

SUMMARY

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STUDY TITLE

Global Dietary and Livestock Assessment of AAD-12 Protein for DAS Soybean Cultivar Based on Event DAS-44406-6

DATA REQUIREMENTS

EFSA GMO guideline, 21 CFR 192.25

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STUDY COMPLETION DATE

28 JUN 2011

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110598

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SUMMARY

This report presents an assessment of the AAD-12 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.

Expression levels of the AAD-12 protein in plant tissues of DAS-44406-6 soybean across environments demonstrate a low dietary exposure risk to humans and animals. The AAD-12 protein has demonstrated low mammalian toxicity. In addition, AAD-12 does not share significant sequence homology with known toxic proteins. Results of this safety assessment indicate that the AAD-12 protein represents a negligible risk to human and animal health.

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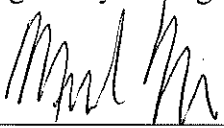
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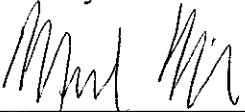
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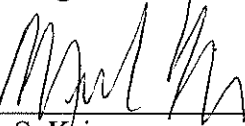
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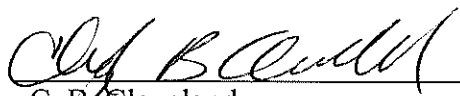
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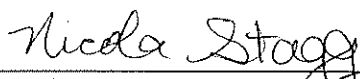
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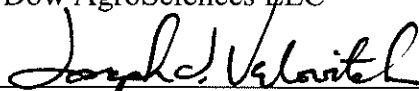
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1. ABSTRACT

This report presents an assessment of the AAD-12 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.

Expression levels of the AAD-12 protein in plant tissues of DAS-44406-6 soybean across environments demonstrate a low dietary exposure risk to humans and animals. The AAD-12 protein does not share significant sequence homology with known toxic proteins, is readily degraded in simulated gastric fluid containing pepsin, and is inactivated after exposure to thermal processing. In addition, the AAD-12 protein was not acutely toxic at the limit dose of 2000 mg/kg-bw. Results of the overall safety assessment demonstrate that the AAD-12 protein expressed in DAS-44406-6 represents negligible risk to human and animal health.

2. BACKGROUND

Dow AgroSciences has produced a transgenic 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 AAD-12 protein for DAS-44406-6 soybean. This report covers several global assessments with a focus on a US,

EU, Taiwan and a high-end global dietary assessment. The 2,4-D herbicide-tolerance trait is commonly known as AAD-12 and is accomplished *via* soybean plants which have been genetically modified to express the aryloxyalkanoate dioxygenase (AAD-12) protein. The *aad-12* gene, which expresses the AAD-12 protein, was derived from *Delftia acidovorans*, a common soil bacterium. *D. acidovorans* is found in several environmental matrices and also has a history of safe use in the food processing industry. Therefore, animals and humans are regularly exposed without adverse consequences to the *D. acidovorans* organism and its components.

3. MAMMALIAN TOXICITY ASSESSMENT

An acute mammalian toxicology study with a single high-dose in mice is summarized here and then used to place potential human or livestock exposure in context. Also, the AAD-12 protein does not share significant sequence homology with known toxic proteins, is readily degraded in simulated gastric fluid containing pepsin, and is inactivated after exposure to thermal processing.

3.1. Mammalian Acute Toxicity

An acute oral toxicity study with AAD-12 protein was conducted in mice at a level of 2000 mg AAD-12 protein/kg-bw (2). 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 AAD-12 in male and female mice was greater than 2000 mg/kg-bw. No mortality was observed, and there were no observable adverse effects with the AAD-12 treated animals, therefore the **NOAEL** (No Observed Adverse Effect Level) **is also considered to be >2000 mg/ kg-bw**. AAD-12 protein has a very low acute toxicity potential.

3.2. Lack of Homology to Known Toxins

For the safety assessment of transgenic crops, evaluation of the protein sequence relative to known toxins was conducted. The toxin search did not identify any matches with safety concerns that might arise from the AAD-12 protein expressed in plants.

The AAD-12 protein does not share meaningful amino acid sequence similarities with known toxins (3). Amino acid homologies were evaluated using a global sequence similarity search against the GenBank non-redundant protein dataset (updated to February 18, 2011). The AAD-12 protein sequence was prepared in FASTA format for use with BLASTp search programs; details of search parameters are contained within Reference 3. All sequence alignments returned by the search were associated with dioxygenase, or similar proteins and none of them were identified any significant sequence similarity to a known toxic protein that is harmful to humans or animals.

3.3. AAD-12 Protein under Heating Conditions

The thermal stability of the AAD-12 protein has been evaluated by heating buffered protein solutions for 30 min at 50, 70 and 95 °C (4). The temperatures of these heat treatments approach (but are conservatively on the lower end of) common temperatures used in food processing research (*e.g.* 50-150 °C (5) and in soybean industrial processing (*e.g.* dry extruder temperatures range from 110 to 170 °C (6)). The AAD-12 protein under heating was assessed for protein immunoreactivity, enzymatic activity and molecular weight. Immunoreactivity was determined using a polyclonal antibody sandwich ELISA specific for the AAD-12 protein; under all test conditions (50 to 95 °C) the protein lost $\geq 99.3\%$ of the immunoreactivity. Enzymatic activity was measured using a colorimetric assay which measures the conversion of dichlorophenoxyacetate to 2, 4-dichlorophenol and glyoxylate, a loss of 100% of enzymatic activity was observed. This result correlates well with the ELISA assay as denatured proteins are not expected to contain enzymatic activity. The stability of the primary protein structure was determined by Coomassie staining of the protein by SDS-PAGE; the molecular mass was essentially unchanged (with the exception of the formation of a minor amount of multimeric protein aggregates). Therefore, it can be concluded that the AAD-12 protein is functionally unstable when heated and that industrial processing of soybean grain will significantly degrade the tertiary structure of the AAD-12 protein, reduce its immunoreactivity and eliminate its enzymatic activity.

4. EXPOSURE INFORMATION FOR EXPRESSED PROTEIN

Human dietary assessments require coupling of field expression information for AAD-12 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 grain and feed exposure levels for use later in the human and livestock assessments.

4.1. Potential Exposure to AAD-12 Protein *via* Soybean

The field expression of AAD-12 protein in DAS-44406-6 soybean has been measured using an AAD-12 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 AAD-12 protein using the Dow AgroSciences validated methods GRM 08.04 (7) using an enzyme-linked immunosorbent assay (ELISA) kit purchased from Acadia BioScience, LLC. The limit of detection (LOD) and limit of quantitation (LOQ) for AAD-12 in soybean tissues were determined during the method validation as 0.5 and 1.0 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 AAD-12 protein was **26.73 ng AAD-12 protein/mg tissue on a dry weight basis** [see Table 2 of Ref (1)]. The full range of applicable values was 9.29 to 42.05 ng/mg tissue [based on review of the 50 values for site/test spray in Appendix E – Table 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 (8). Hence, the maximum level is reported per the EU guidance, but is not used further in the dietary assessment. Expression values for AAD-12 were similar for all treatments irrespective of the herbicide regime.

Expression information is available for soybean forage with a range of applicable values from 45 to 114.38 ng/mg tissue [range of the 50 values for site/test spray in Appendix E – Table 14 thru 18 of Ref (1)]. For forage, although an average expression of 73.3 ng/mg is available, a highest residue (HR) for soybean forage is recommended in the OECD guidance (8) 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 114.38 ng/mg is appropriate and used for AAD-12 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 and additionally soybean forage has been disallowed on the US 2,4-D label for use with AAD-12 soybeans. 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 AAD-12 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 (9, 10). Hence a concentration value of 1.1X is used below for soybean meal. Soybean hulls contain ~ 12% crude protein (11), 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.

Use of these values are protective estimates for exposure to the AAD-12 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 AAD-12 will be mixed with non-

AAD-12 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 (4). It is also known that soybean oil contain very little protein (12).

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 greater than 10000 (Table 2), indicating negligible risk for adverse effects from dietary exposure to AAD-12 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 (13). Table 3 includes 97.5th percentile values for all possible commodities associated with soybean. For AAD-12 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 AAD-12 average field expression level of 26.73 ng/mg tissue for soybean grain, the potential acute exposure to AAD-12 protein *via* soybean is estimated as:

- 0.081 mg protein/kg-bw/day, for general population (i.e. adults)
- 0.148 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 (14). Nonetheless, to place the AAD-12 protein exposure estimate in context, a comparison of the exposure information to the lower limit NOAEL has been made to provide Margins of Exposure (MOE) in Table 2 for AAD-12 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 AAD-12 protein in soybean are extremely large (>10000), 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 (15). 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 20 mg/kg-bw was used as estimated input into the ARfD cell based on a NOAEL of >2000 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 26.73 ng/mg = 26.73 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 0.0163 mg/kg-bw. For children, the “DE diet” (Germany) indicates a maximum of 2.31 g/kg-bw and a protein exposure therefore of 0.0617 mg/kg-bw. Using Equation 1, the resulting EU STI MOEs are also presented Table 2. The resulting MOE values are all greater than 10000, indicating there is negligible concern for dietary risk to AAD-12 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 (16). 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 AAD-12 average field expression level of 26.73 ng/mg tissue for soybean grain, the potential exposure to AAD-12 protein *via* soybean is estimated as:

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

And the resulting MOE is >90000 indicating no concern for dietary risk to AAD-12 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 26.73 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 2000 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. As observed in Table 2, at the 97.5th percentile level, the WHO predicted exposure values for all relevant soy commodities are much greater than those predicted from the DEEM 97.5th percentile for the *VD 541 Soya bean* exposure 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 most exposed subpopulation is the non-nursing infants (<1 yr old) with an estimated exposure of 0.092951 mg/kg-bw and an MOE value of 21516 at the 97.5th percentile. MOE values >100 are typically considered acceptable. All relevant MOE values are greater than 10000, 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 AAD-12 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 AAD-12 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 AAD-12 protein) is substantially equivalent to conventional soybean (17), per the general OECD and ILSI guidance (12, 18). In addition, results from a broiler study indicated that when feed was prepared with AAD-12 protein from DAS-68416-4 soybean meal, it was nutritionally similar to feed prepared with non-transgenic near isogenic soybean meal (non-AAD-12 control) for the production of broiler chickens (19).

6.1. Animal Feed Exposure Assumptions for Soybean

In this assessment for AAD-12 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 AAD-12 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 (8). 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 1.38 mg AAD-12/kg-bw. Other estimates are 0.25, 0.45, 0.47 mg/kg-bw for beef, dairy cattle and swine, respectively. When these estimated intakes are compared to the mammalian NOAEL of >2000 mg/kg-bw, there is an adequate margin of safety for livestock (See Table 8). Variations in calculated livestock feed diets or references 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 AAD-12 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 (20). 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 26.73 ng/mg (or ppm) for AAD-12 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 11.48 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 (20):

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 0.57 mg AAD-12/kg-bw estimate. Lower estimates for dairy cattle, swine and poultry were 0.24, 0.12 and 0.20 mg/kg-bw, respectively (Table 8). For the worse-case diet with forage, the highest exposed US animal is the dairy cow with a 2.81 mg AAD-12/kg-bw estimate. When any of these values are compared to the acute mammalian NOAEL of >2000 mg/kg-bw, there is an adequate margin of safety for livestock; typical MOEs are >3500 and the worse case MOE is >700. 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 AAD-12 protein in soybean.

7. CONCLUSIONS

Results of the overall safety assessment of the AAD-12 protein indicate that it is unlikely to cause adverse effects in humans or animals. AAD-12 protein poses a low risk of toxicity to

mammals with a short term NOAEL value of >2000 mg/kg-bw. This safety threshold is established based on the highest dose achieved in the study. In addition, the homology search to known toxins did not identify any matches with safety concerns that might arise from the AAD-12 protein expressed in plants.

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 AAD-12 protein are compared to the safety thresholds established *via* the toxicity testing. Large Margins of Exposure are demonstrated for humans: greater than 10000-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. Adequate MOEs are predicted for livestock animals. 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 AAD-12 will be mixed with non-AAD-12 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. Mark Krieger of Dow AgroSciences for his continued insight.

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Table 1. Summary of Mean Concentration Levels of AAD-12 Protein Applicable for Human and Animal Exposure^a

	Treatment	Mean ^b AAD-12 (ng/mg)	Std. Dev.	Observed Range for test spray by site AAD-12 (ng/mg)	~HAFT ^c AAD-12 (ng/mg)
Soybean Grain	DAS-44406-6 Unsprayed	27.37	9.7	9.29-41.75	
	DAS-44406-6 + 2,4-D	27.34	10.35	9.72-40.5	
	DAS-44406-6 + glufosinate	27.34	10.02	11.32-42.05	
	DAS-44406-6 + glyphosate	25.77	6.79	14.29-36.85	
	DAS-44406-6 + glyphosate, glufosinate and 2,4-D	25.83	6.51	17.35-35.55	
	Overall mean	26.73			42.05
Soybean Forage	DAS-44406-6 Unsprayed	73.47	20.77	46.75-112.63	
	DAS-44406-6 + 2,4-D	72.53	22.59	45.0- 114.38	
	DAS-44406-6 + glufosinate	73.75	20.39	45.63-101.88	
	DAS-44406-6 + glyphosate	76.04	19.36	47.13-102.38	
	DAS-44406-6 + glyphosate, glufosinate and 2,4-D	70.73	21.88	42.25-101.5	
	Overall mean	73.3			114.38
	Animal Feed Estimates	~STMR			
Soybean meal	Estimated as 1.1X	29.4			
Soybean hull	Estimated as 0.28X	7.48			
Aspirated Grain	Estimated as 20X	534.6			

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

^b AAD-12 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 E in Ref (1).

Table 2. Summary of Human Dietary Margins of Exposure for AAD-12 Protein in Soybean Based on Various Consumption Model for Short Term and Repeat Exposure

Time frame		Food Intake ^b g/kg-bw	Exposure (mg AAD-12 /kg-bw/day)	NOAEL (mg/kg-bw)	MOE
Short Term					
	WHO 97.5th (Japan)^a				
	General Population	3.03	0.081	>2000	>24694
	Children <6 year	5.55	0.148	>2000	>13481
	EU PRIMO				
	Adult	0.61	0.0163	>2000	>122699
	Child	2.31	0.0617	>2000	>32415
	Taiwan				
	Adult (60 kg)	0.81	0.0217	>2000	>92373
	US DEEM 97.5th				
	U.S. Population	<i>0.1302</i>	0.003479	>2000	>574854
	All infants	<i>3.1816</i>	0.085045	>2000	>23516
	Nursing infants	<i>1.6401</i>	0.043840	>2000	>45620
	Non-nursing infants	<i>3.4774</i>	0.092951	>2000	>21516
	Children 1-2 yrs	<i>0.4405</i>	0.011775	>2000	>169850
	Children 3-5 yrs	<i>0.2342</i>	0.006261	>2000	>319437
	Children 6-12 yrs	<i>0.1689</i>	0.004516	>2000	>442906
	Youth 13-19 yrs	<i>0.0969</i>	0.002591	>2000	>771995

^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 (13). 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 AAD-12 Protein Endpoints

AAD-12			
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):	20
Source of ADI:		Source of ARfD:	2000 NOEL/100
Year of evaluation:		Year of evaluation:	

Table 5. Summary of PRIMO INPUT File Assumptions for AAD-12 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	26.73	26.73	2.31	97.5	DE	16.15	37.30	0.0617	0.3
Acute_overview_ Adults									
Soya bean	26.73	26.73	0.61		PL	62.80	38.60	0.0164	0.1

Table 6. DEEM Input File for Soybean Exposure to AAD-12 protein

Cheryl Cleveland

Ver. 2.16

DEEM-FCID Acute analysis for AAD12 PROTEIN IN SOYBEAN

Residue file name: C:\Users\U099010\Documents\DEEM residue files - ata\AAD12soybean.R98

Analysis Date 06-13-2011

Residue file dated: 06-13-2011/17:20:13/118

Reference dose (NOEL) = 2000 mg/kg bw/day

EPA Code	Crop Grp	Food Name	Def Res (ppm)	Adj. Factors #1	Adj. Factors #2
06003470	6	Soybean, seed	26.730	1.0	1.0
06003480	6	Soybean, flour	26.730	1.0	1.0
06003481	6	Soybean, flour-babyfood	26.730	1.0	1.0
06003490	6	Soybean, soy milk	26.730	1.0	1.0
06003491	6	Soybean, soy milk-babyfood	26.730	1.0	1.0

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

Feedstuff	Type	Dry Matter (%)	Dietary Contribution (%)				AAD-12 (ppm)	Animal Dietary Burden (ppm)			
			Beef	Dairy	Poultry	Pig		Beef	Dairy	Poultry	Pig
EU											
Soybean seed		89	10	10	20	20	26.73	3.00	3.0	6.01	6.01
Soybean meal		92	20	25	40	30	29.4	6.39	7.99	12.78	9.59
Soybean hulls		90	10	10	10	0	7.48	0.83	0.83	0.83	0.00
							Total	10.23	11.82	19.62	15.59
US (typical)											
Soybean Hulls	R	90	15	20	Nu	Nu	7.48	1.25	1.66	-	-
Aspirated grain	CC	85	5	Nu	Nu	Nu	534.6	31.45	-	-	-
Soybean seed	PC	89	<i>Meal used</i>	15	<i>Meal used</i>	<i>Meal used</i>	26.73		4.51	-	-
Soybean meal*	PC	NA	5	<i>Seed used</i>	25	15	29.4	1.60	-	7.35	4.41
							Total	34.29	6.17	7.35	4.41
US (worse case)											
Soybean Hulls	R	90	15	20	Nu	Nu	7.48	1.25	1.66	-	-
Aspirated grain**	CC	85	5	Nu	Nu	Nu	534.6	31.45	-	-	-
Soybean seed	PC	89	<i>Meal used</i>	15	<i>Meal used</i>	<i>Meal used</i>	26.73		4.51	-	-
Soybean meal*	PC	NA	5	<i>Seed used</i>	25	15	29.4	1.60	-	7.35	4.41
Soybean Forage	R	35	Nu	20	Nu	Nu	114.38	-	65.36	-	-
							Total	34.29	71.53	7.35	4.41

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 AAD-12 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 AAD-12 intake (mg/kg feed)	10.23	11.82	15.59	19.62
	Maximum intake (mg/kg bw)	0.25	0.45	0.47	1.38
	MOE vs Mammalian NOAEL	8000	4444	4255	1449
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 AAD-12 intake (mg/kg feed)	34.29	6.17	4.41	7.35
	Maximum intake (mg/kg bw)	0.57	0.24	0.12	0.20
	MOE vs Mammalian NOAEL	3509	8333	16667	10000
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 AAD-12 intake (mg/kg feed)	34.29	71.53	4.41	7.35
	Maximum intake (mg/kg bw)	0.57	2.81	0.12	0.20
	MOE vs Mammalian NOAEL	3509	713	16667	10000

10. Appendix A— DEEM Output Files for Soybean Acute Exposure to AAD-12 Protein

DEEM Output File for Soybean Acute Exposure to AAD-12 Protein

Cheryl Cleveland Ver. 2.16
DEEM-FCID ACUTE Analysis for AAD12 PROTEIN IN SOYBEAN (1994-98 data)
Residue file: AAD12soybean.R98 Adjustment factor #2 NOT used.
Analysis Date: 06-13-2011/22:14:39 Residue file dated: 06-13-2011/17:40:00/118
NOEL (Acute) = 2000. 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:	0.001904	>1000000	0.003479	574854	0.012794	156319
All infants:	0.064674	30924	0.085045	23516	0.110325	18128
Nursing infants (<1 yr old):	0.010632	188105	0.043840	45620	0.065383	30588
Non-nursing infants (<1 yr old):	0.074448	26864	0.092951	21516	0.115671	17290
Children 1-2 yrs:	0.005662	353245	0.011775	169850	0.047116	42448
Children 3-5 yrs:	0.003780	529065	0.006261	319437	0.012006	166583
Children 6-12 yrs:	0.002412	829108	0.004516	442906	0.008978	222775
Youth 13-19 yrs:	0.001676	>1000000	0.002591	771995	0.004448	449665