Review of literature for 305423 and 305423x40-3-2 soybeans in the scope of their authorisations for food and feed uses, import and processing in the EU (2021 update)

PHI-R131-Y21

Table of contents

1. Summary

An updated systematic search and review of peer-reviewed literature was conducted for 305423 soybean and 305423 x 40-3-2 soybean. This exercise was performed in line with the EFSA Guidance on conducting a systematic review (EFSA, 2010) and taking into account the explanatory note on literature searching (EFSA, 2019), with the following review question "Does 305423 soybean or 305423 x 40-3-2 soybean and derived food/feed products, or the intended traits (the newly expressed proteins or their combination) have adverse effects on human and animal health and the environment in the scope of their authorisation?".

The current systematic search complements the searches previously performed in 2020. All portions of the search were conducted according to the methodologies outlined in the previous searches.

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 305423 soybean nor 305423x40-3-2 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 (2020) is still relevant and no updates were identified.

3. Results of the Scoping Exercise

3.1. Outcome of literature searches

In July 2021, 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 1.

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[$]	7 July 2021	2020-7 July 2021	None	27
CAB Abstracts[$]	7 July 2021	2020-7 July 2021	None	13
MEDLINE[$]	7 July 2021	2020-7 July 2021	None	16
Europe PMC[$]	7 July 2021	2020-7 July 2021	None	15
Screening reference lists	NA	-	NA	NA

[$] The search syntaxes used for electronic bibliographic databases are reported in Appendix 1.
NA: Not applicable as no publications relevant for screening reference lists were identified.

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 305423 or 305423x40-3-2 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 2020 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

Review question: "Does 305423 soybean or 305423 x 40-3-2 soybean and derived food/feed products, or the intended traits (the newly expressed protein(s) or their combination), have adverse effects on human and animal health and the environment in the scope of their authorisation?"	**Number of records**
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 2020 monitoring reports)	21
Number of publications excluded from the search results after rapid assessment for relevance based on title and abstract	19
Total number of full-text documents assessed in detail	2
Number of publications excluded from further consideration after detailed assessment for relevance based on full text	1
Total number of unobtainable/unclear publications	0
Total number of relevant publications	1

The 21 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.

In the first stage of screening, entries were screened based on title/abstract. Records that were deemed to be irrelevant were not further retained. In cases where the record seemed relevant, or if 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.

Publications assessed at full text level and found not to be relevant were not further assessed and a justification was provided. Records that are relevant were summarized and their potential to influence the initial risk assessment was evaluated in the format laid out by the Commission decision 2009/770/EC (EC, 2009).

In this literature search exercise, one peer-reviewed publication relevant to the risk assessment was identified (Hemingway et al., 2021) (see Appendix 4, Table 4.1 and Table 3). Publications excluded after assessment of the full-text are presented in Table 4.2 in Appendix 4 and a reason for exclusion based on the eligibility/inclusion criteria is provided. No unclear publications were identified (see Appendix 4, Table 4.3).

Table 3: Review of a recent relevant paper in 2009/770/EC format. Agronomic, phenotypic and compositional characterisation of the GM plant (Hemingway et al., 2021):

Publication	Summary of research and results	Protection goal	Observed parameter	Adverse effects	Feedback on initial risk assessment
Hemingway J, Schnebly SR and Rajcan I, 2021. Agronomic and seed traits of high-oleic soybean lines containing the DP-305423-1 transgene in four backcross populations. Crop Science 61, 500-518.	The DP-305423-1 transgene elevates oleic acid concentration and reduces polyunsaturated fatty acids in soybean [Glycine max (L.) Merr.] oil, resulting in increased oxidative stability. In light of previous work suggesting that seed yield might be negatively influenced by the HO phenotype induced by the DP-305423-1 event across multiple genetic backgrounds, the authors performed research to investigate the effect of the DP-305423-1 transgene on agronomic and seed traits using four backcross derived soybean populations to provide further understanding of the transgene's influence across varying genetic backgrounds and environments. The authors tested more elite populations developed with more modern HO parents compared with those used by previous work and created with one cycle of backcrossing to the elite NO parent to increase the genetic contribution of the superior parent. Additionally, comparisons were made using high-oleic, low-linolenic (HOLL) and normal-oleic, low-linolenic (NOLL) progeny, which previously had not been done. The authors found a significantly negative correlation between oil concentration and oleic acid concentration and significantly positive correlation between protein concentration and oleic acid concentration across all genotypes. The results of this study demonstrate no influence of the DP-305423-1 transgene on seed yield and suggest that the negative association between seed yield and the HO trait in progeny containing the DP-305423-1 transgene, as shown by previous work, may be attributed to the genetic background and population structure of the populations tested rather than the transgene itself.	Agronomic, phenotypic and compositional characterisation of the GM plant	Comparative assessment of agronomic and phenotypic characteristics	None	No change

4. Conclusion

One publication was identified as relevant for the molecular characterisation, food/feed and environmental safety of 305423 or 305423 x 40-3-2 soybean within the scope of the authorisations for the defined time period. No safety concerns have been identified for 305423 or 305423 x 40-3-2 soybeans by this literature search exercise.

References

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 authorized soybeans

Web of Science Core collection

Search Part	Search Syntax
Event #1	TS=(3ø5423* OR 3-circle-divide-5423* OR 3empty-set5423* OR 305423* OR dp305423* OR dp3ø5423* OR dp3-circle-divide-5423* OR dp3empty-set5423* OR plenish*)
Stack #2	TS=(*DP-3Ø5423-1xMON-Ø4Ø32-6* OR *DP-3-circle-divide-5423-1xMON-circle-divide-4-circle-divide-32-6* OR *DP-3empty-set5423-1xMON-empty-set4empty-set32-6* OR *305423x40-3-2* OR *3Ø5423x40-3-2* OR Plenish*)
#3	#1 OR #2
Proteins #4	TS=((gm-fad2 OR gmfad2 OR gm-hra OR gmhra OR Glycine-max-HRA OR fad2 OR fatty-acid-desaturase-2-1 OR omega-6-fatty-acid-desaturase OR (hra AND acetolactate-synthase)) AND (soy* OR soja* OR glycine OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna 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 OR stack))
Traits #5	TS=(((high NEAR/1 oleic) OR (oleic NEAR/1 acid) OR sul*onylurea* OR ALS-inhibiting-herbicide*) AND (toler* OR resist* OR protec* OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna) 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* OR stack))))
#6	#3 OR #4 OR #5
Reporting Period #7	PY=(2020-2100)
Final Results #8	#6 AND #7

CAB Abstracts

Search Part	Search Syntax
Event #1	TS=(3ø5423* OR 3<o>5423* OR 305423* OR dp305423* OR dp3ø5423* OR dp3<o>5423* OR plenish*)
Stack #2	TS=(*DP-3Ø5423-1xMON-Ø4Ø32-6* OR *DP-3-circle-divide-5423-1xMON-circle-divide-4-circle-divide-32-6* OR *DP-3empty-set5423-1xMON-empty-set4empty-set32-6* OR *305423x40-3-2* OR *3Ø5423x40-3-2* OR Plenish*)
#3	#1 OR #2
Proteins #4	TS=((gm-fad2 OR gmfad2 OR gm-hra OR gmhra OR Glycine-max-HRA OR fad2 OR fatty-acid-desaturase-2-1 OR omega-6-fatty-acid-desaturase OR (hra AND acetolactate-synthase)) AND (soy* OR soja* OR glycine OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna 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" OR stack))
Traits #5	TS=(((high NEAR/1 oleic) OR (oleic NEAR/1 acid) OR sul*onylurea* OR ALS-inhibiting-herbicide*) AND (toler* OR resist* OR protec* OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna) AND (soy* OR soja* OR Glycine OR max) AND (GMHT OR transgen* OR engineer* OR lmo or lmos OR ge OR manipulat* OR transform* OR stack OR "genetically engineered foods"))
#6	#3 OR #4 OR #5
Reporting Period #7	PY=(2020-2100)
Final Results #8	#6 AND #7

MEDLINE

Search Part	Search Syntax
Event #1	TS=(3ø5423* OR 305423* OR dp305423* OR dp3ø5423* OR plenish*)
Stack #2	TS=(*DP-3Ø5423-1xMON-Ø4Ø32-6* OR *305423x40-3-2* OR *3Ø5423x40-3-2* OR Plenish*)
#3	#1 OR #2
Proteins #4	TS=((gm-fad2 OR gmfad2 OR gm-hra OR gmhra OR Glycine-max-HRA OR fad2 OR fatty-acid-desaturase-2-1 OR omega-6-fatty-acid-desaturase OR (hra AND acetolactate-synthase)) AND (soy* OR soja* OR glycine OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna 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" OR stack))
Traits #5	TS=(((high NEAR/1 oleic) OR (oleic NEAR/1 acid) OR sul*onylurea* OR ALS-inhibiting-herbicide*) AND (toler* OR resist* OR protec* OR Rnai OR rna-interference OR siRNA OR small-RNA OR *silencing OR double-stranded-rna OR dsrna) AND (soy* OR soja* OR Glycine OR max) AND (GMHT OR transgen* OR engineer* OR lmo or lmos OR ge OR manipulat* OR transform* OR stack OR "Food, Genetically Modified"))
#6	#3 OR #4 OR #5
Reporting Period #7	PY=(2020-2100)
Final Results #8	#6 AND #7

Europe PMC

(plenish OR 305423x40-3-2 OR dp305423 OR dp3ø5423 OR 305423 OR 3ø5423) AND (FIRST_PDATE:[2020-01-01 TO 2100-12-31])

Appendix 2. Eligibility/Inclusion Criteria[1]

Concept	Criteria
Population (taking into account scope of the authorisation)	Publication addressing human and animal health, and/or the environment relevant for the scope of the authorisation. 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 **I**ntervention/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).
Intervention/exposure	305423, 305423x40-3-2 soybeans and derived food/feed products, and/or the intended traits (newly expressed protein(s) or their combination).
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	Effects/impacts on human and animal health, and/or the environment are addressed. 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.
Reporting format	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). However, risk assessments from relevant risk assessment bodies (excluding EFSA) will not be excluded.

[1] This table is provided for ease of reference, no updates have been introduced since the previous report.

Appendix 3. Entries retrieved by the performed searches to literature databases for the authorised soybeans 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 2021a in one appendix and EFSA 2021b in another)

1. Entries retrieved using Web of Science Core collection

al Amin N, Ahmad N, Wu N, Pu XM, Ma T, Du YY, Bo XX, Wang N, Sharif R and Wang PW, **2020**. CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal omega-6 desaturase in soybean (Glycine max.L) (vol 19, 9, 2019). Bmc Biotechnology 20, 2. 10.1186/s12896-020-00634-x

Chen GQ, Johnson K, Nazarenus TJ, Ponciano G, Morales E and Cahoon EB, **2021**. Genetic Engineering of Lesquerella with Increased Ricinoleic Acid Content in Seed Oil. Plants-Basel 10, 14. 10.3390/plants10061093

Darr L, Cunicelli M, Bhandari H, Bilyeu K, Chen F, Hewezi T, Li ZL, Sams C and Pantalone V, **2020**. Field Performance of High Oleic Soybeans with Mutant FAD2-1A and FAD2-1B Genes in Tennessee. Journal of the American Oil Chemists Society 97, 49-56. 10.1002/aocs.12306

Demeke T, Eng M, Holigroski M and Lee SJ, **2021**. Effect of Amount of DNA on Digital PCR Assessment of Genetically Engineered Canola and Soybean Events. Food Analytical Methods 14, 372-379. 10.1007/s12161-020-01889-y

Deol P, Kozlova E, Valdez M, Ho C, Yang EW, Richardson H, Gonzalez G, Truong E, Reid J, Valdez J, Deans JR, Martinez-Lomeli J, Evans JR, Jiang T, Sladek FM and Curras-Collazo MC, **2020**. Dysregulation of Hypothalamic Gene Expression and the Oxytocinergic System by Soybean Oil Diets in Male Mice. Endocrinology 161, 21. 10.1210/endocr/bgz044

Dong LM, Long LK, Xing ZJ, Li CC, He YX, Yan W, Xia W and Li FW, **2020**. Establishment of Multi-Fluorescence Real-Time PCR Assay for GM Soybean Detection. International Journal of Agriculture and Biology 24, 292-298. 10.17957/ijab/15.1437

Gaskin EL, Carrero-Colon M and Hudson KA, **2021**. Combination of the Elevated Stearic Acid Trait with Other Fatty Acid Traits in Soybean. Journal of the American Oil Chemists Society 98, 221-226. 10.1002/aocs.12446

Hemingway J, Schnebly SR and Rajcan I, **2021**. Agronomic and seed traits of high-oleic soybean lines containing the DP-305423-1 transgene in four backcross populations. Crop Science 61, 500-518. 10.1002/csc2.20341

Jarvis BA, Romsdahl TB, McGinn MG, Nazarenus TJ, Cahoon EB, Chapman KD and Sedbrook JC, **2021**. CRISPR/Cas9-Induced fad2 and rod1 Mutations Stacked With fae1 Confer High Oleic Acid Seed Oil in Pennycress (Thlaspi arvense L.). Frontiers in Plant Science 12, 18. 10.3389/fpls.2021.652319

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Lakhssassi N, Lopes-Caitar VS, Knizia D, Cullen MA, Badad O, El Baze A, Zhou Z, Embaby MG, Meksem J, Lakhssassi A, Chen PY, AbuGhazaleh A, Vuong TD, Nguyen HT, Hewezi T and Meksem K, **2021**. TILLING-by-Sequencing(+) Reveals the Role of Novel Fatty Acid Desaturases (GmFAD2-2s) in Increasing Soybean Seed Oleic Acid Content. Cells 10, 20. 10.3390/cells10051245

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Oil Biosynthesis Pathway in Soybeans: A New and Effective Platform for High-Throughput Gene Functional Analysis. International Journal of Molecular Sciences 22, 21. 10.3390/ijms22084219

Lee MW, Padilla CS, Gupta C, Galla A, Pereira A, Li JM and Goggin FL, **2020**. The FATTY ACID DESATURASE2 Family in Tomato Contributes to Primary Metabolism and Stress Responses. Plant Physiology 182, 1083-1099. 10.1104/pp.19.00487

Naegeli H, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firbank 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, **2020**. 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. 10.2903/j.efsa.2020.6111

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Wu H, Qian C, Wu C, Wang Z, Wang DC, Ye ZZ, Ping JF, Wu J and Ji F, **2020**. End-point dual specific detection of nucleic acids using CRISPR/Cas12a based portable biosensor. Biosensors & Bioelectronics 157, 7. 10.1016/j.bios.2020.112153

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Yeom WW, Kim HJ, Lee KR, Cho HS, Kim JY, Jung HW, Oh SW, Jun SE, Kim HU and Chung YS, **2020**. Increased Production of alpha-Linolenic Acid in Soybean Seeds by Overexpression of Lesquerella FAD3-1. Frontiers in Plant Science 10, 14. 10.3389/fpls.2019.01812

Zhang L, Wang YZ, Li T, Qiu HM, Xia ZJ and Dong YS, **2021**. Target-specific mutations efficiency at multiple loci of CRISPR/Cas9 system using one sgRNA in soybean. Transgenic Research 30, 51-62. 10.1007/s11248-020-00228-5

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2. <u>Entries retrieved using CAB Abstracts</u>

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Lee M, Padilla CS, Gupta C, Galla A, Pereira A, Li J and Goggin FL, **2020**. The FATTY ACID DESATURASE2 family in tomato contributes to primary metabolism and stress responses. Plant Physiology 182, 1083-1099.

Naegeli H, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firbank 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, Sanctis Gd, Dumont A, Devos Y, Gennaro A, Ruiz JAG, Lanzoni A, Neri FM, Papadopoulou N and et al., **2020**. Assessment of genetically modified soybean MON 87705 * MON 87708 * MON 89788, for food and feed uses, under regulation (ec) no 1829/2003 (application EFSA -GMO -NL -2015-126). Efsa Journal 18. 10.2903/j.efsa.2020.6111

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Narayanan S, Zoong-Lwe ZS, Gandhi N, Welti R, Fallen B, Smith JR and Rustgi S, **2020**.

Comparative lipidomic analysis reveals heat stress responses of two soybean genotypes differing in temperature sensitivity. Plants 9. 10.3390/plants9040457

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Niu Y, Zhang G, Wan F and Zhang Y, **2020**. Integration of RNA-seq profiling with genome-wide association study predicts candidate genes for oil accumulation in soybean. Crop & Pasture Science 71, 996-1009. 10.1071/cp20358

Zhang L, Wang Y, Li T, Qiu H, Xia Z and Dong Y, **2021**. Target-specific mutations efficiency at multiple loci of CRISPR/Cas9 system using one sgRNA in soybean. Transgenic Research 30, 51-62. 10.1007/s11248-020-00228-5

3. <u>Entries retrieved using MEDLINE</u>

Al Amin N, Ahmad N, Wu N, Pu X, Ma T, Du Y, Bo X, Wang N, Sharif R and Wang P, **2020**. Correction to: CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal omega-6 desaturase in soybean (Glycine max.L). Bmc Biotechnology 20, 42. 10.1186/s12896-020-00634-x

Chen GQ, Johnson K, Nazarenus TJ, Ponciano G, Morales E and Cahoon EB, **2021**. Genetic Engineering of Lesquerella with Increased Ricinoleic Acid Content in Seed Oil. Plants (Basel, Switzerland) 10. 10.3390/plants10061093

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5. Entries retrieved using reference lists of opinions of regulatory bodies and screened on full text

None

6. New entries retrieved using all search strategies (excluding duplicates and studies retrieved by the previous searches conducted in 2020)

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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 info/ data requirement(s)	Publication
Agronomic, phenotypic and compositional characterisation of the GM plant	Hemingway J, Schnebly SR and Rajcan I, 2021. Agronomic and seed traits of high-oleic soybean lines containing the DP-305423-1 transgene in four backcross populations. Crop Science 61, 500-518.

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
Gaskin EL, Carrero-Colon M and Hudson KA, 2021. Combination of the Elevated Stearic Acid Trait with Other Fatty Acid Traits in Soybean. Journal of the American Oil Chemists Society 98, 221-226.	Intervention/ exposure (not on 305423 or 305423x40-3-2)

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