A.105, Cry2Ab2, Cry3Bb1 and EPSPS at the Monsanto laboratories (MSL0021070). Phillips (2008) investigated the expression rate of Cry1F, Cry34Ab1, Cry35Ab1 and PAT at the Dow AgroSciences laboratories (Sub-Report ID: 61026.05).

In 2006, plants were grown at five US field sites. Only four sites were used for further studies because contaminated seeds were found in one field site. During cultivation, the stacked events SmartStax were grown alongside parental events such as MON88017 (for Cry3Bb1 and EPSPS), MON89034 (for Cry1A.105 and Cry2Ab2), TC 1507 (for Cry1F and PAT), DAS 59122 (for Cry34Ab1, Cry35Ab2 and PAT). Particular information about the environmental impact during cultivation of the plants is not given.

The teams both worked with ELISA, but appear to have used different protocols. The protocols used by Dow AgroSciences have not been published, the company refers to its own unpublished reports. Some of the protocols used by Dow AgroSciences are even characterized as “under development” (Phillips, 2008, page 22). Monsanto published more details about their methods but they did not involve any external laboratory to evaluate their methods. There was no attempt to compare the results of one laboratory with the other, none of the samples were analysed in the laboratories. Thus, it is not possible to decide if the protocols used by the different companies render similar results when applied to the same material. The results were even expressed on different basis: The Monsanto labs provided data on dry tissue weight (dwt) and on fresh tissue weight (fwt). Dow AgroSciences only provided data on a dry tissue weight basis. Samples were taken from leaves (over season leaf, OSL), roots (over season root, OSR), whole plants (over season whole plant, OSWP), from pollen and grain.

The data presented by the companies showed some differences between the stacked events and the parental lines (for example in Cry1Ab.105) that EFSA considered “comparable” although there was no definition of what this meant. Especially the PAT enzyme showed a higher expression rate in SmartStax. This finding was explained away with the doubling of the gene construct in the stacked events.

Additional evaluation of the data by Testbiotech actually shows huge variations. By mixing the raw data of the particular Bt proteins from single events with those from stacked events, a much broader range of variation (within the different parts of the plants) emerges than is summarized by EFSA and the applicants (see table 1). In several cases, the maximum Bt content exceeds the minimum Bt content by more than tenfold, but there were also results found where the data showed a twenty fold or an even higher range of variation.

No explanation is given concerning the content of Bt toxin in the different parts of the plants regarded as technically acceptable or necessary to provide resistance against pest insects. By summing up the overall minimum and maximum content of Bt toxins within the different parts of the plants, the data showed a such a huge range of variation that the genetically engineered maize plants should not be seen as sufficiently defined in their technical qualities, they might even show genetic instability under certain environment conditions (see graphic 1).

Adding up the Bt content of the different parts of the plant shows that the overall Bt content in the stacked events is much higher and not “comparable” with the Bt content in the single events.

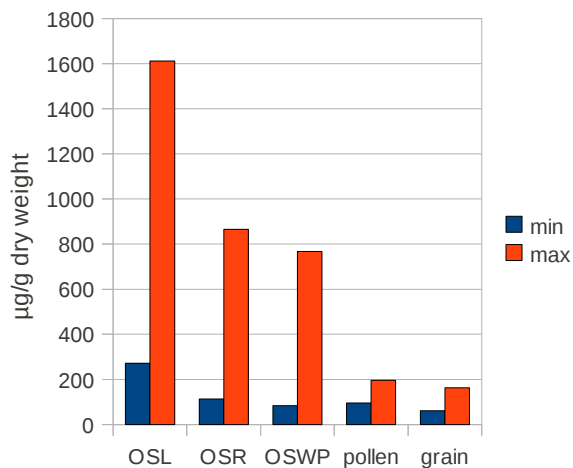
No data is given concerning the life cycle of the Bt protein such as degradation in soil and water, persistence during the passage through the gut of the animals, and to which extent the environment is exposed to Bt proteins through manure.

Table 1 Overview: Ranges of the Bt toxin content in different parts of the plant, using the data from parental lines as well as from SmartStax (µg/g dry weight tissue)

	OSL	OSR	OSWP	Pollen	Grain
Cry1A.105	39 - 210	11 – 240	3,8 – 86	5,1 – 21	1,7 – 4,9
Cry1F	9,84 – 34,3	3,19 – 14,7	2,71 – 15,8	14,3 - 32,2	2,12 – 7,43
Cry2Ab2	60 - 350	4 – 120	3,6 – 130	0,18 – 2,3	2,7 – 7,5
Cry3Bb1	53 – 580	23 – 260	6,9 – 220	7,5 – 24	10 – 38
Cry34Ab1	71,5 – 279	65 – 150	64,1 – 233	68 – 117	43,6 – 102
Cry35Ab2	38,5 – 158	13,8 – 80,5	2,54 – 82,3	–	1,24 – 2,65
Overall content	271,84 – 1611,30	113,61- 865,20	83,65 - 767,1	95,08 – 196,50	61,36 – 162,48

OSL: over season leaf, OSR: over season root, OSWP: over season whole plant

Fig. 2: Overview of ranges of the Bt toxin content in different parts of the plant, using data from parental lines as well as from SmartStax ($\mu\text{g/g}$ dry weight tissue)



OSL: over season leaf, OSR: over season root, OSWP: over season whole plant

3. Assessment of the investigations

3.1 Evidence of insufficient testing

The investigations show severe deficiencies in quality control, essential data are completely missing.

The measurements were only conducted by Monsanto and Dow AgroScience laboratories. No independent institutions were involved to prove that testing was done in reliable manner. The protocols used by the two labs differ but there was no double check to find out if the results were comparable. Findings were not peer reviewed and published. Not even the protocols used by the labs have been fully published, therefore the investigations cannot be repeated by independent institutions and results cannot be checked.

The Dow AgroSciences files show that the method for measuring particular Bt proteins was not even fully established. In the case of Cry34Ab1, “draft” methods were used instead. In addition, the Dow AgroSciences report lists several relevant deviations from the protocol that could have influenced the outcome of the measurements.

Regarding the data, a lot more investigations is necessary. There is no information concerning environmental impacts on the plants that might have influenced the expression rate (such as climate, soil, fertiliser, overall use of pesticides). Data was only collected during one period of vegetation. The range of the possible variations and the impact factors on the rate of expression were not determined, despite the fact that Bt content in the plants showed huge variations. What missing is some kind of a stress test under defined conditions that could help to identify those impact factors and the true range of possible variations in Bt expression.

The life cycle of the Bt proteins was not explored. No information was given concerning the rate of degradation or potential accumulation in the soil, not even in the case of the synthetic protein Cry1A.105, whose biological properties cannot be derived from comparison with naturally occurring Bt toxins.

3.2. Assessment by EFSA and the experts of EU Member States

EFSA has not dealt with these figures in particular. It only addresses the content of foreign proteins in the grain. However, it should be noted that the application is not restricted to grain (even if grain is the most likely product to be imported) but to the whole plant and all of its uses in food and feed. In the event that only the Bt content in grains is assessed by EFSA, then the usage of the plants in food and feed must be restricted to the grains only.

The opinion as published (EFSA 2010 a) is also relevant for the upcoming discussion on the application for cultivation of these crops, as the environmental risk assessment will refer to the opinion on food and feed for the use of the material in food production.

Further data such as Bt content in leaves and roots are necessary to gather sufficient information about the overall technical quality of the plants and their reaction to environmental conditions. Thus, the narrow approach of only assessing the grains is unacceptable. The range of variation and life cycles of the proteins were not sufficiently explored.

In its opinion (EFSA, 2010a), EFSA makes the following statement:

“Therefore, protein expression data related to the grain (F2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 5912222 are considered most relevant, and are summarised in Table 1. Levels of proteins in the grain (F2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122 are comparable to those in the single events, although the mean level of Cry1A.105 was lower in maize MON 89034 compared to maize MON 89034 x 1507 x MON 88017 x 59122. The levels of the newly expressed proteins do not pose a safety concern (also see section 5.1.4.1, 5.1.5.1 and 6.1.2). The same conclusions were reached by the EFSA GMO Panel for the parental maize stacks 1507 x 59122 (EFSA, 2009c) and MON 89034 x MON 88017 (EFSA, 2010).”

Experts from several member states such as Austria, Belgium and Germany requested more data on Bt expression (EFSA 2010 b). For example, data from more seasons as well as information on environmental impact and on measurements in Bt plants that were not sprayed with herbicides.

In their response to the Member States, EFSA (EFSA 2010 b) referred to their interpretation of the data as “comparable” with those from the single events. Rates of degradation of the Bt toxins are considered relevant, but hardly any data is provided.

Table 2: Relevant comments from the experts of Member States and the response from EFSA 2010b

Member State	Statement	answer from EFSA
Austria	The information submitted by the notifier does not assess the expression of the transgenic proteins in plants not treated with herbicides containing glyphosate and glufosinate-ammonium. According to the EFSA guidance how to conduct field trials for comparative assessment, "in case of herbicide-tolerant GM-plants it is advisable to include both blocks of genetically modified plants exposed to the intended herbicide and blocks not exposed to the herbicide. This design would allow assessment of	Expression levels of recombinant proteins in maize plants with or without treatment of herbicides were previously assessed for the single events and do not have to be repeated for the stacked lines. Moreover, only grains from treated plants will be imported. In addition, none of the newly expressed proteins is considered to be toxic to the consumers. (page 3)

Member State	Statement	answer from EFSA
	whether the expected agricultural condition might influence the expression of the studied parameters” (EFSA 2006). Since such a comparison between expression of transgenic components in treated vs. untreated plants was not conducted, the submitted information is considered insufficient. Additional information addressing this issue is requested from the notifier in line with EFSA guidance. (page 3 and 4)	
Austria	Furthermore, expression is only assessed for a single growing season. An assessment of expression over several growing seasons would be more adequate to establish baseline exposure data. The notifier, thus, is requested to present data from at least 2 consecutive growing seasons. (page 4)	Expression levels of the single events were already assessed. For stacked lines expression data for one season are considered to be sufficient. (page 4)
Austria	Thus, the notifier does not adequately address other exposure routes of products derived from GM maize MON88017x MON89034x 1507x59122 and of transgenic constituents of this GM maize hybrid. Additional exposure may, for instance, result from feed use (leading for instance to exposure to non-target organisms especially in the soil via organic fertiliser) or from waste materials and sewage from the feed industry (which may lead to the exposure of non-target organisms in aquatic eco-systems (Rosi-Marshall et al. 2007)). A number of studies indicate the presence of immunoreactive parts of cry-proteins in the faeces of ruminants fed GM-feed (Einspanier et al. 2004; Lutz et al. 2006) and the possibility for sustained presence of these cry-toxins in soil material (Lee et al. 2003; Bayerische Landesanstalt für Landwirtschaft 2005). (page 29)	Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny) during transportation and processing. (page 29)
Belgium	In part 1 of the technical dossier we can read p.45 “For the PAT protein, expression was higher in the combined trait product as compared to 1507 and 59122” but the Table 12 (PAT) shows that the values for PAT protein levels in grain collected from MON 89034 x 1507 x MON 88017 x 59122 (0.050 µg/g dw) are similar to those of 59122 (0.049). Is there no contradiction between the statement “... the levels of Cry1A.105...are comparable to the protein levels in the positive levels of newly expressed proteins between stacked controls...” (Technical dossier, part I, page 45) and the data provided Table 6 for this protein (4.3 vs 2.8 in the control)? (almost no overlap in range; means are about 3 SD different). (page 35)	The EFSA GMO Panel takes note of this comment. Considering the scope of the application and the safety of the newly expressed proteins, the values reported can be considered “comparable” (whether statistically significantly different or not). It should be noted that differences in expression in lines and the single events are not uncommon. (page 35)
Germany (BfN)	MON89034x1507xMON88017x59122 maize also differs from the parental lines with regard to the absolute amount of toxin produced which is far greater than in the parental lines. We advise to reflect this stronger when assessing both health and environmental effects. (page 50)	In addition, the EFSA GMO Panel evaluated whether the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed grain produced by maize MON 89034 x

Member State	Statement	answer from EFSA
		<p>1507 x MON 88017 x 59122. Due to the specific insecticidal selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins belong to the same or closely related taxonomic groups as those of the target organisms. Data supplied by the applicants suggest that only low amounts of the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins enter the environment due to low expression in grain. Moreover, these Cry proteins are degraded by enzymatic activity in gastrointestinal tracts of animals fed GM maize or derived feed products (see section 5.1.1), meaning that only low amounts of these proteins would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab. It is expected that there would subsequently be further degradation of Cry proteins in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1 Cry34Ab1 and Cry35Ab1 proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localised. While Cry proteins may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). Compared to the Cry1Ab protein, the Cry3Bb1 protein of GM maize was found to be degraded more rapidly in soil under similar conditions (Baumgarte and Tebbe, 2005; Miethling-Graff et al., 2010).</p> <p>Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny), it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins is likely to be very low and of no ecological relevance. (page 52/53)</p>
Germany (BfN)	Expression analysis must be regarded as an important part of the GMO risk assessment because it allows reflecting on the stability of the genetic modification, and indicates possible interactions between the GMO and environmental factors such as climate, soil or agricultural practice (e.g. fertilisation). Expression data should provide reliable estimates on the quantity of expression in different plant tissues with regard to biotic and	The scope of the application covers food and feed uses, import and processing, therefore protein data related to the grain are considered most relevant and information on other tissues was provided. Expression data were supplied from trials conducted in 2006 at five location in the major USA maize growing regions that represent different environmental conditions. Expression levels were comparable to those of the single events. The EFSA

Member State	Statement	answer from EFSA
	<p>abiotic factors.</p> <p>The data presented in the dossier do not meet the above objectives. Expression data were submitted from five North American sites for only one growing season (2006) (MSL-0021078; MSL-0021070; 061026.05). No criteria were given for selecting the presented field sites which should be representative for a diversity of climatic and agronomic conditions.</p> <p>To complete the assessment of expression the notifier is asked to:</p> <ul style="list-style-type: none"> • Provide information and selection criteria which allow to establish, that the chosen field sites are representative and cover a range of environmental and agronomic variables • Describe the chosen experimental sites in full detail indicating not only the region but the location of the field site. • Test differences between the stacked event and each of the parental lines in a statistically reliable design. • Test the influence of environmental factors such as climate or soil on expression in a statistically reliable design. • Test the influence of different genetic backgrounds on the expression pattern in a statistically reliable design. <p>We strongly recommend comparing and analysing expression data with other data already available. We also recommend increasing sample size to allow analysing data with a higher statistical power. We also recommend testing the influence of the application of glyphosate and glufosinate on the expression. The expression data presented indicate that Cry1A.105 is expressed higher (twofold) in some tissues (pollen and grain) of the stacked GMO compared to the parental line 89034. Data also show that expression of PAT is markedly higher in all tissues of the stacked GMO than in the parental lines 59122 and 1507. While the increased expression of PAT can be explained by the additive action of the multiple gene copies present in the stacked GMO, the different expression pattern of Cry1A.105 in pollen and grain should be checked and further analysed. (page 54/55)</p>	<p>GMO Panel is of the opinion that these data are sufficient from a safety point of view.</p> <p>The plants were treated with glufosinate-ammonium and glyphosate-based herbicides and this is considered sufficient as only grains from treated plants will be imported. The mean Cry1A.105 levels are indeed higher in grain of MON 89034 x 1507 x MON 88017 x 59122 compared to MON 89034. However there is an overlap in the range of Cry1A.105 levels measured in the stacked event and the single event MON 89034 and levels are low in grain and comparable to previously obtained results. It should be noted that differences in the levels of newly expressed proteins between stacked lines and the single events are not uncommon and do not necessarily pose a safety concern. (page 54)</p>
Germany (BfN)	<p>The notifier is requested to submit a detailed exposure analysis including the exposure of the environment via the food-feed chain, including the exposure of soil and water to Bt proteins. Data on the quantity and the degradation of the mixture of Bt proteins in all relevant media such as organic waste, waste water, and manure are required.</p>	<p>Considering the proposed uses of maize MON 89034 x 1507 x MON 88017 x 59122, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed grain (F2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122 and with the accidental release into the environment</p>

Member State	Statement	answer from EFSA
	Following this, the potential accumulation of the Cry proteins in the environment should be assessed. (page 58)	of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny see section 3.1) during transportation and processing. (page 58)
Germany (BfN)	Data on the degradation of Cry toxins during processing and the use of food/feed for MON89034x 1507x MON88017x 59122 are missing. With respect to studies on the degradation of microbially derived Cry1A.105, CryAb2, Cry1F, Cry3Bb1 and Cry34Ab1/ Cry35AB1 the notifier is requested to refer to scientific studies and not to other EFSA dossiers (e.g. as done in page 143 of the dossier). As stated in the EFSA guidelines applications need to be stand-alone documents. (page 61) To assess the degradation of Cry Proteins detailed description of the used methodology is necessary. The cited half-lives of Cry Toxins by the notifier seem to be in conflict with results from peer reviewed literature. (Page 61)	It is noted that Codex alimentarius recommends the performance of in vitro resistance test against proteolysis by pepsin, which has been performed for the newly expressed proteins in each single event (see the EFSA GMO Panel"s opinions on each of these events) . (Page 61)
Germany (BVL)	Moreover, information on known or anticipated human/animal exposure to other sources of analogous GM food/feed and from other routes of exposure to the new gene products is missing and should be provided. In this context, an exposure assessment regarding the new gene products taking into account all sources of exposure is lacking and might be requested from the applicant. (Page 68)	Taking into account that no risk has been identified and that a Pan-European database on consumption data is not yet available, and that the estimated exposure is very low, a more detailed exposure assessment appears not to be warranted. The data in the dossier containing an estimate of potential exposure to the transgenic proteins can be summarized as follows: Based on the expression levels of the newly expressed proteins measured during the field trials in the USA in 2006, and on human and animal consumption data for maize and derived products, the applicants estimated the potential intake of the newly expressed proteins by humans and animals consuming maize. Whilst the estimates were conservative, assuming a 100% - substitution scenario and no losses of newly expressed proteins during processing, the outcomes show that these levels were several orders of magnitude below the levels having no adverse effects in the acute oral toxicity studies previously performed with these proteins. (page 68)
Norway	The expression of the cry1A.105 gene in MON 89034 x 1507 x MON 88017 x 59122 is about 100 % higher in pollen and about 50 % higher in corn compared to MON 89034. The applicant is asked to explain these differences.	The scope of the application covers food and feed uses, import and processing, therefore only protein data related to the grain are considered relevant. The mean Cry1A.105 levels are indeed higher in MON 89034 x 1507 x MON 88017 x 59122 compared to MON 89034. However there is an overlap in the range of Cry1A.105 levels measured in stacked event and MON 89034. It should be noted that differences in the levels of newly expressed proteins between stacked lines and the single events are not uncommon and do not necessarily pose a safety concern. (page 68)

4. Conclusions:

The data presented are insufficient and the risk assessment performed by EFSA is not acceptable. The data are not produced in a reliable way, the protocols for determining the Bt content have not been published, the results have not been peer reviewed.

Substantial data are missing and industry failed to determine the true range of expression and the relevant impact factors. Possible accumulation of Bt toxins in soil through manure was mentioned by EFSA as relevant, but no specific investigations were requested. EFSA largely ignored the fact that the stacked events with their Bt toxins pose a much higher risk to the food chain and the environment than the parental events.

Since selectivity of the Bt toxins and possible synergies between the Bt toxins and with other external factors have also not been fully investigated (see Levine et al., 2008), the huge range of variation in the content of the Bt toxins is a matter of serious concern in the overall risk assessment of SmartStax.

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