

SUMMARY

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STUDY TITLE

Global Dietary and Livestock Assessment of PAT Protein for DAS Soybean Cultivar Based on Event DAS-44406-6

DATA REQUIREMENTS

EFSA GMO guideline, 21 CFR 192.25

AUTHOR(S)

C. B. Cleveland

STUDY COMPLETION DATE

28-JUN-2011

PERFORMING LABORATORY

Dow AgroSciences LLC
Regulatory Laboratories-Indianapolis Lab
9330 Zionsville Road
Indianapolis, Indiana 46268

STUDY NUMBER

110599

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SUMMARY

This report presents an assessment of the phosphinothricin acetyltransferase (PAT) protein in soybeans in the context of a dietary exposure assessment for humans and livestock. Dietary assessments involve comparison of a projected exposure based on dietary consumption patterns to a safety threshold based on toxicity testing. This report includes several human dietary global assessments for several national consumption patterns. The report also includes livestock exposure assessments.

Very low expression levels of the PAT protein in plant tissues of DAS-44406-6 soybean across environments translate to a very low dietary exposure risk to humans and animals. PAT has a long history of safe use in commerce. The overall safety assessment indicates that the PAT protein expressed in DAS-44406-6 represents a negligible risk to human and animal health.

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AUTHOR(S)

C. B. Cleveland
[(317) 337-3532; cbcleveland@dow.com]

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Title: Global Dietary and Livestock Assessment of PAT Protein for DAS Soybean
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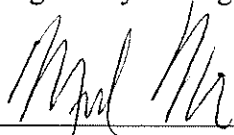
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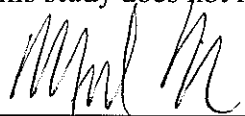
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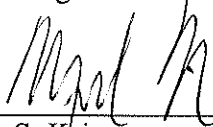
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M. S. Krieger
Sponsor
Dow AgroSciences LLC

28 June 2011


Date



M. S. Krieger
Submitter
Dow AgroSciences LLC

28 June 2011

Date



C. B. Cleveland
Study Director/Author
Dow AgroSciences LLC

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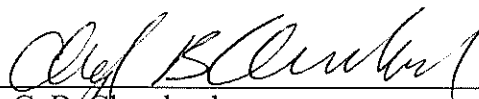
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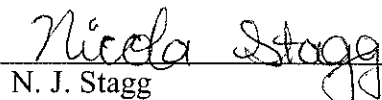
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C. B. Cleveland
Author, Human Health Assessment
Dow AgroSciences LLC

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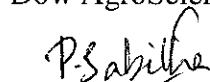
Date



N. J. Stagg
Peer Reviewer, Human Health Assessment
Dow AgroSciences LLC

27 June 2011

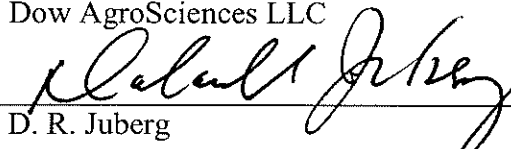
Date



S. Papineni
Peer Reviewer, Human Health Assessment
Dow AgroSciences LLC

27, June, 2011

Date



D. R. Juberg
US Manager, Human Health Assessment
Dow AgroSciences LLC

24 June 2011

Date

STUDY PERSONNEL

Title: Global Dietary and Livestock Assessment of PAT Protein for DAS Soybean
Cultivar Based on Event DAS-44406-6

Study Director: C. B. Cleveland

Other Study Personnel:

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1. ABSTRACT

This report presents an assessment of the phosphinothricin acetyltransferase (PAT) protein in soybeans in the context of a dietary exposure assessment for humans and livestock. Dietary assessments involve comparison of a projected exposure based on dietary consumption patterns to a safety threshold based on toxicity testing. This report includes several human dietary global assessments with a focus on US, EU, Taiwan and a high-end global consumption from Japan. The report also includes livestock exposure assessments with US and EU animal feed assumptions. Other national dietary consumption patterns may result in slightly different numeric values, but they would not result in different overall conclusions.

DAS-44406-6 soybean exhibits very low expression levels of the PAT protein in plant tissues across environments. The PAT protein is a well-understood protein which provides tolerance to glufosinate-based herbicides and has a history of approvals and safe use without reports of adverse effects to humans. The overall safety assessment indicates that the PAT protein expressed in DAS-44406-6 represents a negligible risk to human and animal health.

2. BACKGROUND

Dow AgroSciences has produced a soybean that provides herbicide tolerance to: a) 2,4-dichlorophenoxyacetic acid (2,4-D), b) glufosinate and c) glyphosate. The herbicide tolerances are conferred respectively *via* genes which encode for a) AAD-12, b) PAT and c) 2mEPSPS proteins. Field expression levels of the proteins associated with DAS-44406-6 are found in the Dow AgroSciences report by Lepping and Maldonado (1); the expressions are summarized and then used within this assessment in conjunction with national consumption estimates.

This report presents a summary of the dietary and livestock assessments for the PAT protein for DAS-44406-6 soybean. This report covers several global assessments with a focus on US, EU, Taiwan and a high-end global dietary assessment. The *pat* gene is derived from *Streptomyces*

viridochromogenes which encodes a phosphinothricin acetyltransferase (PAT) protein which detoxifies L-phosphinothricin, the active ingredient in the herbicide glufosinate ammonium.

3. MAMMALIAN TOXICITY ASSESSMENT

The regulatory history and safety assessments for the PAT trait are reviewed first. Then an acute mammalian toxicity study with a single high-dose of protein in mice is summarized here for use later in the dietary and livestock assessments that follow.

3.1. Regulatory History of PAT

The *pat* gene, originally obtained from *Streptomyces viridochromogenes* has already been reviewed and found “not to raise safety concerns” to human health during the assessment of glufosinate-ammonium tolerant maize by EFSA (2) and as noted by OECD (3): “Governmental regulatory authorities in the United States, Canada, Japan and European Union have made decisions that the presence of the PAT protein in plants does not render them unsafe for consumption as food or feed”. These assessments are confirmed by a toxicity study consisting of feeding rats with the PAT protein reviewed by Health Canada (4) and EFSA (2). The PAT protein is readily degradable in simulated digestive juice as noted in several regulatory reviews: CFIA (5), EPA (6, 7), OECD (3) and EFSA (2) and there is a lack of glycosylation when expressed in soybeans as shown in immunoblot detection analyses as cited by EFSA (2). There have been no studies, reports or observations with regards to adverse effects of PAT protein exposure in humans. In conclusion, there is a reasonable certainty of no harm resulting from the inclusion of the PAT proteins in human food or in animal feed.

3.2. Mammalian Acute Toxicity

An acute oral toxicity study with PAT protein was conducted in mice at a level of 5000 mg PAT protein/kg-bw (8). All animals survived and no clinical signs were observed during the study. All animals gained weight by study termination on day 15. There were no treatment-related gross pathological observations. The report concludes that under the conditions of this study, the acute oral LD₅₀ of PAT in male and female mice was greater than 5000 mg/kg-bw. No mortality

was observed and there were no observable effects with the PAT-treated animals, except one female was noted as not gaining body weight over the duration of the study. Therefore the **NOAEL** (No Observed Adverse Effect Level) **is also estimated to be 5000 mg/kg-bw**. PAT protein has a very low acute toxicity potential.

4. EXPOSURE INFORMATION FOR EXPRESSED PROTEIN

Human dietary assessments require coupling of field expression information for PAT protein with conservative (i.e. protective) dietary consumption data for soybean. Similarly, a livestock assessment also requires estimates of levels in feeds to be used in the calculation of an animal dietary burden. This section reviews and summarizes the appropriate field expression information to derive appropriate grain and feed exposure levels for use later in the human and livestock assessments.

4.1. Potential Exposure to PAT Protein *via* Soybean

The field expression of PAT protein in DAS-44406-6 soybean has been measured using a PAT specific enzyme linked immunosorbent assay (ELISA) in several plant tissues at various growth stages of soybean (1). Field expression data is available for trials conducted at multiple test sites located within the major soybean-producing regions of the U.S. Protein expression was analyzed in leaf at V5 and V10-12 growth stages, with root, forage, and grain tissues collected throughout the growing season from DAS-44406-6 soybean plants treated with or without the herbicides 2,4-D, glufosinate, and/or glyphosate. Samples of soybean plant tissues were analyzed for the amount of PAT protein using the Dow AgroSciences validated methods GRM 08.05 (9) using an enzyme-linked immunosorbent assay (ELISA) kit purchased from Envirologix, Inc. The limit of detection (LOD) and limit of quantitation (LOQ) for PAT in soybean tissues were determined during the method validation as 0.06 and 0.12 ng/mg, respectively (1).

The components relevant for the dietary and livestock assessments are reproduced in Table 1. For the human diet, only the grain is relevant. In soybean grain, the average value of PAT protein was **2.12 ng PAT protein/mg tissue on a dry weight basis** [see Table 1 of Ref (1)]. The full range of applicable values was 1.60 to 2.97 ng/mg tissue [based on review of the

50 values for site/test spray in Appendix D – Tables 26 thru 30 of Ref (1)], but the use of an average expression value is most appropriate, because grain is a blended commodity, making consumption of single-servings of grain at the maximum expression-level highly unlikely. This approach is supported by the Harmonized Feedstuffs Table of the OECD guidance overview for residue studies which instructs the use of a STMR (~ mean residue value) for animal feeds associated with soybean meal and soybean seed grain (10). Hence, the maximum level is reported per the EU guidance, but is not used further in the dietary assessment. Expression values for PAT were similar for all treatments irrespective of the herbicide regime.

Table 1 also includes the optional information on soybean forage. Expression information is available for soybean forage with a range of applicable values from 3.94 to 9.35 ng/mg tissue [range of the 50 values for site/test spray in Appendix D – Tables 14 thru 18 of Ref (1)]. For forage, although an average expression of 6.32 ng/mg is available, a highest residue (HR) for soybean forage is recommended in the OECD guidance (10) and the US typically uses a highest average field trial (HAFT) value for animal feed inputs. Therefore as bolded in Table 1, a **value of 9.35 ng/mg is appropriate and used for PAT forage** in the calculation of animal burdens. However, it is also noted that soybean forage is a possible yet uncommon animal feed in the US. Therefore the US livestock assessment presents calculations with and without soybean forage as representative and worse-case assessments. Given there are no plans to grow PAT soybean in the EU, soybean forage is not considered as a viable animal feed in the EU assessment.

Soybean meal and hulls are potential animal feeds. Numerically, there is little concentration of protein from raw soybean into soybean meal, because the crude protein content of whole soybeans is ~43% and commercial soybean meal is typically sold as either 44 or 48% protein concentrate with a full range from 38 to 48% crude protein depending on the process used (11, 12). Hence a concentration value of 1.1X is used below for soybean meal. Soybean hulls contain ~ 12% crude protein (13), therefore the ratio of 12/43 is used to estimate the 0.28X reduction of protein in hulls relative to the whole seed. A 20X concentration of the seed residue has been assumed for potential aspirated grain exposure used as animal feed in the US.

These input values are protective estimates for exposure to the PAT protein from soybean; actual dietary exposure and impact of the protein will be lower because: 1) there may be protein degradation during transport and storage, 2) grain containing PAT will be mixed with non-PAT grain and 3) for humans, consumption of soy products is primarily in food forms which are cooked and heat is known to denature this protein (7). It is also known that soybean oil contain very little protein (14).

5. HUMAN DIETARY ASSESSMENT

For a dietary risk/safety assessment, the short term intake (STI) consumption is compared to an acute toxicity endpoint. Estimates of single serving (acute, or STI) soybean exposure have been used in this report based on available consumption patterns for the EU consumer, the US diet, Taiwan and the global maximum as calculated by the World Health Organization (WHO), which covers Japan for soybeans. National consumption values from other countries could change the numeric calculations slightly, but would not change the overall conclusions. Regardless of which method is used, all acute Margins of Exposure (MOE) are extremely large and greater than 400000 (See Table 2), indicating negligible risk of adverse effects from dietary exposure to PAT protein.

5.1. WHO Consumption Estimate

A conservative acute consumption (i.e. exposure) estimate is made based on global data published by the WHO. WHO has established a maximum consumption of each food commodity for acute exposures for the entire world, based on maximum inputs from multiple countries (15). Table 3 includes 97.5th percentile values for all possible commodities associated with soybean. For PAT soybean, the appropriate maximum human consumption value is associated with the “VD 541 Soya bean dry” group with an upper limit for soybean reported by Japan. Information for soybean oil is presented here for completeness only, because it is understood that the oils and other highly refined fractions do not contain significant amounts of protein. Moreover, total acute consumption across immature and dry entities cannot be

calculated, because it is not appropriate to add 97.5th percentile values for individual commodities for survey results from different countries.

When the WHO “VD 541 Soya bean dry” acute consumption information is coupled to the PAT average field expression level of 2.12 ng/mg tissue for soybean grain, the potential acute exposure to PAT protein *via* soybean is estimated as:

- 0.0064 mg protein/kg-bw/day, for general population (i.e. adults)
- 0.0118 mg protein/kg-bw/day, for children of 6 years or younger

5.2. Acute Margin of Exposure Calculation Based on WHO Consumption Estimate

Acute risk assessments are typically not required for substances with acute NOEL (No Observed Effect Level) values above 500 mg/kg-bw/day or for compounds which have no associated mortalities below 1000 mg/kg-bw in single dose studies (16). Nonetheless, to place the PAT protein exposure estimate in context, a comparison of the exposure information to the lower limit NOAEL of >5000 mg/kg has been made to provide Margins of Exposure (MOE) in Table 2 for PAT protein where:

$$\text{MOE} = \frac{\text{NOAEL}}{\text{Exposure}} \quad \text{Equation 1}$$

The larger the MOE value, the less likelihood there is for adverse effects, because the exposure is well below the established NOAEL threshold. The calculated MOE values of Table 2 for PAT protein in soybean are extremely large (>400000), indicating no concern for adverse effects from protein in soybeans based on the available safety threshold information.

5.3. EU PRIMo Model

For potential dietary exposures specific to EU diets, the European Food Safety Authority (EFSA) PRIMo, version 2 was reviewed for soybean consumption (17). The PRIMo model is typically used to conduct dietary assessment for assessing the maximum residue limits (MRLs) for active ingredients in crop protection products. For this acute assessment, the portion of the PRIMo

model which calculates the International Estimated Short Term Intake (IESTI) for EU children and adults was used. In order to employ the spreadsheet, a value of 50 mg/kg-bw was estimated input into the ARfD cell based on a NOAEL of >5000 mg/kg-bw and an UF of 100; see Table 4 for a screen shot of the PRIMo Input assumptions for endpoints. The observed protein expression value of 2.12 ng/mg = 2.12 mg/kg was input as an STMR for soybean in the dietary model and the resulting detailed calculations for children and adult consumption are copied in Table 5.

For adults, the critical food intake for soybean is from the “PL diet” (Poland) at 0.61 g/kg-bw resulting in an estimated exposure of 0.00129 mg/kg-bw. For children, the “DE diet” (Germany) indicates a maximum of 2.31 g/kg-bw and a protein exposure therefore of 0.00490 mg/kg-bw. Using Equation 1, the resulting EU STI MOEs are also presented Table 2. The resulting MOE values are all greater than 1000000, indicating there is negligible concern for dietary risk to PAT protein in soybeans based on the available safety threshold information.

5.4. Taiwan Dietary Assessment

Dietary consumption data for Taiwan is available on a national website (18). Reported 2008 consumption for Taiwan is found in the “10. Food Balance Sheet” pdf. Soybeans are categorized under 4(1) as a subset of Pulses and oilseeds. The reported daily consumption of soybeans is 48.38 g. When this number is divided by the typical body weight of 60 g, the daily consumption becomes 0.806 g soybean/kg-bw. Per Table 2, when the Taiwan consumption information is coupled to the PAT average field expression level of 2.12 ng/mg tissue for soybean grain, the potential exposure to PAT protein *via* soybean is estimated as:

- 0.00171 mg protein/kg-bw/day, for general population (i.e. adults)

And the resulting MOE is >2900000 indicating no concern for dietary risk to PAT protein in soybeans.

5.5. US DEEM Assessment

For a US assessment, the MOE values were calculated using the DEEM dietary exposure model program (DEEM-FCID version 2.16, Exponent, 2007). In this exercise, the residue value of 2.12 ppm was conservatively applied to all commodities listed in DEEM that are associated with soybean, per Table 6. This is a conservative approach for processed flour and soymilk. No residues were assigned to oil. Using this residue file as input, MOE values were calculated against the acute NOAEL of >5000 mg/kg for several subpopulations with the results listed in Appendix A. The output MOE values at the 97.5th percentile have been included in the Summary Table 2 for comparison purposes. The values are congruent with the values derived from use of the 97.5th WHO data, but DEEM allows for a deeper segmentation of the populations and also for several cuts of percentile. At the 97.5th percentile level, the WHO predicted exposure values for all relevant soy commodities is an order of magnitude higher those predicted from the DEEM 97.5th percentile for the general population. This is understood, because the WHO data represents global worse case consumption and is not based on US consumption. In this DEEM estimation, the highest exposed subpopulation is the non-nursing infant (<1 yr old) with an estimated exposure of 0.00737 mg/kg-bw and an MOE value of >678235 at the 97.5th percentile. MOE values >100 are typically considered acceptable. All relevant MOE values are extremely large, indicating there is no concern.

6. LIVESTOCK DIETARY EXPOSURE ASSESSMENT

A dietary exposure estimate for novel feed in livestock diets based on traditional use of the unmodified feeds is provided here by coupling field expression information for PAT protein from DAS-44406-6 soybeans with livestock dietary consumption assumptions for soybean animal feeds. Livestock assessments are conducted in this report using US and EU reference animals to calculate animal dietary burdens in representative livestock. National animal consumption models from other countries could change the numeric calculations slightly, but would not change the overall conclusions. In addition, the relevance of the exposure estimate is placed into context, based on the mammalian toxicity information.

The presence of PAT protein in general soybeans is not anticipated to have impact for feed ration formulation, because nutrient composition analyses have shown that DAS-68416-4 soybean (which also contains the PAT protein) is substantially equivalent to conventional soybean (19), per the general OECD and ILSI guidance (14, 20). Also, a study of the nutritional and metabolic value of feed containing PAT soybeans relative to commercially available soybeans was conducted with broiler chickens (21) and although the study title highlights the AAD-12 protein, the PAT gene was also present in the DAS 68416-4 soybean tested. The study results indicate feed prepared with PAT-containing DAS-68416-4 soybean meal was nutritionally similar to feed prepared with non-transgenic near isogenic soybean meal (control).

6.1. Animal Feed Exposure Assumptions for Soybean

In this assessment for PAT soybean, grain, hulls and meal (plus aspirated grain and/or forage when applicable) are employed as animal feeds. Because soybean forage is an uncommon animal feed in the US, the US livestock assessment presents calculations with and without soybean forage as representative and worse-case assessments. With no plans to cultivate PAT soybean in the EU, soybean forage is not considered as a viable animal feed in the EU assessment. An estimate for the slight concentration of protein from raw soybean seed to soybean meal is found in Table 1 along with a projected reduction of protein in the hulls and concentration for aspirated grain. The livestock diets are built based on the traditional use of the unmodified counterpart per regulatory procedures and estimates of dietary exposure are conservative (and protective) in that they have assumed 100% replacement of the unmodified counterpart.

6.2. EU Animal Dietary Burdens

The EU livestock assessment is constructed based on the traditional use of the unmodified counterpart per EU diets listed in the Annex 4 Harmonized Feedstuffs Table of the 2009 OECD Guidance for residue studies (10). It is noted that animal diets across the EU may vary by region, but for this assessment maximum potential exposure to soybean for EU reference animals was assumed. The resulting intake dietary burden for animal feeds is totaled in Table 7 for the

four typical representative animals of Beef and Dairy Cattle, Poultry (broiler) and Swine (finishing). Use of the reference animal weight and feed consumption allows for a translation to daily dose by animal and the final results are found in Table 8.

The highest potentially exposed EU reference animal is poultry with an estimate of 0.11 mg PAT/kg-bw. Other estimates are ≤ 0.4 mg/kg-bw for beef, dairy cattle and swine. When these estimated intakes are compared to the mammalian NOAEL of >5000 mg/kg-bw, there is an adequate margin of safety for livestock (See Table 8). Variations in calculated livestock feed diets or reference animals could result in slight changes in the calculated values, but would not alter the conclusion regarding the large margin of safety afforded livestock animals for PAT protein in soybean.

6.3. US Animal Dietary Burdens

A US livestock assessment is presented here based on the Maximum Reasonably Balanced Diet (MRBD) animal burden procedures of US EPA (22). This US assessment includes several soybean commodity forms as potential animal feeds: seed, meal, hulls and aspirated grain fractions, and optional forage. The US MRBD guidance is used to construct a maximum soybean feed contribution for swine, poultry and cattle based on the average value of 2.12 ng/mg (or ppm) for PAT protein in DAS-44406-6 soybean seed. This value for soybean seed has also been used to estimate exposure to soybean feeds for which there was no direct expression measurement. The value for the seed is substituted for the meal and hull feeds and a 20X concentration of the seed residue has been assumed for potential aspirated grain exposure (Table 1). Because meal and seed are both protein concentrates, they are not simultaneously used in a US feed diet. When forage was included, a value of 9.35 ng/mg was used as the Highest Residue as the animal feed; forage is only an input for dairy cattle in the US model.

US EPA currently assumes the following for reference animals for dietary assessments based on animals in finishing or feedlots (22):

Beef: Finishing or feedlot beef (body weight at slaughter, 1200 lb or **544 kg**, daily feed intake of 20 lb or **9 kg** dry matter feed). Feedlot rations in the finishing stage consist of high

amounts of grain or grain supplements (80% CC), forages (15% R), and protein sources (5% PC) in last 120 to 180 days (4 to 6 months) before slaughter at **16 to 18 months of age**.

Dairy: Mature lactating cow (body weight, 1350 lb or **612 kg**, daily feed intake of 53 lb or **24 kg dry matter feed**, and producing average of 90 lb of milk a day). Feed rations include forages (45% R), grain or grain supplements (45% CC), and protein source (10% PC). Dairy cows generally calve at **24 to 28 months of age**. The usual length of lactation is 250 to 450 days, with a 305 day lactation being the standard. Dairy cows are usually slaughtered after 2 or 3 calves. The average productive life span of the mature lactating dairy cow is 3 to 4 years.

Poultry: Chicken: Laying hen (body weight, 4.2 lb or **1.9 kg**, average daily intake of 52 grams or **0.052 kg of feed**). Laying hens are usually slaughtered **after 18 months**. A daily ration includes grain or grain supplement (75% CC) and protein source (25% PC). Alternate poultry would be frying and rotisserie chickens weighing 3 to 4 lb, with an average life span of 38 to 42 days. The broiler diet contains 85% CC and 15% PC.

Swine: Finishing or Market hog (body weight, up to 250 lb or **113 kg**, average daily intake of 6.8 lb or **3.1 kg of feed**). Hogs are slaughtered in **5 to 8 months**. In general, daily ration consists of high grain or grain supplement (85% CC) and oilseed meal (15% PC).

The resulting US intake dietary burden for animal feeds is totaled in Table 7. Because only soybean feeds are considered, the nutritional balance of the diets is assumed to be comprised of unmodified feeds. Use of the reference animal weight and feed consumption allows for a translation to daily dose by animal and results are found in Table 8.

For the typical US diet, the highest exposed US animal is the beef cow with 0.05 mg PAT/kg-bw estimate. Lower estimates for dairy cattle, swine and poultry were ≤ 0.02 mg/kg-bw (Table 8). For the worse-case diet with forage, the highest exposed US animal is the dairy cow with a 0.23 mg PAT/kg-bw estimate. When any of these values are compared to the acute mammalian NOAEL of >5000 mg/kg-bw, there is an adequate margin of safety for livestock because typical MOEs are >100000 and the worse case MOE is >21000 . Variations in livestock feed diets

elsewhere in the world could result in slight changes in the calculated values, but these global variations in diet are not expected to alter the conclusion regarding the large margin of safety afforded livestock animals for PAT protein in soybean.

7. CONCLUSIONS

Results of the overall safety assessment of the PAT protein indicate that it is unlikely to cause adverse effects in humans or animals. PAT protein poses a very low risk of toxicity to mammals with a short term NOAEL value of >5000 mg/kg-bw. This safety threshold is established based on the highest dose achieved in the study. The PAT protein is a well-understood protein which provides tolerance to glufosinate-based herbicides and has a history of approvals and safe use without reports of adverse effects to humans.

Human consumption for soybean for representative EU, US, Taiwan and a high-end global diet (Japan) is presented and the projected dietary exposures to the PAT protein are compared to the safety thresholds established *via* the toxicity testing. Very large Margins of Exposure are demonstrated for humans: greater than 400000-fold or greater. Livestock dietary assessments have conservatively predicted the maximum exposed EU reference animal would be poultry for EU livestock model, the beef cow for the typical US model without forage, or the dairy cow for the worse-case exposure with forage. Large MOEs are predicted for livestock animals as well. The assessments presented here are known to be conservative and an over prediction of actual dietary exposure to the protein. Actual exposure and impact will be lower because: 1) there may be protein degradation during transport and storage, 2) soybean containing PAT will be mixed with non-PAT soybean and 3) for humans, consumption of soybean products is often in food forms which are cooked and heat is known to denature this protein.

8. RETENTION OF RECORDS AND ACKNOWLEDGEMENT

The original study report, supporting calculations and key references will be archived in the Dow AgroSciences R&D archive in Indianapolis, IN upon issuing the final report. Thank you to Dr. N. Stagg of Dow AgroSciences for her valued teamwork.

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Table 1. Summary of Mean Concentration Levels of PAT Protein Applicable for Human and Animal Exposure^a

	Treatment	Mean ^b PAT (ng/mg)	Std. Dev.	Observed Range for test spray by site PAT (ng/mg)	~HAFT ^c PAT (ng/mg)
Soybean Grain	DAS-44406-6 Unsprayed	2.12	0.49	1.60-2.97	
	DAS-44406-6 + 2,4-D	2.13	0.36	1.61-2.71	
	DAS-44406-6 + glufosinate	2.11	0.44	1.51-2.82	
	DAS-44406-6 + glyphosate	2.15	0.39	1.59-2.73	
	DAS-44406-6 + glyphosate, glufosinate and 2,4-D	2.11	0.38	1.43-2.70	
	Overall mean	2.12			2.97
Soybean Forage	DAS-44406-6 Unsprayed	6.19	1.79	3.95- 9.35	
	DAS-44406-6 + 2,4-D	5.9	1.4	4.11-8.44	
	DAS-44406-6 + glufosinate	6.72	1.67	3.94-8.76	
	DAS-44406-6 + glyphosate	6.48	1.87	4.56-9.33	
	DAS-44406-6 + glyphosate, glufosinate and 2,4-D	6.33	1.54	4.51-8.41	
	Overall mean	6.32			9.35
	Animal Feed Estimates	~STMR			
Soybean meal	Estimated as 1.1X	2.33			
Soybean hull	Estimated as 0.28X	0.59			
Aspirated Grain	Estimated as 20X	42.4			

^a Bolded values used as input in human and livestock dietary calculations, when appropriate.

^b PAT ng/mg Tissue Dry Weight from measured protein expression in field trials

^c Maximum reported per EU guidance, for grain it is improbable that humans or animals are exposed to maximums due to blending of stored grain, ~HAFT is chosen as maximum from the range of 50 values across testspray/site results of Appendix D in Ref (1).

Table 2. Summary of Human Dietary Margins of Exposure for PAT Protein in Soybean Based on Various Consumption Model for Short Term Exposure

Time frame		Food Intake ^b g/kg-bw	Exposure ^a (mg PAT /kg-bw/day)	NOAEL (mg/kg-bw)	MOE
Short Term					
	WHO 97.5th (Japan)^a				
	General Population	3.03	0.0064	>5000	>778380
	Children <6 year	5.55	0.0118	>5000	>424953
	EU PRIMO				
	Adult	0.61	0.0013	>5000	>3866378
	Child	2.31	0.0049	>5000	>1020922
	Taiwan				
	Adult (60 kg)	0.81	0.0017	>5000	>2911717
	US DEEM 97.5th			>5000	
	U.S. Population	<i>0.1302</i>	0.000276	>5000	>1000000
	All infants	<i>3.1816</i>	0.006745	>5000	>741281
	Nursing infants	<i>1.6401</i>	0.003477	>5000	>1000000
	Non-nursing infants	<i>3.4774</i>	0.007372	>5000	>678235
	Children 1-2 yrs	<i>0.4406</i>	0.000934	>5000	>1000000
	Children 3-5 yrs	<i>0.2344</i>	0.000497	>5000	>1000000
	Children 6-12 yrs	<i>0.1689</i>	0.000358	>5000	>1000000
	Youth 13-19 yrs	<i>0.0967</i>	0.000205	>5000	>1000000

^a Based on WHO 97.5th percentile consumption of soybean under commodity **VD 541**.

^b Consumption for DEEM has been back-calculated based on the exposure estimate results and noted in italics.

Table 3. Estimates of Acute Soybean Consumption from the GEMS/Food Highest 97.5th Percentile “Eater-Only” Worldwide

Commodity	Country with Reported Maximum	Consumption ^a (g/kg/day)	
		General Population	Children ≤6 years
VP 541 Soya bean (immature seeds)	Thailand	2.41	3.86
<i>VD 541 Soya bean (dry)</i>	<i>Japan</i>	<i>3.03</i>	<i>5.55</i>
OR 541 Soya bean oil ^b , refined	USA	1.51	2.36

^a Total acute consumption across these entities cannot be calculated because, it is not appropriate to add 97.5th percentile values for individual commodities survey results from different countries; REF (15). For completeness only information on soybean oil consumption information is provided, however it is understood that oil will not contain appreciable amounts of protein.

Table 4. Copy of PRIMO INPUT File Assumptions for PAT Protein Endpoints

PAT			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):		ARfD (mg/kg bw):	50
Source of ADI:		Source of ARfD:	5000 NOEL/100
Year of evaluation:		Year of evaluation:	

Table 5. Summary of PRIMO INPUT File Assumptions for PAT Protein Consumption

Acute_overview_ Children									
Examples of individual products within the groups to which the MRLs apply	pTMRL input (mg/kg)	individual pTMRL	Maximum or Critical food intake reported (g/kg bw)	Percentile	MS	body weight (kg)	Large portion g/person	IESTI 1 (calculation with VF 5, 7 and 10) µg/kg bw/day	% ARfD (alternatively % ADI)
Soya bean	2.12	2.12	2.31	97.5	DE	16.15	37.30	0.00049	0.0
Acute_overview_ Adults									
Soya bean	2.12	2.12	0.61		PL	62.80	38.60	0.0013	0.0

Table 6. DEEM Input File for Soybean Exposure to PAT Protein

Cheryl Cleveland Ver. 2.16
DEEM-FCID Acute analysis for PAT PROTEIN IN SOYBEAN
Residue file name: C:\Users\U099010\Documents\DEEM residue files - ata\PATsoybean.R98
Analysis Date 06-13-2011 Residue file dated: 06-13-2011/17:40:00/118
Reference dose (NOEL) = 5000 mg/kg bw/day

EPA Code	Crop Grp	Food Name	Def Res (ppm)	Adj. Factors #1	Adj. Factors #2
06003470	6	Soybean, seed	2.120	1.0	1.0
06003480	6	Soybean, flour	2.120	1.0	1.0
06003481	6	Soybean, flour-babyfood	2.120	1.0	1.0
06003490	6	Soybean, soy milk	2.120	1.0	1.0
06003491	6	Soybean, soy milk-babyfood	2.120	1.0	1.0

Table 7. EU and US Intake Animal Dietary Burdens for Livestock from Soybean

Feedstuff	Type	Dry Matter (%)	Dietary Contribution (%)				PAT (ppm)	Animal Dietary Burden (ppm)			
			Beef	Dairy	Poultry	Pig		Beef	Dairy	Poultry	Pig
EU											
Soybean seed		89	10	10	20	20	2.12	0.24	0.24	0.48	0.48
Soybean meal		92	20	25	40	30	2.33	0.51	0.63	1.01	0.76
Soybean hulls		90	10	10	10	0	0.59	0.07	0.07	0.07	0.00
							Total	0.81	0.94	1.56	1.24
US (typical)											
Soybean Hulls	R	90	15	20	Nu	Nu	0.59	0.1	0.132	-	-
Aspirated grain	CC	85	5	Nu	Nu	Nu	42.4	2.49	-	-	-
Soybean seed	PC	89	<i>Meal used</i>	15	<i>Meal used</i>	<i>Meal used</i>	2.33		0.36	-	-
Soybean meal*	PC	NA	5	<i>Seed used</i>	25	15	29.4	0.13	-	0.58	0.35
							Total	2.72	0.49	0.58	0.35
US (worse case)											
Soybean Hulls	R	90	15	20	Nu	Nu	0.59	0.1	0.132	-	-
Aspirated grain	CC	85	5	Nu	Nu	Nu	42.4	2.49	-	-	-
Soybean seed	PC	89	<i>Meal used</i>	15	<i>Meal used</i>	<i>Meal used</i>	2.33		0.36	-	-
Soybean meal*	PC	NA	5	<i>Seed used</i>	25	15	29.4	0.13	-	0.58	0.35
Soybean Forage	R	35	Nu	20	Nu	Nu	9.35	-	5.34	-	-
							Total	2.72	5.83	0.58	0.35

Note Input for Feedstuff values are found in Table 1.

* estimate based on measured value for seed

**based on theoretical estimate of 20X the value in soybean seed

Table 8. EU and US Livestock Daily Dose Estimates of PAT Protein from Soybean Animal Feeds

		Cattle		Swine	Poultry
		Beef	Dairy	Finishing	Broiler
EUASSESSMENT					
	Body weight (kg)	500	650	100	1.7
	Daily Maximum Feed [kg Dry Matter (DM)]	12	25	3	0.12
	Maximum PAT intake (mg/kg feed)	0.81	0.94	1.24	1.56
	Maximum intake (mg/kg bw)	0.02	0.04	0.04	0.11
	MOE vs Mammalian NOAEL	257113	138754	134823	45552
USASSESSMENT					
(Without Forage)	Body weight (kg)	544	612	113	1.9
	Daily Maximum Feed [kg Dry Matter (DM)]	9	24	3.1	0.052
	Maximum PAT intake (mg/kg feed)	2.72	0.49	0.35	0.58
	Maximum intake (mg/kg bw)	0.05	0.02	0.01	0.02
	MOE vs Mammalian NOAEL	111111	260204	520737	314987
USASSESSMENT					
(With Forage)	Body weight (kg)	544	612	113	1.9
	Daily Maximum Feed [kg Dry Matter (DM)]	9	24	3.1	0.052
	Maximum PAT intake (mg/kg feed)	2.72	5.83	0.35	0.58
	Maximum intake (mg/kg bw)	0.05	0.23	0.01	0.02
	MOE vs Mammalian NOAEL	111111	21870	520737	314987

10. Appendix A— DEEM Output Files for Soybean Acute Exposure to PAT Protein

DEEM Output File for Soybean Acute Exposure to PAT Protein

Cheryl Cleveland Ver. 2.16
 DEEM-FCID ACUTE Analysis for PAT PROTEIN IN SOYBEAN (1994-98 data)
 Residue file: PAT 44406_6 soybean.R98 Adjustment factor #2 NOT used.
 Analysis Date: 06-16-2011/10:36:01 Residue file dated: 06-16-2011/10:34:55/118
 NOEL (Acute) = 5000. mg/kg body-wt/day
 Run Comment: ""

Summary calculations--per capita:

	95th Percentile		97.5th Percentile		99th Percentile	
	Exposure	MOE	Exposure	MOE	Exposure	MOE
U.S. Population:	0.000151	>1000000	0.000276	>1000000	0.001015	>1000000
All infants:	0.005129	974776	0.006745	741281	0.008750	571422
Nursing infants (<1 yr old):	0.000843	>1000000	0.003477	>1000000	0.005186	964198
Non-nursing infants (<1 yr old):	0.005905	846801	0.007372	678235	0.009174	545016
Children 1-2 yrs:	0.000449	>1000000	0.000934	>1000000	0.003737	>1000000
Children 3-5 yrs:	0.000300	>1000000	0.000497	>1000000	0.000952	>1000000
Children 6-12 yrs:	0.000191	>1000000	0.000358	>1000000	0.000712	>1000000
Youth 13-19 yrs:	0.000133	>1000000	0.000205	>1000000	0.000353	>1000000