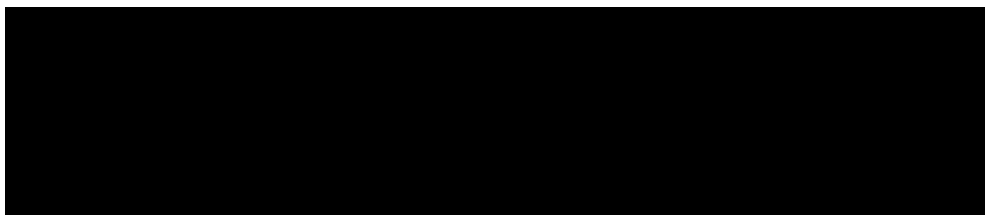


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**Review of literature of DAS-44406-6 soybean in the scope of the  
authorisation for food and feed uses, import and processing in the EU  
(2020 update)**



**PHI-R106-Y20**

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## 1. Summary

An updated systematic search of peer-reviewed literature in line with the EFSA Guidance on conducting a systematic review (EFSA, 2010) and taking into account the explanatory note on literature searching conducted in the context of GMO applications (EFSA, 2019), was conducted with the following review question “Does DAS-44406-6 soybean and derived food/feed products, or the intended traits (the newly expressed protein(s)), have adverse effects on human and animal health and the environment in the scope of the authorisation?”.

The current systematic search complements the search previously performed in 2019. Unless outlined below, all portions of the search were conducted according to the methodologies outlined in the previous search.

The outcome of this analysis showed that one publication relevant for the review question was identified during the selected time period. No safety concerns were identified for DAS-44406-6 soybean by this literature search exercise.

## 2. Confirmation of the Suitability of the Search Strings

All portions of the search were conducted according to the methodologies outlined in the previous searches. It was confirmed that the search strategy utilized in the previous literature search report (2019) is still relevant and no updates were identified.

## 3. Results of the literature search exercise

### 3.1. Outcome of literature searches

In October 2020, searches against electronic bibliographic databases and manual searches in view of screening of reference lists were performed. The search process is reported in line with EFSA guidance (EFSA, 2010 Appendix B4(2)) in Table 2.

**Table 1.** Documenting and reporting the search process

Resources	Date of search	Period searched*	Other restrictions	Number of records retrieved
Web of Science Core collection <sup>#</sup>	7 Oct 2020	2019-7 Oct 2020	None	70
CAB Abstracts <sup>#</sup>	7 Oct 2020	2019-7 Oct 2020	None	36
MEDLINE <sup>#</sup>	7 Oct 2020	2019-7 Oct 2020	None	32
Europe PMC <sup>#</sup>	7 Oct 2020	2019-7 Oct 2020	None	13
Screening reference lists	7 Oct 2020	-	2019-7 Oct 2020 <sup>§</sup>	0 **

<sup>#</sup> A justification for choosing these databases was provided in Section 2.2 of the previous literature search report (2019). The combination of these sources allows having a broad coverage of publications related to GMO risk assessment.

<sup>§</sup> The search syntaxes used are reported in Appendix 1 for electronic bibliographic databases.

<sup>§</sup> The time period was applied post-hoc.

\*\* Number of records screened on full text.

The publications retrieved across all methods of searching (Web of Science Core collection, CAB Abstracts, MEDLINE, Europe PMC, and screening of reference lists) can be found in Appendix 3.

In the framework of the reference list screening exercise, no detailed risk assessments regarding DAS-44406-6 soybean were retrieved that contained information on food and feed safety. Considering that no opinions were published within the selected time period no further screening was performed.

The publications grouped in the Endnote® library were deduplicated. Publications retrieved by the previous searches conducted in the frame of the 2019 annual monitoring report were also removed (see Appendix 3, Section 6).

The results of the publication selection process are presented in Table 2.

**Table 2.** Results of the publication selection process, for the review question

<b>Review question: “Does DAS-44406-6 soybean and derived food/feed products, or the intended traits (the newly expressed protein(s)), have adverse effects on human and animal health and the environment in the scope of the authorisation?”</b>	<b>Number of records</b>
Total number of publications retrieved after all searches of the scientific literature (excluding duplicates and publications retrieved by the previous searches conducted in the frame of the 2019 monitoring reports)	63
Number of publications excluded from the search results after rapid assessment for relevance based on title and abstract	54
Total number of full-text documents assessed in detail	9
Number of publications excluded from further consideration after detailed assessment for relevance based on full text	8
Total number of unobtainable/unclear publications	0
Total number of relevant publications	1

The 63 unique entries present in the Endnote database (Table 2) were manually screened for relevance to the review question by two independent reviewers using the a priori eligibility/inclusion criteria described in Appendix 2.

Entries that are deemed to be irrelevant based on title/abstract were not further retained. In cases where the title/abstract did not contain sufficient information, the publication was progressed to the second stage and assessed for relevance at the level of the full text (as listed in Appendix 4). The reason for excluding a result from the second screening is documented and a justification for not further assessing a reference is provided in Table 4.2 in Appendix 4. No unobtainable/unclear publications were identified (see Appendix 4, Table 4.3).

In this literature search exercise, one peer-reviewed publication relevant to the risk assessment of DAS-44406-6 soybean was identified (Cicchillo et al., 2020) (see Section **Error! Reference source not found.** and Table 4.1 in Appendix 4). Details are provided in **Error! Reference source not found.** in the format laid out by the Commission decision 2009/770/EC (EC, 2009).

**Table 3:** Review of relevant peer-reviewed publication: Food/Feed safety (Cicchillo et al., 2020)

Publication	Summary of research and results	Protection goal	Observed parameter	Adverse effects	Feedback on initial risk assessment
Cicchillo RM, Beeson WT, McCaskill DG, Shan G, Herman R and Walsh TA, <b>2020</b> . Identification of iron-chelating phenolics contributing to seed coat coloration in soybeans ( <i>Glycine max</i> (L.) Merr.) expressing aryloxyalkanoate dioxygenase-12. <i>Phytochemistry</i> 172, 11. <sup>1</sup>	As indicated by the authors, soybeans genetically modified to express aryloxyalkanoate dioxygenase-12 (AAD-12), an enzyme that confers tolerance to the herbicide 2,4-D, can sometimes exhibit a darker seed coat coloration than equivalent unmodified soybeans. The authors investigated the biochemical basis for this coloration “in a non-commercial transgenic event, DAS-411Ø4-7 that exhibited more pronounced AAD-12-associated seed coat coloration than the commercial event, DAS-444Ø6-6.” <sup>2</sup> The authors report that “Analysis of color-enriched seed coat fractions from DAS-411Ø4-7 showed that the color was due to localized accumulation of iron-chelating phenolics, particularly the isoflavone genistin, that are associated with seed coat pectic polysaccharide and produce a brown chromophore. The association between genistin, iron, and pectic polysaccharide was characterized using a variety of analytical methods. Darker seeds from commercial soybean event DAS-444Ø6-6 also show higher genistin content localized to the darker colored portions of the seed coat (with no increase in whole seed genistin levels).”	Food/Feed safety	Darker seed coat coloration	None	No change

<sup>1</sup> This paper was included in the Review of literature of DAS-44406-6 soybean in the scope of the authorisation for food and feed uses, import and processing in the EU (2019 update), while still under review

<sup>2</sup> Text between double quotes is an excerpt from the above-mentioned paper.

#### **4. Conclusion**

One publication was identified as relevant for the molecular characterisation, food/feed and environmental safety of DAS-44406-6 soybean within the scope of the authorisation for the defined time period. No safety concerns have been identified for DAS-44406-6 soybean by this literature search exercise.

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- EC, **2009**. Commission Decision 2009/770/EC of 13 October 2009 establishing standard reporting formats for presenting the monitoring results of the deliberate release into the environment of genetically modified organisms, as or in products, for the purpose of placing on the market, pursuant to Directive 2001/18/EC of the European Parliament and of the Council. *Official Journal of the European Union* 275, 9-27.
- EFSA, **2010**. Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal* 8(6):1637. [90 pp.].
- EFSA, **2019**. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publication 2019:EN-1614. [62 pp.].

**Appendix 1. Detailed search syntaxes for the DAS-44406-6 soybean****Web of Science Core collection**

Set	Search query
Event #1	TS=(DAS44406* OR DAS-44406 OR DAS-444Ø6-6 OR DAS-444-circle-divide-6-6 OR DAS-444empty-set6-6 OR ((44406 OR Enlist*) AND (soy* OR soja* OR Glycine OR Dow OR Corteva OR herbicid*)))
Proteins #2	TS=((2m-epsps OR 2mepsps OR ((5-enolpyruvylshikimate-3-phosphate-synthase OR epsps OR 5-enol-pyruvyl-shikimate-3-phosphate-synthase OR EPSP-synthase) AND modified AND protein AND (maize OR corn OR zea OR mays)) OR (phosphinothricin AND (acetyltransferase OR acetyl-transferase)) OR (pat AND phosphinothricin) OR aad-12 OR aryloxyalkanoate-dioxygenase-12) AND (Streptomyces OR viridochromogenes OR Delftia OR acidovorans OR soy* OR soja* OR glycine OR (((herbicid* AND (genetical* NEAR/3 modif*)) OR GMHT) AND (crop OR plant OR food OR feed)) OR gmo OR gmos OR lmo OR lmos OR gm OR ge))
Traits #3	TS=((glyphosate* OR Roundup OR "Round-up" OR glyfosate* OR glyphosate* OR glifosate* OR ((2-4-D OR AOPP) AND herbicid*) OR 2-4-dichlorophenoxyacetic-acid OR 2-4-dichlorophenoxy-acetic-acid OR aryloxyphenoxypropionate OR aryloxyphenoxy-propionate OR (fop AND (herbicid* or aryloxyphen*)) OR quizalofop OR haloxyfop OR glufosinate* OR gluphosinate* OR (liberty* AND herbicid*)) AND (toler* OR resist* OR protect*) AND (soy* OR soja* OR Glycine OR max) AND (gmo OR gmos OR lmo OR lmos OR living-modified OR transgen* OR GMHT OR ((GM OR GE OR genetic*) NEAR/5 (modif* OR transform* OR manipulat* OR engineer*))))
#4	#1 OR #2 OR #3
Reporting Period #5	PY=(2019-2100)
<b>Final Results</b> #6	#4 AND #5

**CAB Abstracts**

Set	Search query
Event #1	TS=(DAS44406* OR DAS-44406 OR DAS-444Ø6-6 OR DAS-444<o>6-6 OR ((44406 OR Enlist*) AND (soy* OR soja* OR Glycine OR Dow OR Corteva OR herbicid*)))
Proteins #2	TS=((2m-epsps OR 2mepsps OR ((5-enolpyruvylshikimate-3-phosphate-synthase OR epsps OR 5-enol-pyruvyl-shikimate-3-phosphate-synthase OR EPSP-synthase) AND modified AND protein AND (maize OR corn OR zea OR mays)) OR (phosphinothricin AND (acetyltransferase OR acetyl-transferase)) OR (pat AND phosphinothricin) OR aad-12 OR



	aryloxyalkanoate-dioxygenase-12) AND (Streptomyces OR viridochromogenes OR Delftia OR acidovorans OR soy* OR soja* OR glycine OR (((herbicid* AND (genetical* NEAR/3 modif*)) OR GMHT) AND (crop OR plant OR food OR feed)) OR lmo OR lmos OR ge OR "genetically engineered foods"))
Traits #3	TS=((glyphosate* OR Roundup OR "Round-up" OR glyfosate* OR glyphosate* OR glifosate* OR ((2-4-D OR AOPP) AND herbicid*) OR 2-4-dichlorophenoxyacetic-acid OR 2-4-dichlorophenoxy-acetic-acid OR aryloxyphenoxypropionate OR aryloxyphenoxy-propionate OR (fop AND (herbicid* or aryloxyphen*)) OR quizalofop OR haloxyfop OR glufosinate* OR gluphosinate* OR (liberty* AND herbicid*)) AND (toler* OR resist* OR protect*) AND (soy* OR soja* OR Glycine OR max) AND (GMHT OR transgen* OR engineer* OR lmo or lmos OR ge OR manipul* OR transform* OR "genetically engineered foods"))
#4	#1 OR #2 OR #3
Reporting Period #5	PY=(2019-2100)
<b>Final Results</b> #6	#4 AND #5

**MEDLINE**

Set	Search query
Event #1	TS=(DAS44406* OR DAS-44406 OR DAS-44406-6 OR ((44406 OR Enlist*) AND (soy* OR soja* OR Glycine OR Dow OR Corteva OR herbicid*)))
Proteins #2	TS=((2m-epsps OR 2mepsps OR ((5-enolpyruvylshikimate-3-phosphate-synthase OR epsps OR 5-enol-pyruvyl-shikimate-3-phosphate-synthase OR EPSP-synthase) AND modified AND protein AND (maize OR corn OR zea OR mays)) OR (phosphinothricin AND (acetyltransferase OR acetyl-transferase)) OR (pat AND phosphinothricin) OR aad-12 OR aryloxyalkanoate-dioxygenase-12) AND (Streptomyces OR viridochromogenes OR Delftia OR acidovorans OR soy* OR soja* OR glycine OR (((herbicid* AND (genetical* NEAR/3 modif*)) OR GMHT) AND (crop OR plant OR food OR feed)) OR lmo OR lmos OR ge OR "Food, Genetically Modified"))
Traits #3	TS=((glyphosate* OR Roundup OR "Round-up" OR glyfosate* OR glyphosate* OR glifosate* OR ((2-4-D OR AOPP) AND herbicid*) OR 2-4-dichlorophenoxyacetic-acid OR 2-4-dichlorophenoxy-acetic-acid OR aryloxyphenoxypropionate OR aryloxyphenoxy-propionate OR (fop AND (herbicid* or aryloxyphen*)) OR quizalofop OR haloxyfop OR glufosinate* OR gluphosinate* OR (liberty* AND herbicid*)) AND (toler* OR resist* OR protect*) AND (soy* OR soja* OR Glycine OR

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	max) AND (GMHT OR transgen* OR engineer* OR lmo or lmos OR ge OR manipul* OR transform* OR "Food, Genetically Modified"))
#4	#1 OR #2 OR #3
Reporting Period #5	PY=(2019-2100)
<b>Final Results</b> #6	#4 AND #5

**Europe PMC**

(DAS44406 OR DAS444Ø6 OR "das-44406" OR "das-444Ø6" OR "44406 soy\*" OR "444Ø6 soy\*" OR "soy\* 44406" OR "soy\* 444Ø6" OR "Enlist E3") AND (FIRST\_PDATE:[2019-01-01 TO 2020-12-31])

## Appendix 2. Eligibility/Inclusion Criteria

Concept	Criteria
Population (taking into account scope of the authorisation)	<p>Publication addressing human and animal health, and/or the environment relevant for the scope of the authorisation.</p> <p>The pathways and level of exposure to the GMO, derived food/feed products, and the intended traits addressed in the study (as assessed under the Intervention/exposure part) are relevant for the intended uses of the GMO and derived food/feed products under regulatory review (e.g. in case of an authorisation for food, food, import, efficacy of the traits, pest susceptibility, etc. are not considered relevant).</p>
Intervention/exposure	DAS-44406-6 soybean and derived food/feed products, and/or the intended traits (the newly expressed protein(s)).
Intervention/exposure Plant species	In case of studies using GM plants, only studies using soybean are considered eligible. This criterion is not employed for studies regarding the newly expressed proteins.
Intervention/exposure Source organism of the protein	In case of publications using the protein of interest, only publications with the protein from the specific source organism will be considered eligible.
Comparator	If the study is a comparative study that uses plant material as test material, eligible publications must report a non-GM variety.
Outcomes	<p>Effects/impacts on human and animal health, and/or the environment are addressed.</p> <p>Publications addressing other issues such as benefits, socio-economics, ethics, crop protection, detection methods, efficacy, public perception and risk communication are to be excluded using this criterion, as they are not relevant to the risk assessment of GMOs.</p>
Reporting format	<p>Original/primary data are presented in the study. This permits the exclusion of publications that do not present original/primary data (e.g., reviews, editorial, position papers).</p> <p>However, risk assessments from relevant risk assessment bodies (excluding EFSA) will not be excluded.</p>

### **Appendix 3. Entries retrieved by the performed searches to literature databases for the DAS-44406-6 soybean within the indicated search period**

Note: the numbering of the references in the different appendixes is independent of each other (e.g. a certain reference might be called EFSA 2020a in one appendix and EFSA 2020b in another)

#### **1. Entries retrieved using Web of Science Core collection**

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None

<sup>3</sup> The time-period is applied post-hoc as described in Table 2

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#### Appendix 4. Publications screened for relevance based on the full text

**Table 4.1.** Report of all relevant publications retrieved after detailed assessment of full-text documents for relevance

Category of information/ data requirement(s)	Reference (Author, year, title, source)
Food/Feed Safety	Cicchillo RM, Beeson WT, McCaskill DG, Shan G, Herman R and Walsh TA, <b>2020</b> . Identification of iron-chelating phenolics contributing to seed coat coloration in soybeans ( <i>Glycine max</i> (L.) Merr.) expressing aryloxyalkanoate dioxygenase-12. <i>Phytochemistry</i> 172, 11.
Molecular characterisation of the genetic modification of GMO	De Cerqueira DT, Fast BJ, Silveira AC and Herman RA, <b>2019</b> . Transgene-product expression levels in genetically engineered breeding stacks are equivalent to those of the single events. <i>GM crops &amp; food</i> 10, 35-43.

**Table 4.2.** Report of publications excluded from the risk assessment after detailed assessment of full-text documents

Reference (Author, year, title, source)	Reason(s) for exclusion based on eligibility/inclusion criteria
Carlson AB, Mukerji P, Mathesius CA, Huang E, Herman RA, Hoban D, Thurman JD and Roper JM, 2020. DP-202216-6 maize does not adversely affect rats in a 90-day feeding study. <i>Regulatory toxicology and pharmacology</i> : RTP 117, 104779.	Intervention/exposure (not on DAS-44406-6 soybean)
De Cerqueira DT, Fast BJ, Silveira AC and Herman RA, 2019. Transgene-product expression levels in genetically engineered breeding stacks are equivalent to those of the single events. <i>GM crops &amp; food</i> 10, 35-43.	Outcome (correlation between expression levels in stack vs single event)
Fast BJ, Shan GM, Gampala SS and Herman R, 2020. Transgene expression in sprayed and non-sprayed herbicide-tolerant genetically engineered crops is equivalent. <i>Regulatory Toxicology and Pharmacology</i> 111, 8.	Intervention/exposure (not on DAS-44406-6 soybean, expression levels on higher order stack DAS-81419–2xDAS-44406-6)
Geng T, Wang YC, Liu L, Li B and Hill RC, 2019. Endogenous Allergens from Genetically Modified Soybean: Background, Assessment, and Quantification. In: <i>Current Challenges and Advancements in Residue Analytical Methods</i> . Eds Schoenau EA, Geng T, Hill R, Houston NL, Saha Mand Zhou X. Amer Chemical Soc, Washington, 73-94.	Reporting format (Not a primary study: book chapter)
Miyazaki J, Bauer-Panskus A, Bohn T, Reichenbecher W and Then C, 2019. Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. <i>Environmental Sciences Europe</i> 31, 21.	Reporting format (Not a primary study: review)
Naegeli H, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firbank L, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogu F, Rostoks N, Serrano JJS, Savoini G, Veromann E, Veronesi F, Alvarez-Alfageme F, Ardizzone M, Dumont AF, Devos Y,	Reporting format (Not a primary study) <sup>4</sup>

<sup>4</sup> The EFSA GMO Panel concludes that SYHT0H2 soybean (containing PAT) is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment

Gennaro A, Ruiz JAG, Lanzoni A, Neri FM, Paraskevopoulos K and Or EPGM, 2020a. Assessment of genetically modified soybean SYHT0H2 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2012-111). Efsa Journal 18, 29.	
Naegeli H, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firkbank LG, Guerche P, Hejatko J, Moreno FJ, Mullins E, Nogue F, Rostoks N, Serrano JJS, Savoini G, Veromann E, Veronesi F, Alvarez F, Ardizzone M, De Sanctis G, Dumont A, Devos Y, Gennaro A, Ruiz JAG, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K, Raffaello T and Modified EPG, 2020b. Assessment of genetically modified soybean MON 87705 x MON 87708 x MON 89788, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-126). Efsa Journal 18, 36.	Reporting format (Not a primary study)
Steinberg P, van der Voet H, Goedhart PW, Kleter G, Kok EJ, Pla M, Nadal A, Zeljenkova D, Alacova R, Babincova J, Rollerova E, Jadudova S, Kebis A, Szabova E, Tulinska J, Liskova A, Takacsova M, Mikusova ML, Krivosikova Z, Spok A, Racovita M, de Vriend H, Alison R, Alison C, Baumgartner W, Becker K, Lempp C, Schmicke M, Schrenk D, Poting A, Schiemann J and Wilhelm R, 2019. Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Archives of toxicology 93, 1095-1139.	Intervention/exposure (not on DAS-44406-6 soybean)

**Table 4.3.** Report of unobtainable/unclear publications

Reference (Author, year, title, source)	Description of (unsuccessful) methods used to try to obtain a copy of the publication
None	Not applicable